



UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM PRODUTOS
NATURAIS E SINTÉTICOS BIOATIVOS



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**Desenho computacional de metabólitos secundários de Annonaceae: Seleção e
atividades antiparasitárias**

**Computer design of secondary metabolites of Annonaceae: Selection and
antiparasitic activities**

JOÃO PESSOA

2022

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Desenho computacional de metabólitos secundários de Annonaceae: Seleção e atividades antiparasitárias

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*Tese de doutorado do Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, do Centro de Ciências da Saúde, da Universidade Federal da Paraíba, como requisito para obtenção do título de Doutora em Produtos Naturais e Sintéticos Bioativos, na área de concentração: **Farmacoquímica.***

Orientador: Prof. Dr. Marcus Tullius Scotti

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JOÃO PESSOA - PB

2022

Catálogo na publicação
Seção de Catalogação e Classificação

M543d Menezes, Renata Priscila Barros de.

Desenho computacional de metabólitos secundários de
Annonaceae : seleção e atividades antiparasitárias -
Computer design of secondary metabolites of Annonaceae
: selection and antiparasitic activities / Renata
Priscila Barros de Menezes. - João Pessoa, 2022.
412 f. : il.

Orientação: Marcus Tullius Scotti.

Coorientação: Suzana Guimarães Leitão.

Tese (Doutorado) - UFPB/CCS.

1. Annonaceae. 2. QSAR. 3. Aprendizado de máquina.
4. Triagem virtual baseada em ligante. 5.
Antiparasitária. 6. Metaboloma. I. Scotti, Marcus
Tullius. II. Leitão, Suzana Guimarães. III. Título.

UFPB/BC

CDU 582.677.5(043)

Desenho computacional de metabólitos secundários de Annonaceae: Seleção e atividades antiparasitárias

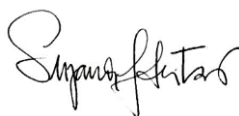
Computer design of secondary metabolites of Annonaceae: Selection and antiparasitic activities

Aprovada em: 25/08/2022

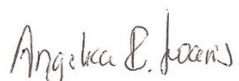
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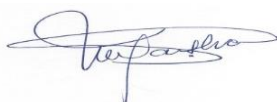
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A Ele que é a razão da minha existência, o ar que eu respiro e o principal responsável pelas minhas conquistas... Dedico a Deus,
o Senhor Soberano de todo o universo!

“Como é precioso o teu amor ó Deus! ”

Salmos 36:7a

AGRADECIMENTOS

Agradeço à Deus, em primeiro lugar, por todo cuidado, infinito amor e maravilhosa graça. Sou grata, meu Deus, por toda essa caminhada do doutorado, por toda experiência vivida, lutas, desafios, vitórias, o amadurecimento, um longo caminho, mas que tanto me fez crescer.

Minha gratidão à minha família, aos meus pais e meus irmãos que tanto me incentivaram e me apoiaram. Ter vocês em minha vida, compartilhando tantos momentos, aquece o coração, me enche de alegria e gratidão. Painha e Mainha, reconheço todo o esforço que fizeram por mim e sou extremamente grata por tudo. Fernanda, minha irmã, obrigada por ouvir pacientemente as histórias e acontecimentos do doutorado e me incentivar quando eu cansava e queria desistir.

Meu muito obrigada a meu marido amado, Anderson, por todo incentivo que me dá, por todo apoio, suporte. Te agradeço por me emprestar seus ouvidos e escutar meus monólogos discutindo os resultados da pesquisa ou um artigo, e você mesmo sem compreender boa parte do que eu estava falando, escutava tudo pacientemente, muito obrigada. Grata sou pela sua vida, por tudo que estamos construindo como família, pelo carinho, amor, companheirismo, você me faz muito feliz.

Ao meu orientador, Prof. Dr. Marcus Scotti, por todo apoio, encorajamento e por acreditar no meu potencial. Obrigada por estar sempre disponível, sempre disposto a ajudar, tirar dúvidas, orientar. Foram várias reuniões no laboratório, chamadas de vídeo, mensagens no WhatsApp, e-mails, que foram fundamentais para a realização dessa tese. A sua orientação, todo o conhecimento compartilhado, não só científico, mas de tantos outros assuntos, porque o senhor também é cultura, irão ficar sempre em minhas memórias. O senhor é um grande mestre, uma referência para mim, muito obrigada por tudo.

A minha coorientadora, Profa. Dra. Suzana Leitão, muito obrigada por abrir as portas do seu laboratório para mim, pelo apoio, por fazer possível nossa colaboração e a realização do trabalho. Apesar do pouco contato presencial, devido a pandemia, a senhora foi inspiradora, sempre muito amável e dedicada.

Agradeço à professor Eugene Muratov por todo conhecimento e pelos ensinamentos que me passou. O senhor não só acreditou no meu potencial, como me incentivou a ir mais longe. Muito obrigada.

Agradeço aos amigos do laboratório de quimioinformática pelo conhecimento compartilhado, pelas conversas, pelos lanches da tarde (um momento para aliviar o estresse). Natália, Andreza, Chonny, Mayara, Gabi, Luana.

Aos amigos do laboratório de “Tia Su”, particularmente a Brendo e Thamirys, vocês são especiais, muito obrigada pela disponibilidade, contribuição ao meu trabalho, e toda ajuda.

Aos meus amigos: Diégina, Amanda, Sallet, Rayanne, Ízola e Vannuty, por todas as conversas, conselhos, apoio, vocês foram fundamentais para tornar essa caminhada mais divertida, mais fácil e mais leve.

Obrigada aos amigos que a cidade maravilhosa me deu: Sávio, Samkya, Camila, Diogo, Laís e Savyo. Vocês ajudaram a aliviar o fardo da vida e do doutorado. Que a amizade e carinho perdure.

Sou grata a todos com quem pude colaborar em trabalhos incríveis, que renderam tantos artigos, o aprendizado e conhecimento trocado foram gratificantes.

Obrigada aos amigos que fiz no doutorado e que torcem pelo meu sucesso, me apoiam e incentivam.

Agradeço também aos professores Dr. Klinger Rodrigues e Dr. Igor Rodrigues que aceitaram a parceria para realização dos testes *in vitro* e tanto contribuiu para a conclusão da minha tese.

Aos professores do PgPNSB pelos ensinamentos e conhecimentos compartilhados.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) por ter financiado a pesquisa.

Enfim, sou muito grata a todos que colaboraram de alguma forma para a concretização deste meu doutorado.

Muito obrigada a todos vocês.

RESUMO

As plantas são fontes ricas de produtos naturais, que por sua vez são fonte potencial de substâncias bioativas para o desenvolvimento de novos medicamentos. A família Annonaceae é extremamente rica em metabolitos secundários, com grande diversidade química, apresentando uma grande variedade de potencial biológico. O uso de técnicas computacionais para a descoberta de novos fármacos tem se tornado cada vez mais comum e necessário, uma vez que leva à redução de custos de pesquisa e tempo. O desenvolvimento de drogas assistido por computador permite a exploração de grandes bases de dados químicos, reduzindo esses bancos a conjuntos de moléculas com potencial elevado de atividade biológica, processo conhecido como triagem virtual. Portanto, este estudo tem como objetivo realizar estudos computacionais com vistas a obtenção de moléculas promissoras com atividade biológica para as doenças negligenciadas, doença de Chagas e leishmaniose a partir de banco de dados de metabólitos secundários de Annonaceae. Além de investigar o potencial leishmanicida de extratos de quatro espécies de *Annona* (*Annona glabra*, *Annona mucosa*, *Annona sylvatica* e *Annona dolabripetala*) por meio de uma abordagem metabolômica usando análise estatística multivariada (PCA e PLS) e dados de LC-MS, para correlacionar dados espectroscópicos com atividade leishmanicida, buscando sugerir compostos ou grupos de compostos responsáveis pela atividade biológica. No capítulo 1 foi realizada uma revisão dos estudos de atividade biológica realizados com espécies da família Annonaceae objetivando evidenciar quão versátil e promissora é essa família na busca por novas drogas. No capítulo 2 foi realizada uma revisão sobre aprendizado de máquina aplicado a QSAR, escrito em português, com intuito de abordar o assunto de forma simples e didática para estudantes e pesquisadores que estão iniciando nesta área tão promissora e importante. No capítulo 3 a partir da construção de um banco de dados com os metabolitos secundários já isolados da família Annonaceae entre os anos de 1970 e 2019, foi realizada uma análise quimiotaxonômica utilizando a classe de diterpenos. Através deste estudo quimiotaxonômico foi possível separar as tribos Annoneae, Xylopieae e Miliuseae de acordo com a separação morfológica e taxonômica da família. Esse fenômeno permite prever a localização de um determinado diterpeno nas tribos Annoneae, Xylopieae e Miliuseae das Annonaceae e buscar esses metabólitos secundários e seus potenciais biológicos de forma mais eficaz. Nos capítulos 4 e 5 são realizados estudos de triagem virtual baseada em ligante em busca de moléculas com potencial atividade antichagásica e leishmanicida utilizando o banco de dados de metabólitos secundários de Annonaceae, constituído por 1860 moléculas. Os modelos preditivos criados para *L. amazonensis* e *T. cruzi* obtiveram uma acurácia acima de 72%. Para os dois protozoários foi possível identificar moléculas potencialmente ativas, selecionar algumas delas e realizar o teste *in vitro*. Para o *T. cruzi* o ácido 13-epicupressico foi o mais promissor, pois foi previsto como composto ativo no estudo *in silico* contra a forma amastigota do *T. cruzi*, além de possuir atividade *in vitro* contra a forma epimastigota. Já para *L. amazonensis* O triterpeno lupeol, apresentou a melhor atividade em ensaios biológicos *in silico* e *in vitro* para a forma promastigota, além de possuir probabilidade de potencial ativo superior a 77% contra a forma amastigota. No capítulo 6 foi realizado uma análise metabolômica utilizando análise estatística multivariada (PCA e PLS) e dados de LC-MS, para correlacionar dados espectroscópicos com atividade leishmanicida, buscando sugerir compostos ou grupos de compostos responsáveis pela atividade biológica de 4 espécies de *Annona*.

Palavras-chave: Annonaceae, QSAR, aprendizado de máquina, triagem virtual baseada em ligante, antiparasitária, metaboloma.

ABSTRACT

Plants are rich sources of natural products, which in turn are a potential source of bioactive substances for the development of new drugs. The Annonaceae family is extremely rich in secondary metabolites, with great chemical diversity, presenting a vast variety of biological potential. The use of computational techniques for the discovery of new drugs has become increasingly common and necessary since it leads to a reduction in research costs and time. Computer-assisted drug development allows the exploration of large chemical databases, reducing these banks to sets of molecules with high potential for biological activity, a process known as virtual screening. Therefore, this study aims to perform computational studies to obtain promising molecules with biological activity for neglected diseases, Chagas disease and leishmaniasis from the Annonaceae secondary metabolite database. In addition to investigating the leishmanicidal potential of extracts from four *Annona* species (*Annona glabra*, *Annona mucosa*, *Annona sylvatica* and *Annona dolabripetala*) through a metabolomics approach using multivariate statistical analysis (PCA and PLS) and LC-MS data, to correlate spectroscopic data with leishmanicidal activity, seeking to suggest compounds or groups of compounds responsible for the biological activity. In chapter 1, a review of the biological activity studies conducted with species of the Annonaceae family was conducted, aiming to show how versatile and promising this family is in the search for new drugs. In chapter 2, a review was conducted on machine learning applied to QSAR, written in Portuguese, to approach the subject in a simple and didactic way for students and researchers who are starting in this very promising and important area. In chapter 3, based on the construction of a database with secondary metabolites already isolated from the Annonaceae family between 1970 and 2019, a chemotaxonomic analysis was performed using the class of diterpenes. Through this chemotaxonomic study it was possible to separate the Annoneae, Xylopieae and Miliuseae tribes according to the morphological and taxonomic separation of the family. This phenomenon makes it possible to predict the location of a particular diterpene in the Annoneae, Xylopiieae and Miliuseae tribes of the Annonaceae and to search for these secondary metabolites and their biological potential more effectively. In chapters 4 and 5, virtual screening studies based on ligand are conducted in search of molecules with potential antichagasic and leishmanicidal activity using the Annonaceae secondary metabolite database, consisting of 1860 molecules. The predictive models created for *L. amazonensis* and *T. cruzi* obtained an accuracy above 72%. For the two protozoa it was possible to identify potentially active molecules, select some of them and perform the *in vitro* test. For *T. cruzi*, 13-epicupressic acid was the most promising, as it was predicted as an active compound in the *in silico* study against the amastigote form of *T. cruzi*, in addition to having *in vitro* activity against the epimastigote form. As for *L. amazonensis*, the triterpene lupeol showed the best activity in *in silico* and *in vitro* biological assays for the promastigote form, in addition to having a probability of active potential greater than 77% against the amastigote form. In chapter 6, a metabolomic analysis was performed using multivariate statistical analysis (PCA and PLS) and LC-MS data, to correlate spectroscopic data with leishmanicidal activity, seeking to suggest compounds or groups of compounds responsible for the biological activity of 4 *Annona* species.

Keywords: Annonaceae, QSAR, machine learning, ligand-based virtual screening, antiparasitic, metabolomic.

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LISTA DE ABREVIATURAS, SIGLAS E FÓRMULAS

5-FU	5-Fluorouracil
5-LOX	5-Lipoxygenase
A-498	Kidney carcinoma
A-549	Lung Carcinoma
AC	Annona crassiflora
ACC	Accuracy
ADMET	Administração, Distribuição, Metabolização, Excreção e Toxicidade
AGS	Gastric cancer cells
AMR	Antimicrobial Resistance
ANN	Artificial Neural Network
APD	Applicability domain
APX	Ascorbate Peroxidase
ASK	Rat Glioma
ATCC	American Type Culture Collection
AUC	Área abaixo da curva/Area under the curve
BAARS	Acid-Resistant Bacilli
BST	Brine Shrimp Lethality Test
CADD	ComputerAided Drug Design
CAT	Catalase
CC₅₀	50% cytotoxic concentration
CD	Chagas disease
Col-2	Human Colon Cancer
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CV	Cross-validation
DI-MS	Direct Injection Mass Spectrometry
DLD1	Colon cancer cells
DMARDs	Disease-Modifying Anti-Rheumatic Drugs
DNDi	Drug for Neglected Diseases
DOX	Doxorubicin
DPPH	1,2-diphenyl-2-picryl-hydrazyl

DT	Decision Tree
EEAM	Acetate extract from <i>Annona muricata</i>
EO	Essential Oil
Epim.	Epimastigote
ESP_{MuDRA}	MuDRA model specificity
ESP_{RF}	RF model specificity
FMLP/CB	formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B
FN	Falso negativo/False negative
FP	Falso positivo/False positive
GC-MS	Gas Chromatography
GNT	Goniothalamine
GU	Gastric ulcer
HA59T	Hepatoma cells
HCT-116	Colon carcinoma
Hek 293	Noncancerous humam embryonic kidney cell
Hep-G2	Hepato G2
HL-60	Human Promyelocytic Leukemia
HPLC	High-performance liquid chromatography
HSC-3	Oral Squamous
HT-29 colon	Adenocarcinoma
HTS	High-Throughput Screening
	concentration of an inhibitor where the response (or binding) is reduced
IC₅₀	by half
IL-6	Interleukine-6
iNOS	Inducible Nitric Oxide Synthase
K1	multi-resistant strains
K-562	Human Chronic myelocytic leukemia
KA	Kauren-19-oic acid
KAH	(-)-Kauran-19-oic acid
KB	Human Epidermoide Carcinoma
KM-12	Colon adenocarcinoma
kNN	k-nearest Neighbors
LBVS	Ligand-Based Virtual Screening

LC-MS	Liquid Chromatography
LD₅₀	Median Lethal Dose
LOX	Lipoxygenase
LPS	Lipopolysaccharide
Lu-1	Human Lung Cancer
MCC	Mathew's coefficient
MCF-7	Breast Carcinoma
MDA	Malondialdehyde
MDA-MD-231	Breast Adenocarcinoma
MIC	Minimum Inhibitory Concentration
ML	Machine Learning
MPO	Myeloperoxidase
MRC-5	Human fetal Lung
MRSA	Methicillin-resistente Staphylococcus aureus
MSSA	Methicillin-susceptible Staphylococcus aureus
MTCC	Microbial Type Culture Collection and Gene Bank
MTT	3-(4,5-dimethylazol-2-yl)-2,5-diphenyltetrazolium bromide
NB	Naïve Bayes
NCI-H460	Non-Small Cell Lung Cancer
NCI-H460	Non-Small Cell Lung Cancer
NF-κB	Nuclear Factor Kappa Beta
NO	Nitric Oxide
NPV	Negative Predictive Value
NSAIDs	Non-Steroidal Antiinflammatory Drugs
NTD	Neglected tropical disease
OVCAR-3	Ovarian
P-388	Mouse Lymphoid Neoplasma
PACA-2	Pancreatic Carcinoma
PC-3	Prostate adenocarcinoma
PCA	Principal Components Analysis
P_{cm}	Combined probability between the models
PDB	Protein Data Bank
PGE2	Prostaglandin E2

PGs	Prostaglandins
PLS	Partial Least Squares
P_{MuDRA}	MuDRA model probability
PPV	Positive Predictive Value
P_{RF}	RF model probability
Q²	Coeficiente de correlação de validação cruzada
QSAR	Quantitative Structure-Activity Relationship
R²	Coeficiente de determinação
RD	Rhabdosarcoma
RF	Randon Forest
RMSE	Root Mean Square Error
ROC curve	Receiver Operating Characteristic curve
ROMs	Reactive Oxygen Metabolites
ROS	Reactive Oxygen Species
RPMI-8226	Multiple myeloma
SBVS	Structure-Based Virtual Screening
SF-268	Glioblastoma
SF-295	Brain tumor
SOD	Superoxide Dismutase
SOM	Self-organizing maps
SVM	Support Vector Machine
TNFα	Tumor Necrosis Factor- α
U-Matrix	Unified distance matrix
VISA	Vancomycin intermediate staphylococcus (staf-i-lo-KOK-us) aureus
VN	Verdadeiros negativos/true negatives
VP	Verdadeiros positivos/true positive
VPN	Verdadeiro preditivo negativo
VPP	Verdadeiro preditivo positivo
VRE	Vancomycin-resistant Enterococcus spp.
VS	Virtual Screening
WHO	World Health Organization

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Introdução

1. Introdução

A humanidade sempre buscou nas plantas propriedades terapêuticas, e seu uso como fonte de substâncias medicinais foi aprimorado e estudado ao longo dos anos ¹. As plantas são fontes ricas de produtos naturais, que por sua vez são fonte potencial de substâncias bioativas para o desenvolvimento de novos medicamentos.²

Os produtos naturais possuem uma grande diversidade estrutural e têm desempenhado um papel notável na descoberta de novas drogas.³ Em uma revisão de literatura recente, Newman e Crag constataram que cerca de 68,8% dos novos medicamentos aprovados para uso terapêutico, entre os anos 1981 e 2019, estão direta ou indiretamente associados a produtos naturais.⁴

A família Annonaceae, descrita por Antonie Laurent de Jussieu (1789), é uma das famílias mais uniformes do ponto de vista anatômico e estrutural.⁵⁻⁸ É a mais rica em espécies da ordem Magnoliades, possuindo cerca de 107 gêneros e aproximadamente 2400 espécies reconhecidas atualmente.⁹ Annonaceae possui distribuição pantropical no mundo, mas requer características específicas de solo, altitude, temperatura e umidade que limitam sua distribuição embora em regiões pantropicais.¹⁰⁻¹²

Diversos estudos científicos apontam para a diversidade estrutural dos produtos naturais provenientes de Annonaceae, além de evidenciarem diversas atividades biológicas, colocando-a como alvo de estudos para a descoberta de novas drogas.¹³⁻¹⁸

Além de apresentarem metabólitos secundários das classes de flavonoides, terpenos, esteroides, entre outras, a família Annonaceae se destaca por seus alcaloides do tipo isoquinolínicos e pela presença de acetogeninas, compostos químicos exclusivos dessa família.^{3,19-21} Assim, as Annonaceae têm sido massivamente estudada para explorar o potencial biológico de seus metabólitos.

Para determinar a ação farmacológica e toxicidade de uma substância, vários ensaios experimentais são necessários, o que requer muito tempo além de consumirem uma grande quantidade de recursos.²² A partir dessas dificuldades, houve a necessidade de aperfeiçoar todo o processo envolvido na descoberta de novos fármacos, buscando uma maior eficiência.

Nessas circunstâncias, surgiram as ferramentas computacionais como uma importante alternativa, uma vez que leva à redução de custos de pesquisa, tempo, diminuição de testes exploratórios, melhorar a compreensão e interpretação de sistemas bioquímicos e alvos terapêuticos.²³⁻²⁶

O uso de técnicas computacionais para a descoberta de novos fármacos tem se tornado cada vez mais comum e necessário. O desenvolvimento de drogas assistido por computador (CADD, do inglês Computer-Aided Drug Design) permite a exploração de grandes bases de dados químicos a fim de reduzir esses bancos a conjuntos de moléculas com potencial elevado de atividade biológica desejada, processo conhecido como triagem virtual (VS, do inglês Virtual Screening).^{25,27-29}

Na VS o objetivo é triar e selecionar compostos potencialmente ativos, além de ser possível identificar e remover compostos tóxicos, ou que apresentem propriedades farmacodinâmicas e farmacocinéticas desfavoráveis, por meio de modelos computacionais e algoritmos para analisar grandes bancos de moléculas de forma automatizada.^{27,30-32}

O processo de VS é orientado pelo conhecimento, e de acordo com o conhecimento/informação a VS pode ser realizada através de duas técnicas: Triagem Virtual Baseada na Estrutura do Receptor (SBVS, do inglês Structure-Based Virtual Screening) e a Triagem Virtual Baseada na Estrutura do Ligante (LBVS, do inglês Ligand-Based Virtual Screening).^{22,24,33}

A técnica de LBVS será a abordada neste estudo, uma abordagem que utiliza o conhecimento de moléculas testadas experimentalmente para a atividade biológica desejada. Nesta técnica, também se utiliza o aprendizado de máquina para gerar modelos capazes de prever a atividade de moléculas que nunca foram testadas para a atividade biológica que se deseja, realizando assim, uma análise de relação quantitativa estrutura-atividade (QSAR, do inglês Quantitative Structure-Activity Relationship).^{24,25,34,35}

Neste contexto, o presente trabalho apresenta estudos computacionais baseado na estrutura do ligante, e aprendizado de máquina, com o objetivo de selecionar metabólitos secundários com potencial atividade antichagásica e leishmanicida, a partir da construção de um banco de dados de produtos naturais isolados de Annonaceae.

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Objetivos

2. OBJETIVOS

2.1 Objetivos gerais

Realizar estudos computacionais com vistas a obtenção de moléculas promissoras com atividade biológica para as doenças negligenciadas, doença de Chagas e leishmaniose a partir de banco de dados de metabólitos secundários de Annonaceae. Além de investigar o potencial leishmanicida de extratos de quatro espécies de *Annona* (*Annona glabra*, *Annona mucosa*, *Annona sylvatica* e *Annona dolabripetala*) por meio de uma abordagem metabolômica usando análise estatística multivariada (PCA e PLS) e dados de LC-MS, para correlacionar dados espectroscópicos com atividade leishmanicida, buscando sugerir compostos ou grupos de compostos responsáveis pela atividade biológica

2.2 Objetivos específicos

- Elaborar um banco de dados com metabólitos secundários encontrados na família Annonaceae;
- Disponibilizar para consulta o banco de dados via ferramenta web (<http://sistemax.ufpb.br>) desenvolvida pelo Laboratório de Quimioinformática;
- Construir um modelo de predição para selecionar moléculas potencialmente ativas para as doenças negligenciadas doença de Chagas e Leishmaniose, utilizando o banco criado da família Annonaceae;
- Realizar estudos de QSAR e estudos *in silico* das propriedades ADME (administração, distribuição, metabolização e excreção) das moléculas mais promissoras;
- Adquirir os metabólitos secundários promissores de Annonaceae, e realizar testes farmacológicos por meio de parcerias com equipes de pesquisa do PGPNSB e externas;
- Confeccionar extratos com alto rendimento a partir do material vegetal coletado das espécies *Annona glabra*, *Annona mucosa*, *Annona sylvatica* e *Annona dolabripetala* da família Annonaceae;
- Desenvolver métodos otimizados para obtenção do perfil químico (*fingerprint*) por DI-MS, LC-MS e GC-MS dos extratos derivados das diferentes espécies da família Annonaceae;

- Realizar análise estatística multivariada exploratória e discriminante dos dados de espectrometria de massas dos extratos etanólicos bruto de cada espécie, bem como das frações obtidas;
- Interpretar os diferentes perfis metabólitos dos extratos e das frações a partir dos dados de análise quimiométricas e listar as variáveis, ou íons discriminantes, que influenciam na separação dos grupos através do PLS;
- Correlacionar os dados de quimiometria e atividade antileishmania com a finalidade de determinar substâncias ou misturas ligadas a estas bioatividades;
- Divulgar os resultados da pesquisa em eventos científicos e periódicos especializados com Qualis no mínimo A4.



CAPÍTULO I

Neste capítulo foi realizada uma revisão dos estudos de atividade biológica realizados com espécies da família Annonaceae objetivando evidenciar quão versátil e promissora é essa família na busca por novas drogas.

Foi observado que a Annonaceae é extremamente rica em metabolitos secundários e com grande diversidade química apresentando uma grande variedade de potencial biológico, além de extratos, fases, frações e óleos essenciais se apresentarem ativos para as mais diversas atividades biológicas.

Grande parte das investigações se limita nas atividades de diferentes extratos vegetais e óleos essenciais, apresentando um déficit de estudos relacionando o potencial ativo de espécies de Annonaceae com os metabolitos secundários presentes nesses extratos e óleos essenciais, bem como a elucidação do mecanismo de ação.

Desta forma, o conteúdo dessa revisão oferece uma grande diversidade de dados aptos para serem explorados em estudos de descoberta de novas drogas. Abrindo um grande leque de exploração dessa família a fim de identificar, isolar e testar substâncias *in vitro* e *in vivo* visando a identificação de novas drogas.

Sweet and useful: Biological activities of Annonaceae family

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Artigo encontra-se em fase de revisão.

Abstract: Annonaceae is a large family under the division of Magnoliophyta which comprising about 107 genera over 2300 species. The most important genera having many species are Annona. Several genera and species of this family are known for their components and their different pharmacological activities. It is considered one of the most promising families, source of bioactive molecules due to its great diversity of secondary metabolites with biological activity. Chemical investigations in the species of Annonaceae have revealed the marked presence of alkaloids, mainly isoquinolinic alkaloids, phenolic compounds, terpenoids, lactones and acetogenins. Being acetogenins exclusive of this family.

Keywords: Annonaceae, Bioactivity, Natural Products, Biological activities.

1. Introduction

In addition to carrying out photosynthesis, an important process for releasing oxygen into the atmosphere, plants are also capable of making secondary metabolites, chemical molecules that can eventually play a biological role in living beings ^{1,2}. Thus, plants are among the main sources for producing new candidates for therapeutic drugs in the last centuries ^{3,4}.

Medicines that are produced from molecules obtained from medicinal plants have some important advantages over medicines that are not based on plant molecules that range from reduced side effects, cost / benefit and accessibility, which leads to the incentive to search for new drugs from plants ⁵⁻⁷. Medicines that have their active ingredients based on plant compounds also have excellent efficacy and safety ^{8,9}.

In this perspective, the Annonaceae family stands out, first described by Antonie Laurent de Jussieu (1789), one of the most uniform families from an anatomical and structural point of view ¹⁰⁻¹³. It is one of the richest families in species of the order Magnoliades, having about 107 genera and 2400 species currently recognized ¹⁴. The Annonaceae has Pantropical distribution in the world, but requires specific characteristics of soil, altitude, temperature and humidity which limits its distribution although in pantropical regions ¹⁵⁻¹⁷.

The Annonaceae is rich in fruit species, most of which are edible, which has made it the target of many studies ^{11,18,19}. Of the several studies carried out in different species, highlighting phytochemical and biological studies, the Annonaceae became known for having a large amount of secondary bioactive metabolites for different pharmacological activities. In addition to the classic secondary metabolite classes such as alkaloids, flavonoids, terpenes, among others, the Annonaceae was characterized by the presence of alkaloids of the isoquinoline type and by the presence of acetogenins, exclusive compounds of this family, which perform important pharmacological activities ²⁰⁻²³. Thus, the Annonaceae has been massively studied to explore the biological potential of its metabolites.

Based on the data presented, the objective of this review is to highlight the biological activities performed by plants of the Annonaceae, highlighting the importance of this family as a source of new drugs.

2. Methodology

Information was collected in the literature regarding the use of plants with biological activity of the Annonaceae, use of extracts, essential oils, and secondary metabolites. The articles were collected in scientific databases: Web of Science, PubMed and ScienceDirect, published until April 2022. Using the keywords "Annonaceae", "Biological activity", "Activity", "Pharmacological activities", "Biological properties".

We included in our review those open access articles with compounds isolated from the Annonaceae family, or papers with extract and essential oils with biological tests. Articles containing synthesis or semi-synthesis, even if inspired by compounds isolated from Annonaceae, were excluded.

3. Results and Discussion

The Annonaceae family has a wide use in traditional medicine, being well known in the tropical regions of the world. Some of its uses in traditional medicine are listed below: treatment of arthritis, rheumatism, and neuralgia ²⁴, asthma ^{25,26}, wound healing ²⁷, cancer ²⁸, parasitic infections ²⁹, fever, diabetes, insomnia, headaches, among others ^{28,30}.

The bibliographic survey on biological activities of the Annonaceae resulted in 266 articles collected. In this review, about 177 species with proven biological activity were reported, which corresponds to approximately 7.37% of the known species of Annonaceae.

These species are distributed in 56 genera, which represents 52.33% of the total Annonaceae genera. These 56 genera are representing the four subfamilies of Annonaceae (Malmeoideae, Annonoideae, Anaxagoreoideae and Ambabavioideae), where 8.92% are part of the subfamilies Ambavioideae and Anaxagoreoideae, which do not have tribes. And 91.08% are represented in seven tribes of the Annonoideae and three tribes of the Malmeoideae. These data are represented in a Venn diagram in Figure 1.

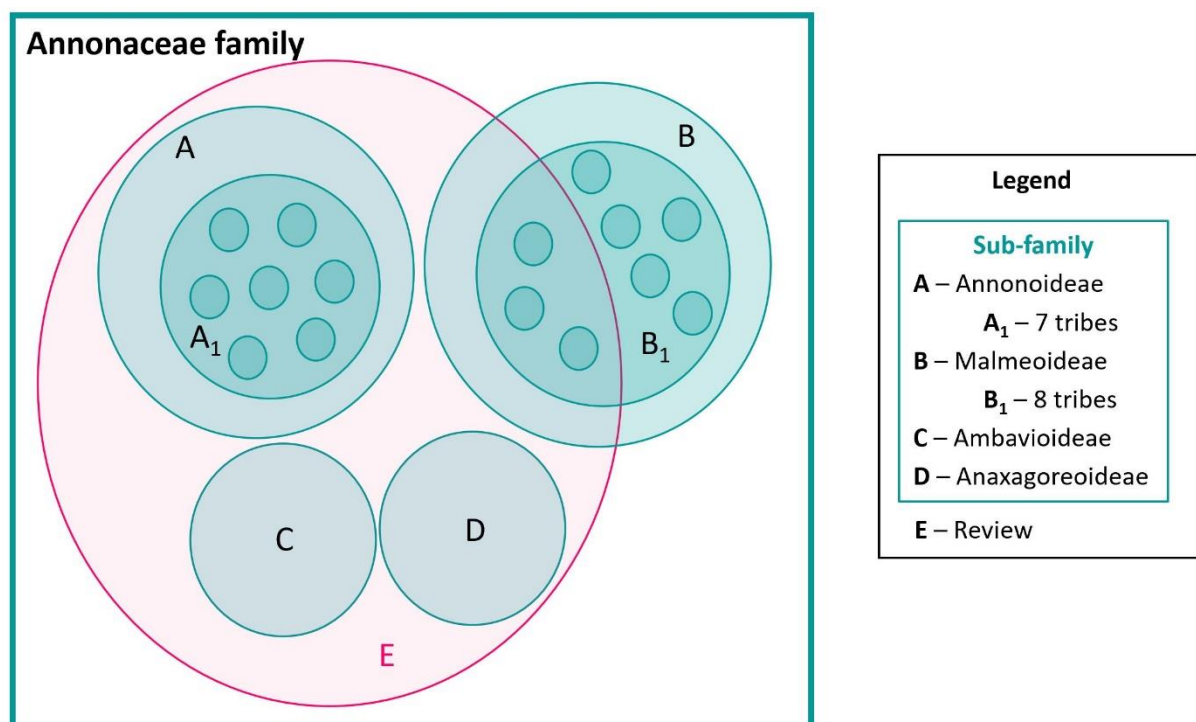


Figure 1: Venn diagram of the species with biological activity reported by the studies covered in this review.

Figure 2 represents the number of articles evaluated in this review grouped by years and, in it, it is possible to observe that the interest in the Annonaceae has grown over the years and since 2011 there has been a large increase in studies evaluating the biological profile of species of the Annonaceae ³¹. As it is an extremely rich family in substances with great structural diversity due to its biogenetic diversity, it is easy to understand this increase in the search for bioactive substances from Annonaceae species ³¹.

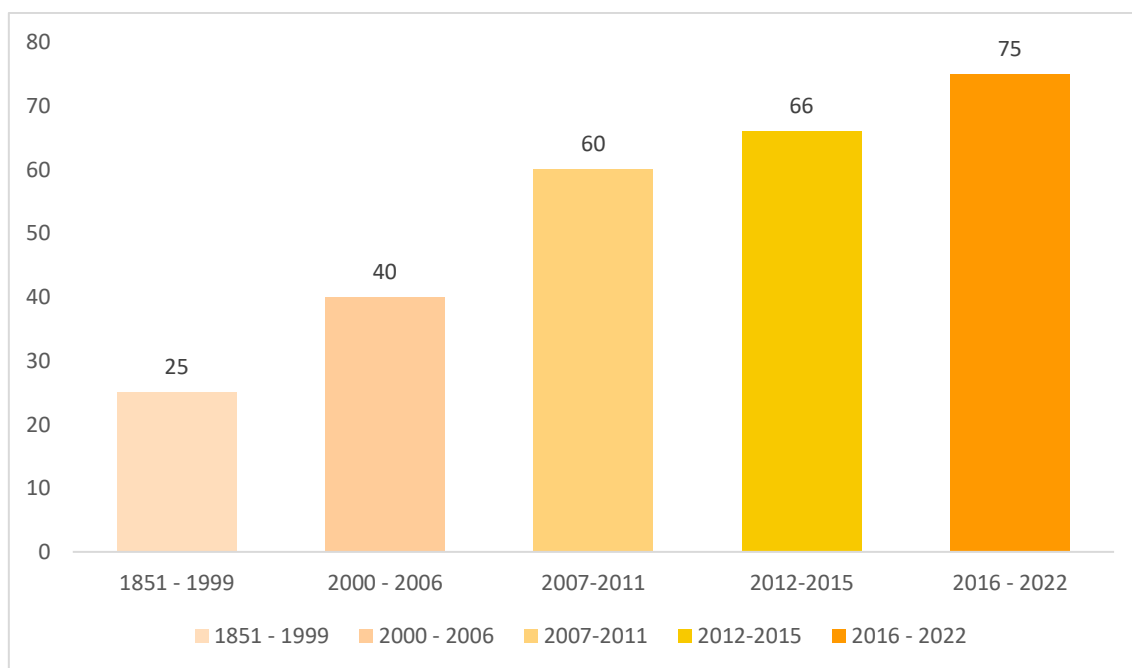


Figure 2: Distribution of papers analyzed in this review over time.

The bibliographic survey on biological activities of the Annonaceae initially resulted in Figure 3, which shows the number of articles collected for each disease already studied in that family. Some biological activities gained a topic for them to be discussed.

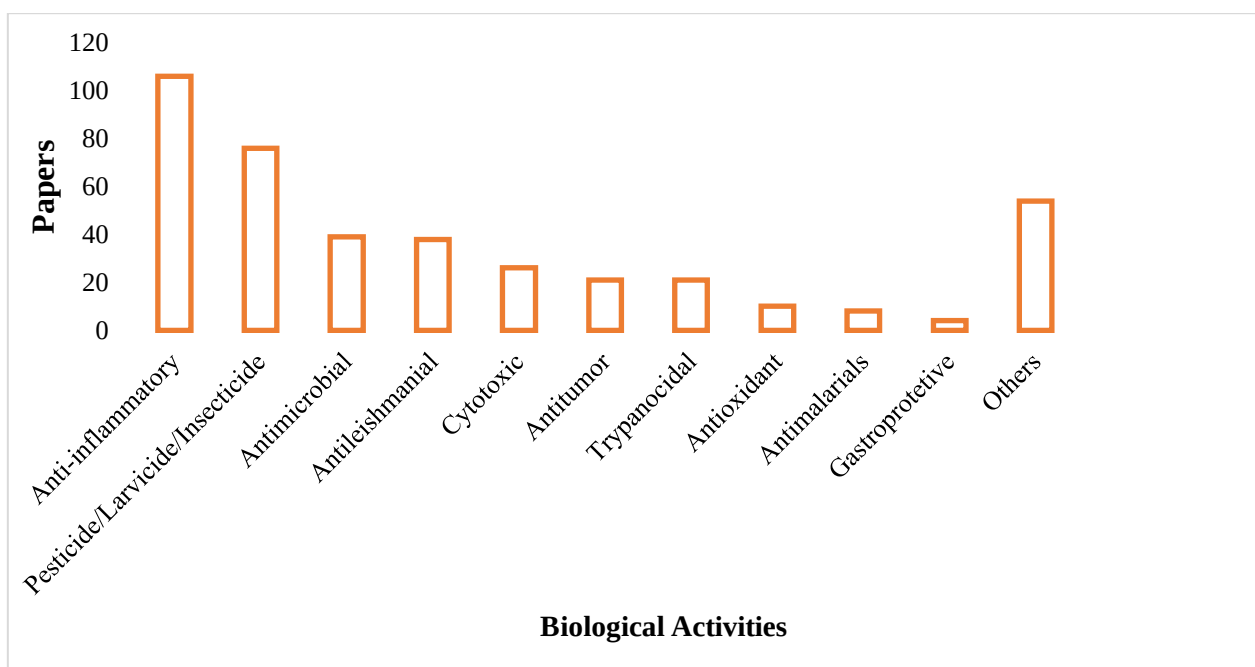


Figure 3: Number of articles collected for each biological activity already studied by the Annonaceae.

In figure 3, other activities include: anti-convulsant activity ³²⁻³⁴, anti-*Onchocerca* ³⁵, anti-hyperprolactinemic ³⁶, α -Glucosidase inhibitory ³⁷, anti-diabetic ³⁸⁻⁵⁴, antidiarrhea ⁵⁵, anti-SARS-CoV-2 ⁵⁶, anti-acetylcholinesterase ⁵⁷, anthelmintic ⁵⁸, antischistosoma ⁵⁹,

anti-Platelet aggregation ⁶⁰, apoptosis induction ^{61–63}, testicular function ⁶⁴, anti-HIV ^{65–74}, antiviral activity ^{75–81}, anxiolytic-like activity ^{82,83}, antidepressant ⁸⁴, anti-hypertensive ⁸⁵.

3.1 Anti-inflammatory

Inflammation is a physiological response triggered by several factors, such as physical trauma, exposure to allergens, chemical stimuli, infections, etc ^{30,86,87}. The inflammatory process can also be understood as an adaptation to a malfunction of body tissues or even an imbalance in homeostasis ⁸⁸. That said, inflammation is a beneficial immune system response with the ability to restore the body's homeostasis, but if unregulated, it can become harmful, which explains its association with several diseases, such as autoimmune diseases (rheumatoid arthritis), hypertension, obesity, cancer, among others ^{30,88–90}.

Inflammation is didactically divided into two phases, the acute phase, and the chronic phase. The acute phase is characterized by the initial stages of inflammation, emergence of the five cardinal signs of inflammation: pain, heat, redness, and swelling ^{91,92}. It also exhibits vasodilation, exudation of protein-rich plasma fluid, and migration of cells to the site of injury. When inflammation is persistent, the chronic phase begins, where there will be changes in the composition of infiltrating leukocytes, that is, instead of neutrophils, high concentrations of lymphocytes and macrophages begin ^{93,94}. The chronic phase has a prolonged duration, lasting several months and/or years and is associated with blood vessel proliferation, fibrosis, and tissue necrosis. The evolution of an inflammation in practice does not usually happen with the acute phase first and then the chronic phase, usually the inflammatory response happens in a progressive way, combining elements of the two phases ^{94,95}.

Inflammation treatment includes several medications such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs) are available for the treatment of painful and life-threatening inflammatory conditions ^{30,96,97}. But a serious problem with these drugs is their toxicity in prolonged use, which can cause serious and even life-threatening side effects such as uncontrolled hypertension, gastric ulcer, acute kidney failure, liver failure, heart failure, glaucoma, etc ^{30,89,90}.

These factors contribute to research being carried out to find drugs to fight inflammation, but which do not have such serious side effects. A rich source of substances

with biological potential are natural products from plants ^{3,98}. And in this context, the Annonaceae family has been listed among the most diverse plant families in tropical forests due to its chemical diversity and abundance in these regions ^{30,99,100}.

Attiq, Jalil and Husain (2017)³⁰ carried out a literature review study highlighting the great potential of the Annonaceae family in fighting inflammation, pointing out isolated substances as great candidates for possible drugs. The authors further highlight terpenes as a promising secondary metabolite class in anti-inflammatory activity. However, there is still much to be studied in the Annonaceae family, especially in the elucidation of substances with biological potential, given the substantial number of studies that analyzed extracts with potent anti-inflammatory activity. Below we highlight some studies, separated by Annonaceae species, after the review by Attiq, Jalil and Husain (2017) ³⁰. Data from these studies are summarized in table 1.

Xylopia genus

Xylopia aethiopica is a plant commonly used in traditional medicine of African for the healing of wounds, inflammatory disorders, and treatment of post-natal pains. Based in this traditional knowledge, in 2010, an oil extract from the fruits of *Xylopia aethiopica* containing carbohydrates, glycosides, flavonoids, saponins, tannins and phytosterols was studied by Ezekwesili et al (2010). Anti-inflammatory effects of this extract were determined. Specifically, the effects on cell membrane stability and prostaglandin synthetase activity were evaluated ¹⁰¹.

X. aethiopica fruit is characterized as a good source of unsaturated fatty acids, this was contrasted through HPLC studies of the lipid extract, being identified: palmitic acid (19.21%), palmitoleic acid (0.81%), stearic acid (4.54%), oleic acid (39.12%), linoleic acid (25.98%) and linolenic acid (1.10%). The *X. aethiopica* extract exhibited a stabilization of erythrocyte membrane which the authors related with the stabilization of lysosomal membrane which is commonly referenced as an indication of anti-inflammatory potentials of drugs. This extract also showed the maintenance of the integrity of the cellular membranes and presented an activity as prostaglandin synthetase substrate and the promotion of the biosynthesis of the prostaglandins ¹⁰¹.

Xylopic acid is the principal constituent isolated from the dried fruit of *X aethiopica*. Anti-inflammatory effects of this acid have been observed, and their properties were related with a H₂S-induced paw edema was because of its intervention on the arachidonic acid pathway ¹⁰². The same authors, through an *in vivo* study, using Sprague Dawley rats treated with xylopic acid or sulphasalazine and challenged

intrarectally with acetic acid (to induce colitis), found that xylopic acid has an effective in suppressing experimentally induced ulcerative colitis by increasing superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) activity ¹⁰³.

At microscopic level, the rats that were treated with xylopic acid showed a reduction of the mucosal injury caused by the acetic acid at 10, 30, and 100 mg kg⁻¹. Additionally, reduced granulomatous inflammation and cellular proliferation with xylopic acid treatment respectively were observed. Respect the enzymes related as prime biomarkers for colon damage, SOD, CAT and APX, the treatment with studied secondary metabolite resulted in significant increase expression in the three enzymes at all administered doses (10-100 mg kg⁻¹). Finally, the levels of myeloperoxidase (MPO) and malondialdehyde (MDA) were determined. MDA and MPO are two reactive oxygen metabolites (ROMs) which are released by immune cells play an important role in the physiopathology of ulcerative colitis. Commonly, the increasement of these two compounds are an indication of oxidative damage and as a marker for free radicals-induced lipid peroxidation ¹⁰⁴. The results showed a reduction of MDA from 2539.0±981.20 M/mg (naïve control) to 2053.0±968.20, 2268.0±987.3, and 1214±513.2 M/mg protein at the doses of 10, 30 and 100 mg/kg, and a reduction at the MPO levels from 31.86±0.61×10⁵ U/mg protein (naïve control) to 13.36±3.36 and 19.59±4.75×10⁵ U/mg protein at 30 and 100 mg kg⁻¹ doses of xylopic acid ¹⁰³.

The anti-inflammatory properties of an aqueous ethanol extract of the fruit of *X. aethiopica* were evaluated through the induction of the mouse carrageenan-induced paw edema, using a 1% of carrageenan suspension in normal saline in 8–10 week old ICR mice (25–30 g). The ethanolic extract (30–300 mg kg⁻¹) showed a dose dependent manner significantly inhibited by 23–62% the mouse pinnal inflammation. After 6 hours, the swellings induced were dose-dependently and significantly suppressed with values between 48% - 75% the inflamed control response ¹⁰⁵.

Recently, in another study, *X. aethiopica* leaves' hydroethanolic extract inhibited the production of TNF- α (Tumor Necrosis Factor- α) in (Lipopolysaccharide) LPS challenged THP-1-derived macrophages. The extract obtained from the leaves of *X. aethiopica*, interfering with TNF- α levels at two concentrations 250 μ g/mL and 500 μ g/mL, leading to a significant inhibition, above 90%, at 500 μ g/mL. The inhibition observed for *X. aethiopica* was much higher than observed for other extracts analyzed of

Parinari excelsa (Sabine), *Cassia sieberiana* DC. and *Neocarya macrophylla* (Sabine), three plants that also related anti-inflammatory properties and are present in Africa ¹⁰⁶.

Additionally, *X. aethiopica* leaves' extract inhibited the production of (interleukine-6) IL-6, a proinflammatory cytokine, in a LPS challenged THP-1-derived macrophages, from 96.25 pg/μg of total protein to 14.84 pg/μg and 3.53 pg/μg, at 250 and 500 μg/mL, respectively. In the same way, the hydroethanolic extract moderately interfered with 5-LOX showing a concentration-dependent effect, being evaluated different concentrations ranging from 16 to 250 μg/mL. The extract proved to display lower a IC₅₀ value of 85 μg/mL, meanwhile the reference inhibitor quercetin reached an IC₅₀ value of 6.55 μg/mL. The anti-inflammatory effects of *X. aethiopica* leaves was related with phenolic compounds identified through HPLC-DAD, among the detected compounds are included: cynaroside, rutin, quercitrin, astragalin, and nicotiflorin, O-caffeoylquinic acids were also detected in the extract and the luteolin monoglycoside cynaroside ¹⁰⁶.

Anti-inflammatory properties of essential oils obtained from eight batches of *X. aethiopica* fruits from Ghana and Nigeria by hydrodistillation were tested using RAW 264.7 macrophage cells. The results showed that the oils inhibit NO (Nitric oxide) production in LPS-stimulated RAW264.7 cells concentration-dependently. A geographical difference was observed in the NO inhibition being that the oils from Ghana presented demonstrated better anti-inflammatory effects than those of Nigerian origin. Additionally, better anti-inflammatory effects were observed for the essential oil respect the control drug, dexamethasone. The authors related these results with synergistic effects of the components that are commonly observed, being identified through gas chromatography (GC-MS) 14 differential metabolites to be potential chemical markers ¹⁰⁷.

Another species of *Xylopi*a genus such as *Xylopi*a *parviflora*, *Xylopi*a *sericea* and *Xylopi*a *vielana* also have been studied by different approaches. The essential oil from striped African pepper (*Xylopi*a *parviflora*) was studied at different concentrations values in RAW 264.7 macrophages which were initially stimulated with LPS (for the increasement of NO production) and co-incubated for 24h with different concentrations of essential oil ¹⁰⁸. The results of this assay showed a dose-dependent decrease in NO production, with a reduction of 37% in NO levels in cells treated with 12 μg/mL of the essential oil respect those cells that were treated with LPS alone, being proposed by the authors that anti-inflammatory activities of the oil have a potential to be utilized.

However, despite the study report a chemical profile of this essential oil (mainly composed by β -pinene (34.0%) and α -pinene (10.3%)), is not reported any compound responsible of the anti-inflammatory activity observed ¹⁰⁸. The ethanolic extract of the Brazilian pepper, also known as “Pindaíba” (*Xylopia sericea*) in LPS-stimulated RAW264.7 cells assay reached a inhibition levels close to 76% and 85% for the production of NO and IL-6, respectively, with an extract dose of 100 μ g/mL. However, the *Xylopia sericea* extract did not reduce the levels of TNF- α and superoxide levels at studied concentrations (6.25 to 100 μ g/ml) ¹⁰⁹.

In other way, an in-silico study approach allowed the prediction of potential anti-inflammatory bioactivity of five guaiane-type sesquiterpene dimers identified as xylopidimers A-E from *Xylopia vielana*. For the molecular docking calculations was used COX-2 protein (Cyclooxygenase-2) as anti-inflammatory target (PDB: 1CX2). All the five evaluated dimers showed a potent inhibitory activity against COX-2 protein receptor, with binding energy values among -10.51 Kcal/mol and -9.23 Kcal/mol, being lower energy values in all cases respect two commercial anti-inflammatory drugs used as control, Ibuprofen and Felbinac, which binding energies were -7.48 and -8.20 Kcal/mol, respectively ¹¹⁰.

Molecular dynamics simulations as well as ADMET properties prediction also were performed being found that theses guaiane-type sesquiterpenes allow a ligand-protein stability, emerging as promissory molecules to be anti-inflammatory. This in silico approach is one of the few studies that report specific secondary metabolites from *Xylopia* together with the Xylopic acid since most anti-inflammatory assays for this genus is related with polar extracts.

Annona genus

In a bioguided fractionation study, the anti-inflammatory activity of the hydromethanolic extracts of the leaves, pulp, and seeds of *Annona cacans* was evaluated in paw edema model, to MPO activity induced by carrageenan in mice. Using a plethysmometer at 2 and 4 h after injection, the edema was determined. The authors found a significant inhibition of edema compared to the control group in the two measured hours; in addition, the extracts inhibited the increase in MPO activity after 6 h, when compared to the dexamethasone and control groups ¹¹¹.

In the same way, a polyphenol-enriched fraction from *Annona crassiflora* collected in Brazil, was studied to evaluate its anti-inflammatory activity, being identified chlorogenic acid, epi-catechin, procyanidins B2 and C1, quercetin-glucoside, kaempferol,

and caffeoyl-glucoside as the main components of this extract. Using male C57BL/6 mice the anti-inflammatory properties of the extract were evaluated considering neutrophils and macrophage activation (MPO and N-acetyl- β -D-glycosaminidase [NAG] activities), respectively ¹¹². Some anti-inflammatory properties were found since all concentrations were able to reduce the activity of neutrophils in the initial period (4 d) and the activity of macrophages in the two evaluated times (4 and 7 d) ¹¹².

Using the paw edema model (300 μ g/paw), the anti-inflammatory properties of an *Annona crassiflora* methanolic extract were determined. Through ¹H NMR studies, kaempferol 3-O- β -glucoside and kaempferol 3-O- β -diglucoside were identified in the extract. Experimentally, male and female Swiss mice (20–30 g) were orally treated with doses of 30, 100, and 300 mg/kg of the extract. The results showed that the methanolic extract of *Annona crassiflora* significantly inhibited the carrageenan-induced edema formation, with values close to 50%. Additionally, the MPO activity showed values of inhibition above of 60% for 100 mg/kg and 300 mg/kg of treatment and a reduction of the total leukocytes, with values of 78% and 90% for 100 mg/kg and 300 mg/kg, respectively ¹¹³. According with the authors, this extract is considered to have anti-inflammatory therapeutic potential because it is capable of inhibiting paw edema, reducing myeloperoxidase activity, reducing the total leukocyte count ¹¹³, being complementary to the observed by De Moura ¹¹² using polar extracts of this species.

Anti-inflammatory and analgesic effects of other species of this genus, *Annona muricata*, which is found in different tropical regions and is commonly used in Africa, was studied in male Sprague–Dawley rats (120–130 g) and 8-week-old Swiss albino mice (20–25 g) using the lyophilized fruit extract in the xylene-induced ear edema test. The topical application of 30 μ L xylene to the inner surface of the right ear increased the ear weight by 34.00 mg for control group. Using the *Annona muricata* extract with 50 and 100mg/kg, the size of the ear was significantly reduced with 82% and 76%, respectively, being observed a high level of inhibition respect 20mg/kg prednisolone treatment, being concluded by the authors that *A. muricata* extract possesses analgesic and anti-inflammatory activities in various models ¹¹⁴.

Several compounds from *Annona squamosa* L. and *Annona reticulata* L. barks were evaluated by Chavan et al. using the carrageenan induced rat paw edema method in Albino rats of Wistar strain, including the sesquiterpene fraction composed by copaene (35.40%), patchoulane (13.49%) and 1H-cycloprop(e)azulene (22.77%), that showed a

significant anti-inflammatory activity at 50 mg kg⁻¹ ¹¹⁵, caryophyllene oxide at the doses of 12.5 and 25mg/kg body weight ¹¹⁶, 18-acetoxy-ent-kaur-16-ene which reached an exhibited significant analgesic along with anti-inflammatory activity at 50 mg/kg ¹¹⁷. In the saponified petroleum ether extract of the *Annona reticulata* L. bark was identified six fatty acids. The composition of saturated and unsaturated fatty acid was 22.10 % and 64.58 %, respectively, this extract showed an anti-inflammatory activity at the doses of 12.5, 25 and 50 mg/kg body weight ¹¹⁸.

Using the fruits of *Annona glabra*, the anti-inflammatory properties of eight ent-kauranes were tested included three novel structures: 7 β ,16 α ,17-trihydroxy-ent-kauran-19-oic acid, 7 β ,17-dihydroxy-16 α -ent-kauran-19-oic acid 19-O- β -D-glucopyranoside ester, and 7 β ,17-dihydroxy-ent-kaur-15-en-19-oic acid 19-O- β -D-glucopyranoside ester. The inhibitory effects were evaluated through the inhibition of Nitrous oxide (NO) in the LPS-stimulated RAW 264.7 macrophages measured by the Griess reaction ¹¹⁹. The authors found an IC₅₀ value of 0.01 $\pm$ 0.01 μ M for the 7 β ,17-dihydroxy-ent-kaur-15-en-19-oic acid 19-O- β -D-glucopyranoside ester. Another two compounds, 7 β ,16 α ,17-trihydroxy-ent-kauran-19-oic acid and 6 β ,17-dihydroxy-ent-kauran-19-al also showed significant IC₅₀ values against NO production: 0.39 $\pm$ 0.12 μ M and 0.32 $\pm$ 0.04 μ M, respectively. In all cases, the tested ent-kauranes showed a higher inhibitory NO production respect the control, dexamethasone which exhibited an IC₅₀ value of 0.80 $\pm$ 0.04 μ M ¹¹⁹.

In this same way, anti-inflammatory activity of five bioactive constituents from seeds of *Annona Senegalensis* Persoon was evaluated through the NO inhibition in LPS-stimulated J774.2 mouse macrophages and the inhibitory effect on oxidative burst where intracellular reactive oxygen species (ROS). N-cerotoyltryptamine, asimicin, ent-19-Carbomethoxykauran-17-oic acid, stigmasterol glycoside and lacceroic acid were separated by column chromatography ¹²⁰. The results showed that among the five tested compounds, only N-cerotoyltryptamine appeared as a potent inhibitor of ROS with an IC₅₀ value of 2.7 μ g/mL being more active respect, ibuprofen (11.2 μ g/mL) which was used as anti-inflammatory reference drug. For the NO inhibition assay, acetogenin (IC₅₀ of 3.9 μ g/mL), was found to be a potent NO inhibitor showing a significant lower inhibitory value compared with the control compound, L-NMMA (IC₅₀ = 24.2 μ g/mL)

¹²⁰.

The anti-inflammatory activity of ethanolic extract of *Annona vepretorum* Mart. at different concentrations (25, 50 and 100 mg/kg) was studied using the paw edema model induced by 2% carrageenan, and 100 µg/paw of histamine, as well as leukocyte migration to the peritoneal cavity. The results showed that the ethanolic extract significantly inhibited the edema volume at 1 and 2 h, initial phase, while the used control (indomethacin, 20 mg/kg), showed the inhibition of edema volume during 5 h ¹²¹.

Histamine-induced hind paw edema was reduced in early hours' respect carrageenan, which the authors related with the involvement of histamine in the anti-inflammatory effect of the *Annona vepretorum* ethanolic extract. In addition, through leukocyte migration to the peritoneal cavity assay was concluded that the anti-inflammatory mechanism of the ethanolic extract is related with the inhibition of the synthesis of many inflammatory mediators whose involvement in the cell migration is well-established ¹²¹.

The paw edema model induced by carrageenan also was used to evaluate the potential anti-inflammatory activity of the hydromethanolic fraction of an *Annona nutans* extract, in an oral treatment with a dose of 300 mg/kg, which was observed a significant reduction of the paw edema 2 h and 4 h after the inflammatory stimulus. Additionally, 50 and 100 mg/kg intraperitoneal doses of this extract fraction were tested in adult male Swiss mice, being observed an inhibition of acute inflammation which was still visible 6 h after carrageenan injection ¹²².

Duguetia genus

The anti-inflammatory activity of *Duguetia furfuracea* essential oil (composed by 24 volatile compounds) was tested with the paw edema induced by LPS model. The oral treatment with 1, 3, and 10 mg/kg extract doses showed a significantly inhibition of the paw edema induced by LPS, being observed that the essential oil inhibits the LPS-induced iNOS (inducible nitric oxide synthase) expression, through immunohistochemical staining for iNOS expression, the control group showed an intense staining respect the naïve group, the essential oil (10 mg/kg) and indomethacin (10 mg/kg). In addition, this essential oil promoted the inhibition of tumor necrosis factor alpha (TNF-α) production, and the recruitment of polymorphonuclear leukocytes ¹²³.

From this tested essential oil which has a 38% of phenylpropanoid concentration, an enriched phenylpropanoid fraction of *Duguetia furfuracea* was obtained, reaching a 64,2% of phenylpropanoid concentration. With this enriched extract another study was performed using the same methodologies to evaluate the anti-inflammatory activity. In

the oral treatment with a dose of 3 mg/kg of this extract, the paw edema was attenuated being observed an inhibition of iNOS expression and the leukocyte recruitment as well as a decrease in the tumour necrosis factor alpha (TNF- α) production ¹²⁴. The authors suggest that the improved anti-inflammatory results observed for the enriched phenylpropanoid extract respect the essential oil is related with the presences of 2,4,5-trimethoxystyrene and α -asarone, this last one, also demonstrated at a dose of 3 mg/kg, a reduction of paw edema as the same parameters evaluated related with the anti-inflammatory activity ^{124,125}.

Using leaves of this same species, a methanolic extract with high contents of phenols (624.37 mg/g), flavonoids (580.21 mg/g), and flavonols (254.44 mg/g) was obtained and its anti-inflammatory activity was determined through the edema assay measured by pletismometer. The authors found that the activity of the extract is dose-dependent, with a dose of 300 mg/kg of the methanolic extract, caused a significantly reduction, reaching an inhibition of 39% at 2 hours and 40% at 4 hours, a lower dose of 100 mg/kg exhibited an inhibition of at 2 hours and 40% at 4 hours. Dexamethasone was used as control and showed significant inhibition at all-time points ¹²⁶.

Dicentrinone, an oxoaporphine alkaloid is related with the observed anti-inflammatory activity, inhibiting both leukocyte migration (69%) and plasmatic leakage (50%) induced by carrageenan in the pouches. In addition, a dose of 300 mg/kg of Dicentrinone reduced in a 27.1% the zymosan induced edema formation with an inhibition, the control group treated with dexamethasone inhibited the edema formation in 73.1%. According with these results, the authors related the presence of the alkaloid dicentrinone in extract with the anti-inflammatory effects ¹²⁶.

The anti-inflammatory properties of alkaloids present in species of *Duguetia* genus also have been tested, i.e. Discretamine, a tetrahydroprotoberberine alkaloid isolated from *Duguetia moricandiana* was studied in a LPS- stimulated macrophages model. For treatments with 100 and 200 μ g/mL of discretamine a LPS-induced NO production was inhibited close to 50%. Also, discretamine treatment with doses of 50, 100 and 200 μ g/mL reduced inflammatory cytokines levels (IL-6, IL1- β and TNF- α). Finally, a reduction of carrageenan-induced paw edema up to 6h was observed in female Swiss albino mice treated with doses of 5, 10, and 20 mg/kg, indomethacin was used as control and exhibited an inhibition at all times evaluated ¹²⁷.

Duguetia staudtii is a species found in Africa, mainly in forest from Sierra Leone to Cameroon which their chemical constituents have been widely studied for several

biological activities. The anti-inflammatory activity of eight compound (one pachypolignan, one bisnorlignan, four flavonoids, one alkaloid, and one triterpenoid) isolated from *Duguetia staudtii* was evaluated on myeloperoxidase dependent (luminol/zymosan) and independent (lucigenin/PMA) oxidative burst. Inhibitory (IC₅₀) values among 6.44 and 14.13 µg/mL were found for pachypodol, kumatakenin, and 5,4'-dihydroxy-3,7,3',5'-tetramethoxyflavone in macrophages, being these three flavonoids the ones that exhibited the highest anti-inflammatory activity ¹²⁸.

Polyalthia genus

Yao et al. in 2019 performed a deep review of *Polyalthia* genus and a series of compounds were identified including: 16-hydroxycleroda-3,13-dien-15,16-olide and 16-oxocleroda-3,13-dien-15,16-oic acid (clerodane diterpenoids), quercetin and rutin (flavonoids), spinasterol and α -spinasterol (phytosterols), goniothalamine and (-)-5-hydroxygoniothalamine (6S-styrylpyrones) which showed IC₅₀ comparable with positive control. Mostly, the authors found that the main anti-inflammatory mechanism includes the inhibition of nuclear factor kappa Beta (NF- κ B), prostaglandins (PGs), pro-inflammatory cytokines, iNOS and ROS¹²⁹.

Recently, Chen et al, in a review study of the pharmacological activities of phytochemicals from *Polyalthia* added some new structures with potential anti-inflammatory activity: polycerasoidol (prenylated benzopyran) 16-Hydroxycleroda-4(18),13-dien-15,16-olide,(-)-3 α ,16 α -dihydroxycleroda-4(18),13(14)Z-dien-15,16-Olide.(4 $\rightarrow$ 2)-abeo-16(R&S)-2,13Z-clerodadien-15,16-olide-3-al which also have a clerodane skeleton and dehydrogoniothalamine which was tested together with the two 6S-styrylpyrones above mentioned ¹³⁰.

Clerodane diterpenoids were obtained for the methanolic extract of *Polyalthia longifolia* and tested against the fMLP/CB induced superoxide generation by neutrophils and phorbol 12-myristate 13-acetate (PMA)-induced action. The compound 16-oxocleroda-4(18),13-dien-15,16-olide obtained an IC₅₀ value of 0.60 µg/mL against fMLP/CB being close to the obtained for a well-known NADPH oxidase inhibitor, used as positive control, diphenyleneiodonium, which showed an IC₅₀ value of 0.11 µg/mL ¹³¹.

The diterpenoid 16(R&S)-3,13-kolavadien-15,16-olide-2-one, at a concentration of 10µg/mL, showed the lowest IC₅₀ value against phorbol 12-myristate 13-acetate (PMA)-induced action. The authors related this action with a critical structural feature such as *E* form double bond at C-13(14) of a 3-ene-clerodane skeleton ¹³¹. Recently, three

of five compounds isolated from a methanolic extract of *Polyalthia longifolia* (16-hydroxy-cleroda-4(18),13-dien-16,15-olide, 3 α ,16 α -dihydroxycleroda-4(18),13(14)Z-dien-15,16-olide, and 16 α -hydroxy-cleroda-3,13(14)Z-dien-15,16-olide) exhibited to be potent against COX-1, COX-2, and 5-LOX inhibitors with the IC₅₀ values similar or lower to Indomethacin (COX-1 and COX-2) and diclofenac (5-LOX) which were used as controls ¹³².

Interestingly, these authors performed an in-silico approach using namely the PDB structures of the three proteins: COX-1 (PDB ID: 2OYU), COX-2 (PDB ID: 4COX), and 5-LOX (PDB ID: 3V99), being found a high accordance with in vitro studies where these three clerodanes showed binding pattern comparable to that of reference drugs (indomethacin and diclofenac. Two structures, 3 α ,16 α -dihydroxycleroda-4(18),13(14)Z-dien-15,16-olide with free energy (MM/GBSA) values of -57.7 and -39.2 kcal/mol, and 16 α -hydroxy-cleroda-3,13(14)Z-dien-15,16-olide showed values of -55.3 and -38.9 kcal/mol, for COX-1 and COX-2, respectively, reported better inhibitors as compared to indomethacin (-47.5 kcal/mol). In this same way, for 5-LOX, these two clerodanes also reported better free energy values compared with the control drug, diclofenac (-26.6 kcal/mol) ¹³².

The anti-inflammatory activity of extracts from other two *Polyalthia* species, *P. simiarum* and *P. suberosa* were evaluated through carrageenan induced paw edema and Xylene-Induced ear edema models, respectively. For *P. simiarum* the ethyl acetate and petroleum ether extracts showed anti-inflammatory activities at 50 mg/kg and 100 mg/kg body in Wister rats being showed a dose dependent inhibition on paw edema respect the control group. Both extracts showed significant inhibition at all-time points ¹³³. Moreover, two extracts (diethyl ether and n-hexane) from leaves of *P. suberosa* showed an inhibition higher than 40% of the xylene-induced ear edema with doses of 200 and 400 mg/kg body in Swiss albino mice. For a dose of 200 mg/kg, inhibition values in the ear edema of 42.70% and 48.54% were obtained for diethyl ether and n-hexane extracts, respectively. Likewise, with 400 mg/kg of the extracts, the percentages of inhibition were significantly higher with values of 62.67% and 65.92% for diethyl ether and n-hexane extracts, respectively. In both extracts with 400mg/kg the inhibition values were close to the obtained for the control drug diclofenac sodium (100mg/kg), which reached an inhibition value of 68.33% ¹³⁴.

Other Annonaceae species

Using spectroscopic techniques seven compounds (flexuvaroxepine A, flexuvarin A–D and flexuvarol A–B) from the methanolic extract of *Uvaria flexuosa* leaves were identified together with four known flavonoids, 6,7-di-O-methyl-baicalein, Chrysin, Negletein, 6-Hydroxy-5,7-dimethoxy-flavone. After, through the superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB, the anti-inflammatory properties of the series were evaluated, being used Genistein as positive control. Only Flexuvarol B with IC₅₀ values of 4.72 μ M and 5.55 μ M, as well as, Chrysin 2.25 μ M and 2.44 μ M showed an anti-inflammatory activity in both models used, superoxide anion and elastase release, being observed in this last model, a higher inhibition rate for these two structures respect Genistein which reached an IC₅₀ values of 6.99 μ M¹³⁵.

In another research, using species of *Uvaria* genus, (-) zeylenol, a polyoxygenated cyclohexene derivative, was isolated from the ethyl acetate extract of *U. grandiflora* stems and its potential anti-inflammatory activity was tested by the EPP-induced rat ear edema using male Sprague-Dawley rats. The authors found that (-) zeylenol produced a significant inhibitory effect on the edema formation at all-times tested, being reached 90% of inhibition at 15 minutes and 52% at 1-2 hours of test, being related with a release or synthesis of various inflammatory mediators. These values were similar to obtained by a dose of 1mg/ear of phenylbutazone, which was used as positive control¹³⁶.

An isoquinoline alkaloid, dactylactone A, was isolated from *Dactylicapnos scandens*, its anti-inflammatory activity at three concentrations, 0.48, 2.4 and 12 μ M was evaluating measuring the inhibition capacity of lipopolysaccharide (LPS)-induced IL-1 β and PGE₂ (prostaglandin E₂) production in RAW264.7 cells, using Dexamethasone as positive control. The results show a dose-dependent inhibition of dactylactone A, without a cytotoxic effect in the RAW264.7 cells, exhibiting better results respect the positive control used¹³⁷.

These same authors also isolated from *Dactylicapnos scandens*, two reconstructed aporphines with unprecedented five-membered carbon ring D, being identified as dactylicapnosines A and B. The evaluation on the production of inflammatory cytokines in LPS-induced RAW 264.7 cells showed an anti-inflammatory and analgesic effects peripherally by inhibition of the expression of TNF- α , IL-1 β , and PGE₂ for dactylicapnosine A. Additionally, the effect on xylene-induced inflammation in mice was evaluated with an intraperitoneal injection of dactylicapnosine A (10.2 mg/kg), being

reached a decrease in the swelling degree of 53.30% and 54.98% respect control group, being higher the inhibition comparing with Parecoxib (10 mg/kg) which was used as positive control ¹³⁸.

Chemical Components from *Phaeanthus vietnamensis* were isolated using different chromatographic methods and 23 compounds were identified, being novel three of them. Anti-inflammatory properties of these molecules were evaluated through the Inhibitory NO production in BV-2 cells assay. BV2 cells were treated with different concentrations from 5.0 to 50 μ M of compounds for 30 min prior to 100 ng/ml of LPS treatment and the NO concentrations were measured in the supernatant of LPS-stimulated BV2 cells by the Griess reaction ¹³⁹.

Among the 12 tested compounds, spathulenol exhibited an IC₅₀ value of 15.7 μ M, being the most potent inhibitor even with respect the L-NMMA which was used as a positive control (22.1 μ M). (8R,80R)-bishydrosyringenin, 1 α H,5 β H-aromandendrane-4 α ,10 α -diol and 1 β H,5 β H-aromandendrane-4 α ,10 β -diol also show similar inhibitory capacities respect L-NMMA with IC₅₀ values from 22.6 to 25.3 μ M. Interestingly, any of the 12 compound tested did not show cytotoxic activities with a cell viability after treatment with 20 μ M of each compound above of 95% ¹³⁹.

The phenolic amide, Melodamide A, together with 12 known compounds were isolated from the leaves of *Melodorum fruticosum* and their inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (FMLP/CB). Among the tested compounds melodamide A showed the more potent inhibition in response to FMLP/CB induced superoxide anion generation with an IC₅₀ value of 5.25 μ M without cytotoxicity. SB202190 a selective p38 MAPK inhibitor, diminished FMLP/CB-induced superoxide anion generation with an IC₅₀ value of 6.73 μ M. Sorafenib was used as positive control with an IC₅₀ value of 3.44 μ M ¹⁴⁰.

To improve the anti-inflammatory properties of Melodamide A, a series of analogues was synthesized and also the inhibitory effects against the release of both superoxide anion and elastase by human neutrophils were tested. From the sixteen analogues, only three compounds which had substitutions in the A-ring showed inhibitory activity on superoxide anion, with IC₅₀ values of 7.49 (2-Cl), 5.59 (3-F), and 5.19 (2-Br) μ M. Finally, respect the elastase release test, all molecules did not present inhibitory activity, the

authors relating these results with the substitutions on ring A which were not of much benefit to the anti-inflammatory activity ¹⁴⁰.

Table 1: Summary of anti-inflammatory activity of species of the Annonaceae

Species	Used material	Substances	Methodology	Inhibition	REF
<i>Xylopi aethiopica</i>	Fruits	Methanolic extract	Prostaglandin synthetase activity	-	101
	Fruits	Xylopic acid	Superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), myeloperoxidase (MPO) and malondialdehyde (MDA) activity	-	102
	Fruits	Ethanolic extract	Mouse pinnal inflammation in carrageenan-induced paw oedema	23% (30 µg/mL) 62% (300 µg/mL)	105
	Leaves	Hydroethanolic extract	TNF-α in (Lipopolysaccharide) LPS challenged THP-1-derived macrophages	>90% (500 mg/kg)	106
	Leaves	Hydroethanolic extract	Inhibition of IL-6 production, in a LPS challenged THP-1-derived macrophages,	84.6 % (250 µg/mL) 96.3 % (500 µg/mL)	106
	Leaves	Hydroethanolic extract	Interferences of 5-LOX in a LPS challenged THP-1-derived macrophages	IC ₅₀ = 85 µg/mL	106
	Fruits	Essential oil	NO production in LPS-stimulated RAW264.7 cells	-	107
<i>Enicosanthum membranifolium</i>				IC ₅₀ µM	
		2β-methoxyhardwickiic acid		65.4	
		(-)-Hardwicckiic acid		38.9	
		2β-acetoxymethoxyhardwickiic acid		16.1	
		2β-hydroxyhardwickiic acid	NO production	82.4	141
		15-methoxyapatogenic acid		28.9	
		Indomethacin (Positive control)		32.2	
<i>Xylopi parviflora</i>	Fruits	Volatile oil	NO production in LPS-stimulated RAW264.7 cells	37% (12µg/mL)	108
<i>Isolona dewevrei</i>	Leaves	Essential oil Nordihydroguaiaretic (Positive control)	Inhibit lipoxigenases (LOX)	0.0020 (mg/mL) 0.013 (mg/ml)	142
<i>Polyalthia viridis</i>		Leaf Essential oil			
	Leaves	Stem Essential oil		80.8 (IC ₅₀ = 76.7 µg/mL) 87.2 (IC ₅₀ = 57.6 µg/mL)	143
	Stem	Butein (Positive control)	NO production in LPS stimulated BV2 cells	91.8 (IC ₅₀ = 16.1 µg/mL)	
<i>Xylopi sericea</i>	Leaves	Ethanolic extract	NO production in LPS-stimulated RAW264.7 cells	76%	109
	Leaves	Ethanolic extract	IL-6 production in LPS-stimulated RAW264.7 cells	85%	109

<i>Annona cacans</i>	Leaves	Hydromethanolic extract (HME)	Myeloperoxidase (MPO) activity carrageenan-induced paw oedema	After 6 h, 28% (300 mg/kg HME- Leaves)	111
	Pulp Seeds	Ethyl acetate fraction (EAF)		53% (100 mg/kg HME- Pulp) 58% (300 mg/kg HME- Pulp) 43% (30 mg/kg EAF), 51% (100 mg /kg EAF)	
<i>Annona crassiflora</i>	Fruit peel	Polyphenol-enriched fraction (PEF)	Wound closure in C57 mice	75% (2% PEF topical) 84%(6% PEF topical)	112
	Leaves	Methanolic extract	Carrageenan-induced edema	53% (100mg/kg) 47% (300mg/kg)	113
	Leaves	Methanolic extract	MPO activity	60% (100mg/kg) 63% (300mg/kg)	113
	Leaves	Methanolic extract	Total leukocytes	78% (100mg/kg) 90% (300mg/kg)	113
	Leaves	Methanolic extract	Carrageenan-induced leukocyte migration	43% (300mg/kg)	113
<i>Annona muricata</i>	Fruits	Lyophilized extract	Xylene-induced ear edema	34.04% (50µg/mL) 63.83(100µg/mL) 80.85(200µg/mL)	114
	Fruits	Lyophilized extract	Cyclooxygenase (COX)-1 activity	39.44% (100µg/mL)	114
	Fruits	Lyophilized extract	Cyclooxygenase (COX)-2 activity	55.71% (100µg/mL)	114
<i>Annona squamosa</i>	Bark	Caryophyllene oxide	Carrageenan-induced paw edema	After 2 hours 45% (12.5mg/kg) 51% (25mg/kg)	117
	Bark	Sesquiterpene fraction (copaene (35.40%), patchoulane (13.49%) and 1H- cycloprop(e)azulene (22.77%))	Carrageenan-induced paw edema	After 2 hours 38% (12.5mg/kg) 34% (25mg/kg)	115
	Bark	18-acetoxy-ent-kaur- 16-ene	Carrageenan-induced paw edema	After 2 hours 51.6% (12.5mg/kg) 60.9% (25mg/kg)	117
<i>Annona glabra</i>	Fruits	7β,16α,17-trihydroxy- ent-kauran-19-oic acid	NO production in LPS-stimulated RAW264.7 cells	IC ₅₀ = 0.39 ± 0.12 µM	119
	Fruits	16β,17-dihydroxy-ent- kauran-19-al	NO production in LPS-stimulated RAW264.7 cells	IC ₅₀ = 0.32 ± 0.04 µM	119
<i>Annona Senegalensis</i>	Seeds	N-cerotoyltryptamine	ROS production in zymosan stimulated human whole blood phagocytes	IC ₅₀ = 2.7 ± 0.1 µg/mL	120
	Seeds	Methanolic extract	ROS production in zymosan stimulated human whole blood phagocytes	IC ₅₀ =8.7 ± 10.2 µg/mL	120
	Seeds	Acetogenin	NO production in lipopolysaccharide (LPS) stimulated J774.2 mouse macrophages	IC ₅₀ = 3.9 ± 0.2 µg/mL	120
<i>Annona vepretorum</i>	Leaves	Ethanolic extract	leukocyte migration to the peritoneal cavity	62 %(25 mg/kg), 76% (50 mg/kg) 98 % (100 mg/kg)	121
	Leaves	Ethanolic extract	Carrageenan-induced paw edema	After 2 hours 58%(25 mg/kg) 45% (50 mg/kg) 72 % (100 mg/kg)	121
	Leaves	Ethanolic extract	Histamine-induced paw edema	After 1 hour	121

				>65% (100mg/kg)	
<i>Annona nutans</i>	Leaves	Hydromethanolic extract	Carrageenan-induced paw edema	For 50 mg/kg 95.45% (2 hours) 74.29% (4 hours)	122
<i>Cyathocalyx pruniferus</i>	Leaves	Spathulenol	PGE ₂	71.4 (IC ₅₀ = 25.8)	144
		Cyclopropa-azulene		8.6 (IC ₅₀ = -)	
		Polycarpol		70.1 (IC ₅₀ = 24.7)	
		Koetjapic acid		80.4 (IC ₅₀ = 13.1)	
		2-Octaprenyl-benzoquinone		86.1 (IC ₅₀ = 11.2)	
		14-methylloctadec-1-ene		3.5 (IC ₅₀ = -)	
		1-Docosene		5.8 (IC ₅₀ = -)	
		β-Sitosterol		21.6 (IC ₅₀ = -)	
		α-Tocopherol		15.9 (IC ₅₀ = -)	
		11-prenol		2.1 (IC ₅₀ = -)	
		Quercetin		79.8 (IC ₅₀ = 15.4)	
		Epicatechin		77.3 (IC ₅₀ = 17.3)	
		Chrysin		73.8 (IC ₅₀ = 21.8)	
		Indomethacin (Positive control)		88.1 (IC ₅₀ = 11.8)	
<i>Cyathocalyx pruniferus</i>	Leaves	Spathulenol	COX-2	21.1 (IC ₅₀ = -)	144
		Cyclopropa-azulene		4.6 (IC ₅₀ = -)	
		Polycarpol		29.6 (IC ₅₀ = -)	
		Koetjapic acid		85.6 (IC ₅₀ = 8.1)	
		2-Octaprenyl-benzoquinone		88.1 (IC ₅₀ = 6.6)	
		14-methylloctadec-1-ene		2.1 (IC ₅₀ = -)	
		1-Docosene		2.4 (IC ₅₀ = -)	
		β-Sitosterol		11.1 (IC ₅₀ = -)	
		α-Tocopherol		10.5 (IC ₅₀ = -)	
		11-prenol		3.3 (IC ₅₀ = -)	
		Quercetin		80.1 (IC ₅₀ = 10.3)	
		Epicatechin		74.7 (IC ₅₀ = 12.5)	
		Chrysin		70.5 (IC ₅₀ = 15.7)	
		Dexamethasone (Positive control)		92.8 (IC ₅₀ = 5.1)	
<i>Duguetia furfuracea</i>	stem bark	Essential oil	LPS-induced paw edema	After two hours 41.67 % (3mg/kg) 86.11 % (10mg/kg)	123
	stem bark	Essential oil	LPS-induced paw edema	After four hours 45.45 % (1mg/kg) 63.64 % (3mg/kg) 92.42 % (10mg/kg)	123
	Leaves	Methanolic extract	Carrageenan-induced paw edema	After two hours 39 % (300mg/kg) 22 % (100mg/kg) 17.5% (30mg/kg)	126
	Leaves	Methanolic extract	Carrageenan-induced paw edema	After four hours 40 % (300mg/kg) 25 % (100mg/kg)	126
	Leaves	Dicentrinone	Carrageenan-induced paw edema	For 100 mg/kg 69.1% (2 hours) 50.4% (4 hours),	126

	Leaves	Methanolic extract	Zymosan-induced edema	38.1% (300mg/kg)	126	
	Leaves	Dicentrinone	Zymosan-induced edema	27.1% (300mg/kg)	126	
	Leaves	Enriched phenylpropanoid extract	LPS-induced paw edema	After two hours 90.91 % (3mg/kg) 92.42 % (10mg/kg)	124	
	Leaves	Enriched phenylpropanoid extract	LPS-induced paw edema	After four hours 77.78 % (1mg/kg) 77.78% (3mg/kg) 81.48 % (10mg/kg)	124	
	Leaves	α -asarone	LPS-induced paw edema	After two hours 62.12 % (3mg/kg) 69.70 % (10mg/kg) 69.70 % (30mg/kg)	125	
	Leaves	α -asarone	LPS-induced paw edema	After four hours 72.22 % (3mg/kg) 81.48 % (10mg/kg) 81.48 % (30mg/kg)	125	
<i>Duguetia moricandiana</i>	Fruits	Discretamine	NO production in LPS-stimulated macrophages	Around 50%. (100 and 200 μ g/mL)	127	
	Fruits	Discretamine	IL-6 production in LPS-stimulated macrophages	74.1% (50 μ g/mL) 76.6% (100 μ g/mL) 75.1% (200 μ g/mL)	127	
	Fruits	Discretamine	IL1-b production in LPS-stimulated macrophages	89.4% (50 μ g/mL) 87.4% (100 μ g/mL) 71.8% (200 μ g/mL)	127	
	Fruits	Discretamine	TNF- α production in LPS-stimulated macrophages	61.0% (50 μ g/mL) 45.2% (100 μ g/mL) 52.6% (200 μ g/mL)	127	
	Fruits	Discretamine	Carrageenan-induced paw edema	After one hour 42 % (10mg/kg) 62 % (20mg/kg)	127	
	Fruits	Discretamine	Carrageenan-induced paw edema	After two hours 44 % (10mg/kg) 67 % (20mg/kg)	127	
	Fruits	Discretamine	Carrageenan-induced paw edema	After four hours 59% (5mg/kg) 49 % (10mg/kg) 48 % (20mg/kg)	127	
<i>Duguetia staudtii</i>	Stem bark	Pachypodol	Myeloperoxidase (luminol/zymosan) burst	dependent oxidative	IC ₅₀ = 8.32 μ g/mL	128
	Stem bark	Kumatakenin	Myeloperoxidase (luminol/zymosan) burst	dependent oxidative	IC ₅₀ = 10.64 μ g/mL	128
	Stem bark	5,4'-dihydroxy-3,7,3',5'-tetramethoxyflavone	Myeloperoxidase (luminol/zymosan) burst	dependent oxidative	IC ₅₀ = 6.44 μ g/mL	128
	Stem bark	Pachypodol	Myeloperoxidase (lucigenin/PMA) oxidative burst	independent	IC ₅₀ = 11.04 μ g/mL	128
	Stem bark	Kumatakenin	Myeloperoxidase (lucigenin/PMA) oxidative burst	independent	IC ₅₀ = 14.13 μ g/mL	128
	Stem bark	5,4'-dihydroxy-3,7,3',5'-tetramethoxyflavone	Myeloperoxidase (lucigenin/PMA) oxidative burst	independent	IC ₅₀ = 8.55 μ g/mL	128
<i>Polyalthia longifolia</i>	Seeds	16-oxo-cleroda-3,13(14)E-dien-15-oic acid	COX-1, COX-2, and 5-LOX inhibitory activities	62.85% (COX-2) 26.41% (LOX-5)	132	
	Seeds	16-hydroxy-cleroda-3,13-dien-15-oic acid	COX-1, COX-2, and 5-LOX inhibitory activities	84.98% (COX-2) 30.51% (LOX-5)	132	

	Seeds	16-hydroxy-cleroda-4(18),13-dien-16,15-olide	COX-1, COX-2, and 5-LOX inhibitory activities	82.97% (COX-2) 12.73% (LOX-5)	132
	Seeds	3 α ,16 α -dihydroxy-cleroda-4(18),13(14)Z-dien-15,16-olide	COX-1, COX-2, and 5-LOX inhibitory activities	75.14% (COX-2) 14.38% (LOX-5)	132
	Seeds	16 α -hydroxy-cleroda-3,13(14)Z-dien-15,16-olide	COX-1, COX-2, and 5-LOX inhibitory activities	92.94% (COX-1) 79.41% (COX-2) 16,94% (LOX-5)	132
	Bark	16-oxocleroda-4(18),13-dien-15,16-olide	fMLP/CB induced superoxide generation by neutrophils	IC ₅₀ = 0.60 mg/mL	131
	Stem bark	Ethyl acetate (EA) extract	Carrageenan-induced paw edema	After four hours 27.5%(50mg/kg) 39.1 % (100mg/kg) 42.70% (200mg/kg) 62.67% (400mg/kg)	133
	Leaves	Diethyl ether extract	Xylene-induced ear edema	48.54% (200mg/kg)	134
	Leaves	n-hexane extract	Xylene-induced ear edema	65.92% (400mg/kg)	134
<i>Uvaria flexuosa</i>	Leaves	Flexuvarol B	Superoxide anion generation assay	IC ₅₀ = 4.72 mM	135
	Leaves	Chrysin	Superoxide anion generation assay	IC ₅₀ = 2.25 mM	135
	Leaves	Flexuvarol B	Elastase release assay	IC ₅₀ = 5.55 mM	135
	Leaves	Chrysin	Elastase release assay	IC ₅₀ = 2.44 mM	135
<i>Uvaria grandiflora</i>	Stems	(-) zeylenol	EPP-induced rat ear edema	90% (15min) 69% (30min) 52% (1 hour) 52% (2 hours)	136
<i>Phaeanthus vietnamensis</i>	Leaves	spathulenol	NO production in LPS-stimulated BV2 cells	IC ₅₀ = 15.7 μ M	139
	Leaves	(8R,80R)-bishydroxyrungenin	NO production in LPS-stimulated BV2 cells	IC ₅₀ = 25.3 μ M	139
	Leaves	1 α H,5 β H-aromandendrane-4 α ,10 α -diol	NO production in LPS-stimulated BV2 cells	IC ₅₀ = 23.0 μ M	139
	Leaves	1 β H,5 β H-aromandendrane-4 α ,10 β -diol	NO production in LPS-stimulated BV2 cells	IC ₅₀ = 22.6 μ M	139
<i>Melodorum fruticosum</i>	Leaves	Melodamide A	Inhibition of superoxide anion generation	IC ₅₀ = 5.25 μ M	140
	Leaves	Melodamide A derivate (2-Cl)	Inhibition of superoxide anion generation	IC ₅₀ = 7.49 μ M	140
	Leaves	Melodamide A derivate (3-F)	Inhibition of superoxide anion generation	IC ₅₀ = 5.59 μ M	140
	Leaves	Melodamide A derivate (2-Br)	Inhibition of superoxide anion generation	IC ₅₀ = 5.19 μ M	140

3.2 Insecticidal, larvicidal and pesticidal

Larvicidal, pesticide and insecticide activities are intricately linked, they may be related to animals that transmit diseases or even harm the planting and breeding of animals. The control of these animals is extremely important for public health as well as in the food sector.

From now on, we will address the term “insecticide” to refer, in a global way, to larvicide, insecticide and pesticide activities. Compounds classified as insecticides are those capable of killing, attracting and repelling insects^{145–147}. However, presenting only toxicity against insects is not enough to be classified as an insecticide. This is because there are other properties that chemical substances must have, associated with their activity, such as being effective at low concentrations, not showing toxicity against mammals and higher animals, not having phytotoxicity, being easy to obtain, handling and application, presenting economic viability and not be cumulative in human and domestic animal adipose tissue^{145–147}. All these properties describe a perfect, ideal insecticide, which will hardly happen in practice.

The use of plants and their isolated compounds for insecticidal activity has been done since ancient times^{148,149}. They were widely used until the 1940s, when synthetic products began to gain space in the market. However, synthetic insecticides are shown to be extremely potent and, on the other hand, are quite unspecific, presenting several toxicity problems¹⁴⁷.

In the search for new insecticides that present the properties of efficacy, safety, and selectivity, are the natural products. There are recorded in the literature more than two thousand species of plants that have the potential for these activities. However, few of these species have been used commercially, with emphasis on pyrethrin's, rotenoids and alkaloids^{146,147,150}.

It has been found that the use of secondary metabolites from plants are a low-cost alternative for pest control^{151,152}. Thus, the search for insecticidal compounds from plants has intensified greatly in recent decades, and it is possible to find in the literature many publications and studies highlighting the insecticidal potential of plants, as well as an economic and ecological source¹⁵².

In this context, the Annonaceae stands out for its structural variability of its secondary metabolites, rich in alkaloids and acetogenins^{152,153}. Acetogenins, a class of natural product exclusive to Annonaceae, have shown to be quite promising as a prototype of insecticidal agents^{147,152,154}. The studies carried out by Colom et al. (2008)¹⁵⁵ and Alvarez et al. (2007)¹⁵⁶ are examples of studies that evaluate the insecticidal potential of acetogenins isolated from Annonaceae.

A literature review carried out by Krinski, Massaroli and Machado (2014)¹⁴⁹ described the great potential of the Annonaceae family in insect control. The authors reported about 42 species of Annonaceae with scientific information of insecticidal

activity for about 60 species of insect pests. Awakening the scientific community for several research still necessary to discover the insecticidal potential of several other species as well as to deepen this knowledge. The review by Krinski, Massaroli and Machado (2014)¹⁴⁹ points out the species *Annona muricata* (soursop) and *Annona squamosa* (fruit-of-conde or pine cone) as the most used in studies of insecticidal potential.

Table 2 summarizes the studies available in the literature that assess the insecticidal potential of Annonaceae species. It is possible to observe that despite promising results proving all the insecticidal potential of Annonaceae, the substantial number of studies evaluate essential oils, extracts, and fractions. Therefore, research is needed to isolate the secondary metabolites of the species already studied to evaluate them separately and prove their insecticidal, larvicidal, pesticidal and cytotoxic potential.

Table 2: Summary of insecticidal, pesticidal and larvicidal activity of species of the Annonaceae.

Annonaceae species	Part of plant	Substances / Extracts	Insect species (Order)	Methods			Ref
				Topical (LC ₅₀ µg/larva)	Topical (LD ₅₀ – 95% CL - µg/nymph)	Oral (LC ₅₀ ppm fresh weight in diet)	
<i>Annona atemoya</i>	Seed	Seed extract	Trichoplusia ni (Lep.)	197.67	-	301.30	157
<i>Annona squamosa</i>	Seed	Seed extract	Trichoplusia ni (Lep.)	382.37	-	167.48	157
<i>Annona cherimola</i>	Seed	Squamocin Molvizarin Almunequin Itrabin Deltamethrin (Positive control)	Oncopeltus fasciatus		0.16 0.34 11.23 14.91 7.4		155
<i>Annona cherimola</i>	Seed	Neoannonin Itrabin Almunequin Asimicin Squamocin	Spodoptera Frugiperda	15.5 18.8 19.7 17.3 Instant death	10 30 30 30 100	0.90 0.59 1.10 0.77 0.16	156

		Motrilin		18.0	20	1.19	
		Cherimolin-1		14.0	0	0.91	
		Cherimolin-2		17.7	10	0.97	
		Tucumanin		14.7	20	0.81	
		Control		12.1	10	1.02	
<i>Annona coriaceae</i>	Seed	Seed Extract	Aedes aegypti (Dip.)	0.01	-	-	158
<i>Annona coriaceae</i>	Seed	Seed Extract	Aedes aegypti (Dip.)				159
		100 ppm		0.50	3.00	3.5	
		50 ppm		0.40	6.00	5.5	
		DMSO 0.1%		0.30	-	-	
		Água		0.20	-	-	
		(Control)					
<i>Annona coriaceae</i>	Seed	Seed Extract	Aedes aegypti (Dip.)				160
		0 ppm		0.0	0.0	10.0	
		50 ppm		0.7	7.5	9.25	
		100 ppm		0.7	7.5	9.25	
		200 ppm		1.0	10.0	9.00	
		300 ppm		2.2	22.5	7.75	
		400 ppm		4.5	45.0	5.50	
		500 ppm		6.2	62.5	3.75	
<i>Annona coriaceae</i>	Seed	Seed Extract	Aedes aegypti (Dip.)				161
		Methanol (des. Hexane)		0.1	100.0	0.007	
		Hexane		0.1	100.0	0.007	
		Dichloromethane		0.1	58.75	0.805	
		Methanol (des. DCM)		0.1	0.0	0.0	
<i>Annona coriaceae</i>	Seed	Diet	Anagasta kuehniella (Lep.)	0.0	26.3	71.8	162
				2.0	16.8	81.9	
<i>Annona coriaceae</i>	Seed	Diet	Corcyra Cephalonica (Lep.)	0.0	23.16	69.7	162
				2.0	32.25	49.3	

<i>Annona coriaceae</i>	Seed	Seed Extract	Dichelops melacanthus (Hem.)				163
		Hexanic					
		8.0%		-	78.00	-	
		4.0%		-	86.00	-	
		2.0%		-	68.00	-	
		1.0%		-	58.00	-	
		0.5%		-	42.00	-	
		Methanolic					
		8.0%					
		4.0%		-	96.00	-	
		2.0%		-	94.00	-	
		1.0%		-	94.00	-	
		0.5%		-	70.00	-	
		Ethanolic		-	40.00	-	
		8.0%					
		4.0%					
		2.0%		-	100.00	-	
		1.0%		-	100.00	-	
		0.5%		-	90.00	-	
				-	84.00	-	
		Distilled water (Positive Control)		-	80.00	-	
		C 01					
		C 02		-	6.00	-	
				-	4.00	-	
				-	6.00	-	
				-	12.00	-	
				-	0.00	-	
				-	2.00	-	
<i>Annona coriaceae</i>	Seed	Seed Extract	Euschistus heros (Hem.)				164
		Preview		3.0	-	-	
		2 Days		4.6	8.91	-	
		5 Days		3.4	-	-	
		7 Days		3.7	26.73	-	

		DMSO 20% (Positive Control 01)		3.1	-	-	
				5.0	-	-	
				3.4	-	-	
		Water (Positive Control 02)		5.0	-	-	
				4.1	-	-	
				4.4	-	-	
				2.8	-	-	
				5.2	-	-	
<i>Annona coriaceae</i>	Seed	Seed Extract	Tuta absoluta (Lep.)				165
		Methanolic					
		0.5		-	8.0	-	
		1.0		-	100	-	
		2.0		-	100	-	
		4.0		-	86.4	-	
		8.0		-	86.6	-	
		DMSO 20%		-			
		Water		-	6.6	-	
					13.2	-	
<i>Annona cornifolia</i>	Leaves	Leave extract	Anticarsia gemmantalis (Lep.)				166
		2.5		-	0.41	-	
		2.0		-	0.38	-	
		1.5		-	-0.10	-	
		1.0		-	-0.25	-	
		0.5		-	0.01	-	
<i>Annona cornifolia</i>	Leaves	Leave extract	Spodoptera frugiperda (Lep.)				166
		2.5		-	0.96	-	
		2.0		-	0.68	-	
		1.5		-	0.55	-	
		1.0		-	0.66	-	
		0.5		-	0.36	-	

<i>Annona Crassiflora</i>	Fruits/ Twigs/ Roots	Extract	<i>Aedes aegypti</i> (Dip.)				167
		Hexanic					
		SB		192.57	-	-	
		RW		154.02	-	-	
		RB		264.15	-	-	
		Ethanollic					
		RW					
		RB		26.89	-	-	
				23.06	-	-	
<i>Annona Crassiflora</i>	Roots	Extract	<i>Aedes aegypti</i> (Dip.)				168
		Root bark		0.71	-	-	
		Root wood		8.94	-	-	
		Stem		16.1	-	-	
<i>Annona Crassiflora</i>	Seeds	Seeds Extract	<i>Aedes aegypti</i>				161
		Methanol (Des. DCM)		1.0	0.0	-	
		Hexanic		1.0	91.25	0.433	
		Hydroalcoholic		1.0	0.0	-	
		Fraction					
		Ethyl Acetate		1.0	0.0	-	
		Chloroform		1.0	0.0	-	
		Crude					
		Methanolic		1.0	11.25	3.189	
<i>Annona Crassiflora</i>	Seeds	Seeds Extract	<i>Euschistus heros</i> (Hem.)				169
		1%		481.50	1.25	353.00	
		2%		542.00	1.50	396.00	
		4%		372.00	2.00	306.00	
		DMSO 40% (Positive control)		683.00	1.25	309.20	
<i>Annona Crassiflora</i>	Seeds	Seeds Extract	<i>Euschistus heros</i> (Hem.)				164

		Preview		2.4			
		2 Days		4.1	17.82		
		5 Days		3.0	13.04		
		7 Days		4.2	16.83		
		DMSO 20% (Positive control 01)					
				3.1			
				5.0	-		
				3.4	-		
		Water (Positive control 02)		5.0	-		
				4.1			
				4.4	-		
				2.8	-		
				5.2	-		
<i>Annona Crassiflora</i>	Seeds	Seed Extract	Tibraca limbativentris (Hem.)				170
		24 h					
		8.0%		70.0	4.46	4.34	
		4.0%		64.0			
		2.0%		44.0			
		1.0%		20.0			
		0.5%		6.0			
		72 h					
		8.0%					
		4.0%		78.0	3.64	3.55	
		2.0%		72.0			
		1.0%		54.0			
		0.5%		30.0			
				10.0			
		120h					
		8.0%					
		4.0%					
		2.0%		81.0	3.30	3.16	

		1.0%		76.0			
		0.5%		58.0			
				34.0			
		Water + Tween 80 (Positive Control 01)		10.0			
				2.0			
		Water (Positive Control 02)		4.0			
				9.0			
				0.0			
				2.0			
				6.0			
<i>Annona dioica</i>	Seeds	Seeds Extract	Aedes Aegypti (Dip.)				161
		Fraction					
		Dichloromethane		1.0	10.00	2.447	
		Methanol		1.0	3.75	5.196	
<i>Annona dioica</i>	Seeds	Seed Extract	Rhodnius neglectus (Hem.)				171
		Topical application method					
		25					
		50			6.2		
		100			85		
		200			90		
					100		
		Cantate application method					
		25					
		50					
		100					
		200			88.2		
					91.6		
		(Control)			95.6		
					96.0		

					0.0 0.0		
<i>Annona diversifolia</i>	Leaves/ Branches	Extract Stem Aqueous Ethanolic Leaf Aqueous Ethanolic	Anastrepa ludens (Dip.)	588.685 409.139 >1000 52.0284			172
<i>Annona foetida</i>	Seeds	Seed Extract Methanolic 24h 48h Hexanic 24h 48h Dichloromethanic 24h 48h	Aedes aegypti (Dip.)	76.15 62.28 15.17 6.72 0.73 0.33			173
<i>Annona glabra</i>	Seeds	Seed Extract	Aedes aegypti (Dip.)	0.06			168
<i>Annona montana</i>	Leaves/ Branches	Annonacin Cis-annonacin-10-one Densicomacin-1 Gigantetronenin Murihexocin-B Tucupentol Control	Spodoptera frugiperda (Hem.)	49.20 44.75 60.00 55.20 55.12 59.00 27.12	50 60 40 70 30 70 0.0	50 40 60 30 70 70 10	174
<i>Annona mucosa</i>	Fruits and branches	Ethanolic extract Rollinacin Rolliniastacin-1	<i>Aedes aegypti</i>	2.60 0.78 0.43		-	175

<i>Annona mucosa</i>	Fruits and branches	Ethanollic extract	<i>Aedes albopictus</i>	0.55			175
		Rollinicin		1.13		-	
		Rolliniastacin-1		0.20			
<i>Annona mucosa</i>	Seeds	Seed Extract	<i>Aedes aegypti</i> (Dip.)	0.010	98.75		161
				-	95.00		
				-	77.50		
				-	75.00		
		Control		-	0		
<i>Annona mucosa</i>	Seeds	Seed Extract	<i>Chrysodeixis</i> <i>inclusens</i> (Lep.)				176
		0.5			< 55%		
		1.0			< 55%		
		2.0			< 55%		
		4.0			86.6%		
		8.0			93.3%		
		Water (Positive control 01)			-		
		Water + Polisorbate (Positive control 02)			-		
<i>Annona mucosa</i>	Seeds/ Branches/ Leaves	Extract	<i>Sitophilus zeamais</i> (Col.)				177
		Seeds					
		300 mg kg		0.80	98.00	7.84	
		1500 mg kg		0.00	100.00	1.12	
		Leaves					
		300 mg kg		36.80	0.50	81.97	
		1500 mg kg		5.60	61.50	57.90	
		Branches					
		300 mg kg					
		1500 mg kg		34.70	1.50	83.71	
				39.10	0.00	64.97	
		Control					
				36.90	0.00	81.94	

				37.00	0.00	81.74	
<i>Annona mucosa</i>	Seeds	Seed Extract	Timbraca limbativentris (Hem.)				170
		24h					
		8.0%		100.0	1.59	0.49	
		4.0%		92.0			
		2.0%		90.0			
		1.0%		76.0			
		0.5%		28.0			
		72h					
		8.0%					
		4.0%		100.0	1.18	-0.24	
		2.0%		96.0			
		1.0%		96.0			
		0.5%		86.0			
				42.0			
		120h					
		8.0%					
		4.0%		100.0	0.91	-0.76	
		2.0%		98.0			
		1.0%		96.0			
		0.5%		88.0			
				56.0			
		Water + Tween80 (Positive Control 01)					
		Water (Positive Control 02)		2.0	-	-	
				4.0			
				9.0			
				0.0	-	-	
				2.0			
				6.0			
<i>Annona muricata</i>	Seeds	Seed Extract	Aedes aegypti (Dip.)	236.23	74.68	-	178

<i>Annona muricata</i>	Seeds	Seed Extract	<i>Aedes aegypti</i> (Dip.)				179
		12h		0.18		0.10	
		24h		0.06		0.05	
		36h		0.04		0.03	
		48h		0.02		0.01	
<i>Annona muricata</i>	Seeds	Seed Extract	<i>Aedes aegypti</i> (Dip.)	900.0		380.0	180
<i>Annona muricata</i>	Leaves/ Branches	Extract	<i>Anastrepha ludens</i> (Dip.)				172
		Leaves					
		Ethanollic					
		24h		831.445	1.0		
					2.2		
		72h					
					22.0		
		Aqueous			68.3		
		24h		>1000			
					0.0		
		72h			2.4		
					70.3		
		Stems			70.3		
		Ethanollic					
		24h		865.0	1.2		
		72h			1.5		
		Aqueous			5.8		
		24h			89.3		
		72h		>1000	0.2		
					0.8		
					44.3		
					74.3		
<i>Annona muricata</i>	Seed	Seed extract	<i>Anopheles albimanus</i> (Dip.)	16.20	0.82		178

<i>Annona muricata</i>	Seed	Food consumption (%) Low dose Medium dose Water (Positive Control 01) 10% Ethanol (Positive Control 02)	Anticarsia gemmantallis (Lep.)				181
					10.0 30.0	25.0 29.3	
					0.0	19.9	
					0.0	19.3	
<i>Annona muricata</i>	Seed	Parviflorin Asimicin Sylvaticin Bullatalicin Annomontacin Gigantetrocin A Cypermethrin Chlorpyrifos Hydramethylnon Propoxur Bendiocarb Parviflorin Asimicin Sylvaticin Bullatalicin Annomontacin Gigantetrocin A Cypermethrin Chlorpyrifos Hydramethylnon Propoxur Bendiocarb	Blatella germanica (Blat.)	0.6 1.8 1.5 6.5 3.6 4.1 0.003 0.3 5.6 39.9 43.2 0.6 1.6 3.1 5.7 2.1 9.5 0.1 2.1 9.5 0.1 - 110.5 88.2	6 10 8 23 23 34 6 3 12 - - 6 10 21 23 10 40 12 14 12 14 12 - -		182
<i>Annona muricata</i>	Leaves	Leaves Extract	Culex Quinquefascintus	20.87		56.47	183

<i>Annona muricata</i>	Seed	Compounds	Leptinotarsa dercemlineata (Col.)		92.18 29.68 100 0.00		184
		1 2 Rotenone (Control)					
<i>Annona muricata</i>	Leaves	Leaves Extract	Plutella xylostella (Lep.)	100.00 53.0 34.8 23.0 1.6	0.00 10.6 10.6 8.8 8.2 8.0	0.00 5.2 5.6 5.4 5.2 4.0	185
<i>Annona muricata</i>	Seeds	Seed Extract	Plutella xylostella (Lep.)	43.0	60.0		186
<i>Annona muricata</i>	Seeds	Seed Extract	Sithophillus zeamais (Col.)				187
		Hexanic 24 48 72 Ethyl Acetathe 24 48 72 Ethanol 24 48 72		11.447 - - - - - - - -		4.009 3.854 3.760 3.280 2.667 2.542 - - -	
<i>Annona muricata</i>	Seeds	Seed Extract	Sithophillus zeamais (Col.)	90.20 91.80 8.96 9.82 16.94			188
		0.0					

				18.04			
		0.05		84.60			
				90.80			
				8.72			
				9.63			
				16.57			
				17.75			
		0.1		80.00			
				89.80			
				8.39			
				8.86			
				14.97			
				16.38			
		0.2		78.80			
				85.40			
				8.08			
				8.32			
				14.67			
				14.98			
		0.4		77.80			
				83.00			
				7.63			
				7.94			
				14.09			
				14.47			
		0.8		45.80			
				62.20			
				6.95			
				7.02			
				11.43			
				13.17			
<i>Annona muricata</i>	Seeds	Seeds Extract	Zabrotes subfasciatus (Col.)	46.0	39.1	36.4	189

<i>Annona reticulata</i>	Seeds	Seed Extract (95% of Methanol) In two periods: 24h and 48h. 1.0 g/l 2.5 g/l 5.0 g/l 7.5 g/l 10.0 g/l 15.0 g/l 20.0 g/l Control	<i>Epilachna vigintioctopunctata</i> (Col.)		(%) 24h and 48h 6.7 - 13.4 40.0 – 53.4 80.0 – 100 100 – 100 100 – 100 100 – 100 100 – 100 0.0 – 0.0		190
<i>Annona reticulata</i>	Uninformed	Petroleum ether extract Ethanollic extract	<i>Rhodnius neglectus</i> (Hem.)		35.0 Not significant		191
<i>Annona reticulata</i>	Seeds	Methanolic extract	<i>Spodoptera litura</i> (Lep.)	301.30 (259.15-326.33)	50.0%	167.48 (110.43-383.65)	157
<i>Annona salzmannii</i>	Barks	Hexanic Extract (8,68 g (0,48 %) Methanolic Extract 143,29 g (7,96%) Fração CHCl ₃ alcaloídica – FCA Fração CHCl ₃ neutra – FCN Óxido de cariofileno Temephós	<i>Aedes aegypti</i> (Dip.)	615.18 >700.00 163.53 15.92 167.00 0.042	(473.71 - 981.44) (00.00-00.00) (107.90-238.82) (13.31-19.40) (130.00-218.00) (0.035-0.050)		192
<i>Annona senegalensis</i>	Root	Root Extract	<i>Callosobruchus maculatus</i> (Col.)	18.7	3.7	0.1	193

		Control		4.7	98.0	79.5	
<i>Annona senegalensis</i>	Fruits	Ethanol extract	Culex quinquefasciatus (Dip.)	0.67	23.42	29.78	183
<i>Annona senegalensis</i>	Uninformed	Extract	Sitophilus zeamais (Col.)	220.71	0.19 – 0.06		194
<i>Annona squamosa</i>	Leaves	Aqueous extract (g/100 ml)	Aedes aegypti (Dip.)				195
		100.0		100.0			
		50.0		100.0			
		25.0		83.3			
		12.5		90.0			
		6.25		70.0			
		3.125		76.6			
		1.5625		73.3			
		Control		5.7			
<i>Annona squamosa</i>	Seeds	Extract (µg/ml)	Aedes albopictus (Dip.)				196
		1		388.3	397.4	486.6	
		2		231.3	240.3	268.5	
		4		162.3	163.8	185.4	
		6		114.0	112.0	121.1	
		8		64.9	72.4	84.9	
		10		45.6	47.8	56.3	
		20		0.0	0.0	0.0	
<i>Annona squamosa</i>	Leaves	Ethanol extract (mg/ml) at 24h, 48h and 72h.	Anopheles gambiae (Dip.)				197
		5			3.33; 6.67; 23.33		
		10			16.67; 33.33; 63.33.		
		20					

		30			40.0; 66.67; 76.67.		
		40			53.33; 73.33; 90.0.		
					70.0; 90.0; 1000.		
<i>Annona squamosa</i>	Whole plant	Extract (ppm)	Anopheles stephensi (Dip.)	(%)	(%)	(%)	198
		50		58	4	52	
		100		60	6	76	
		150		70	16	86	
		200		74	18	92	
		Control		0	4	8	
<i>Annona squamosa</i>	Leaves	Extract (mg/l)	Anopheles subpictus (Dip.)		(%)		199
		500			100 – 0.0		
		250			82.6 – 2.46		
		125			63.0 – 1.84		
		62.5			48.2 – 4.62		
		31.25			16.4 – 2.04		
		15.63			92.0 – 3.28		
		7.82			4.6 – 4.60		
<i>Annona squamosa</i>	Leaves	Ethanol extract	Bemisia tabaci (Hem.)		100 – 0.0		200
		Aqueous extract			99.3 – 1.05		
<i>Annona squamosa</i>	Seeds	Extract (mg/ml)	Callasobruchus chinensis (Col.)		(%)		201
		0.01			9.66		
		0.03			9.66		
		0.05			41.00		
		0.07			81.33		
		0.09			99.00		
<i>Annona squamosa</i>	Seeds	Extract (µg/cm ²)	Ceratitis capitata (Dip.)	(%)	(%)		202
		Petroleo ether		0.031	198.57		
		Ethanol		0.632	614.26		
		Acethone		0.591	1000.40		

		Methanol		4.038	135.25		
<i>Annona squamosa</i>	Seeds	Extract (%)	Crocidolomia pavonana (Lep.)				203
		0.05			10.4 ± 1.2	32.0 ± 6.9	
		0.1			6.3 ± 2.7	69.0 ± 4.8	
		Deltamethrin			11.0 ± 3.6	62.4 ± 5.2	
		Control			14.0 ± 2.0	48.0 ± 6.6	
<i>Annona squamosa</i>	Seeds	Aqueous extract (25%)	Culex quinquefasciatus (Dip.)		33.6 %		204
<i>Annona squamosa</i>	Leaves	Ethanol extract (mg/ml) at 24h, 48h and 72h.	Culex quinquefasciatus (Dip.)		(%)		197
		5			0.0; 13.33; 46.67.		
		10			33.33; 56.67; 73.33.		
		20			56.67; 70.0; 90.0.		
		30			80.0; 96.67; 100.		
		40			93.33; 100; 1000.		
<i>Annona squamosa</i>	Leaves	Aqueous extract (g/100 ml)	Culex quinquefasciatus (Dip.)		(%)		195
		100.0			100.0		

		50.0			60.0		
		25.0			50.0		
		12.5			36.7		
		6.25			26.7		
		3.125			33.3		
		1.5625			10.0		
		Control			4.2		
<i>Annona squamosa</i>	Leaves	Extract	Culex quinquefasciatus (Dip.)	0.64	14.69		183
<i>Annona squamosa</i>	Leaves	Methanol Extract (mg/l)	Culex tritaeniorhynchus (Dip.)		(%)		199
		500			100.0-00.0;		
		250			88.4-1.64;		
		125			52.6-4.63;		
		62.5			34.2-2.84;		
		31.25			20.6-1.67;		
		15.63			12.4-2.45;		
		7.82			6.8-1.87.		
<i>Annona squamosa</i>	Seeds	Diet (µg/2g)	Drosophila melanogaster (Dip.)			62.5	205
		48h					
<i>Annona squamosa</i>	Seeds	Extract (g/l)	Epilachna vigintioctopunctata (Col.)	24h e 48h (%)	(%)		190
		1.0		6.7-13.4	64.8		
		2.5		40.0-53.4	83.4		
		5.0		80.0-53.4			
		10.0		100-100	92.3		
		15.0		100	95.9		
		20.0		100	95.9		
					100.0		
<i>Annona squamosa</i>	Branches	Extract (%)	Musca domestica (Dip.)		41.00		206

<i>Annona squamosa</i>	Leaves	Extract (mg/l)	Musca domestica (Dip.)	(%)		(%)	207
		0		100		100	
		200		80		62.5	
		400		65		53.85	
		600		50		40.0	
		800		30		33.33	
		1000		0		0.0	
<i>Annona squamosa</i>	Leaves	Extract (% plant poder)	Oryctes rhinoceros (Col.)	(%)	(%)		208
		5		10	10		
		10		30	20		
		20		50	20		
		Control		00	00		
<i>Annona squamosa</i>	Leaves	Extract (%)	Periplaneta americana (Blat.)		(%)	Average	209
		Positive Control			100	5.00±0.00	
						4.00±0.0	
		100			80	3.00±0.0	
		75			60	2.5±0.71	
		50			50	1.00±0.0	
		25			20	0.5±0.701	
		10			10	0.5±0.701	
		5				0.0±0.0	
		0.1			10		
						0.0±0.0	
		Negative control			0		
					0		
<i>Annona squamosa</i>	Seeds	Aqueous extract Larval instar (Time h)	Plutella xylostella (Lep.)		(%)	(%)	210
		3rd:					
		24h			5.2 (3.1-8.5);	2.5±1.4	
		48h			1.7 (1.3-2.2);	10.0±6.8	

		72h			0.9 (0.7-1.2).	12.5±6.0	
		4th:					
		24			8.7 (6.6-11.3);	0	
		48			4.2 (3.5-5.1);	1.3±1.3	
		72			2.0 (1.7-2.4).	5.0±2.0	
<i>Annona squamosa</i>	Seeds	Extract (mg/ml)	Plutella xylostella (Lep.)		(%)		¹⁸⁶
		5			46.7		
		10			70.0		
<i>Annona squamosa</i>	Seeds	Aqueous extract	Sitophilus oryzae (Col.)		(LD ₅₀ Min)	(% min)	²¹¹
		1%			23.1 (22.1-23.9)	39.6±1.4	
		5%			11.4 (10.7-12.2)	14.5±1.1	
		Control 1 (Acetone)			0.0	-	
		Control 2 (Methanol)			0.0	-	
		Control 3 (Without solvente)			0.0	-	
<i>Annona squamosa</i>	Seeds	Extract (%)	Spodoptera litura (Lep.)				²¹²
		0.5		21.66±1.66	0.0	28.33±1.66	
				23.33±1.66		33.33±1.66	
		1			0.0		
				38.33±1.66		51.66±1.66	
		5		48.33±1.66	0.0	58.33±1.66	

		10		56.66±1.66	0.0	78.33±3.33	
				51.66±1.66		75.00±0.0	
		15		61.66±1.66	0.0	80.00±0.0	
		20		0.0	1.66±1.66	6.66±1.66	
		25			1.66±1.66		
		Absolute control			1.66±1.66		
<i>Annona squamosa</i>	Seeds	Extract	Tribolium castaneum (Col.)	LD ₅₀ (µg/cm ²)	95% (Lower and Upper)		²¹³
		Petroleum ether		0.031	0.006 and 0.150;		
		EtOH		0.632	0.315 and 1.265;		
		Acetone		0.591	0.285 and 1.224;		
		Methanol		4.038	1.727 and 9.440.		
<i>Annona squamosa</i>	Seeds	Extracts using two methods of application:	Trichoplusia ni (Lep.)	301.30 (25915-326.33)		167.48 (110.43-383.65)	¹⁵⁷
		Topical (µg/larva)					
		Oral (ppm fresh weight in diet.					

<i>Annona squamosa</i>	Seeds	Extract (ppm)	Trogoderma granarium (Dip.)	(%) 10 th day and 15 th day.	(mg) 10 th day and 15 th day.	214
		Hexane extract				
		250		0.0 and 11.13;	66.1 and 80.5;	
		500		4.47 and 11.13;	75.1 and 84.6;	
		750		11.13 and 17.8;	64.9 and 70.9;	
		1000		17.8 and 28.87;	63.6 and 71.8;	
		1250		48.87 and 53.33;	64.9 and 70.1;	
		1500		75.33 and 82.2.	58.3 and 61.5.	
		Ethyl Acetate extract				
		50		4.47 and 20.00;	73.0 and 86.2;	
		250		24.47 and 33.33;	65.6 and 73;	
		500		26.67 and 55.53;	72.4 and 81.0;	
		750		51.13 and 57.8;	62.9 and 69.5;	
		1000		64.47 and 80.00;	59.8 and 66.0;	

		1250		84.47 and 91.13		56.0 and 61.0.	
		Methanol extract 500		6.67 and 8.87;		78.0 and 96.6;	
		750		13.34 and 17.8;		74.1 and 93.1;	
		1000		22.2 and 24.47;		75.2 and 98.2;	
		1250		20.0 and 26.67;		74.6 and 92.4;	
		1500		48.87 and 57.73;		58.0 and 85.0;	
		2000		66.67 and 77.73.		53.4 and 62.5.	
		Acetone control		0.0 and 0.0;		100.9 and 150.6.	
		Control		0.0 and 0.0.		94.9 and 161.6.	
<i>Artabotrys odoratissimus</i>	Bark	Larval instar and exposure periods (h)	Culex quinquefascintus (Dip.)	LC ₅₀			215
		Second 12		52.92	95% (Lower and Upper)		
		24		42.03	33.59 and 83.87;		
		Third 12		110.03	26.18 and 67.47;		

		24		99.13	72.51 and 166.97;		
		Fouth 12		170.12	60.17 and 163.29;		
		24		110.41	137.64 and 210.26;		
					89.66 and 135.95.		
<i>Asimina triloba</i>	Roots	Ethanollic extract (Fraction n°.)	Acalymma vittatum (Col.)	LC ₅₀ (p.p.m.)			²¹⁶
		017		7.56			
		018		>1000			
		019		1.67			
		020		0.04			
		021		715			
		Asimicin		0.03			
<i>Asimina triloba</i>	Barks	Aqueous methanol extract fraction	Cerotoma trifurcata (Col.)	LC ₅₀ (ppm)	(%)		²¹⁷
				0.077 (0.04- 0.12);	2.71		
				0.102 (0.06- 0.15);	1.96		
				0.104 (0.03- 0.20);	2.52		
				0.135 (0.09- 0.21);	2.72		

				0.202 (0.14- 0.31).	1.60		
<i>Asimina triloba</i>	Uninformed	Ethanollic extract FO20 (µg) Asimicin (nmol)	Eurytides marcellus (Lep.)		Pretreatment and Treated 3.36±0.40 and 2.97±0.26; 3.27±0.18 and 2.83±0.84; 3.10±0.79 and 2.74±0.56; 5.26±0.45 and 3.69±0.71; 4.03±0.73 and 3.51±0.73; 5.31±1.21 and 2.47±0.18; 4.43±0.10 and 2.47±0.66; 3.97±0.89 and 5.47±1.27;		218

					3.66±0.09 and 5.11±0.94; 3.92±0.35 and 7.13±1.10; 3.61±0.28 and -; 3.15±0.19 and -.		
<i>Cardiopetalum calophyllum</i>	Seeds	Methanolic extract (1.0 mg/mL)	<i>Aedes aegypti</i> (Dip.)	(%) 5.00	(mg/mL) 1.789		161
<i>Dennettia tripetala</i>	Leaves and roots	Ethanol extract (5mL/100g) in 1, 3 and 7 days. 0.0 2.50 5.00 7.50	<i>Dermestes maculatus</i> (Col.)		(%) In 1, 3 and 7 days. 0.0±0.0; 0.0±0.0; 0.0±0.0. 26.67±0.88; 71.67±0.88; 100.0±0.0. 26.67±0.67; 75.0±1.16; 100.0±0.0. 48.33±0.88; 91.67±0.33; 100.0±0.0.		219

		10.0			51.67±0.66; 98.33±0.33; 100.0±0.0.		
<i>Dennettia tripetala</i>	Seeds	Concentration of plant powder/25g fish	Dermestes maculatus (Col.)	(%)		(g)	220
		Extract		87.0		0.25	
		Untreated control		100		0.50	
<i>Dennettia tripetala</i>	Uninformed	Extracts (ppm)	Ostrinia nubialis (Lep.)				221
		0		5.79			
		10		3.13			
		100		3.87			
		1000		3.84			
<i>Dennettia tripetala</i>	Seeds	Extract (ml/25g)	Necrobia rufipes (Col.)		(%)		220
		0.1			100		
		0.2			100		
		0.3			100		
		Untreated control			10		
<i>Dennettia tripetala</i>	Leaves and roots	Extract (%) at 24h, 48h and 72h.	Sitophilus zeamais (Col.)		(%) at 24h, 48h and 72h.		222
		0%			1.83; 1.09; 1.75.		
		1%			1.50; 2.09; 2.58.		
		5%			1.50; 2.09; 3.08.		

		10%			2.83; 3.58; 4.67.		
<i>Duguetia furfuraceae</i>	Leaves/barks and roots	Extract	<i>Aedes aegypti</i> (Dip.)	(µg/ml) 56.6			167
<i>Duguetia furfuraceae</i>	Leaves	Extract	<i>Sitophilus zeamais</i> (Col.)	(Proportion of insects in the treated area (SDM)) 0.500 (0.113).			223
<i>Guatteria blephrophylla</i>	Leaves	Extract (ppm)	<i>Aedes aegypti</i> (Dip.)	85.74	(74.05 – 112.78)	4.48±0.89	224
<i>Guatteria friesiana</i>	Leaves	Extract (ppm)	<i>Aedes aegypti</i> (Dip.)	52.60	(50.11 – 55.17)	6.48±0.55	224
<i>Guatteria hispida</i>	Leaves	Extract (ppm)	<i>Aedes aegypti</i> (Dip.)	85.74	(74.05 – 112.78)	4.48±0.89	224
<i>Mikilua fragans</i>	Aerial parts	Essential oil	<i>Anopheles gambiae</i> (Dip.)		RC ₇₅ (x10 ⁻⁵ mgcm ⁻²) 481 (11, 145).		225
<i>Oxandra cf xylopioides</i>	Leaves	Extract 24h 48h 72h	<i>Spodoptera frugiperda</i> (Lep.)		ppm 319.61 311.47 294.13		226
<i>Rollinia occidentalis</i>	Seeds	Extract (ppm) 50 100 250	<i>Spodoptera frugiperda</i> (Lep.)	(%) 5 35 50	(%) 30 45 50	(%) - 20 -	227

<i>Uvaria</i> <i>scheffleri</i>	Roots	Essential oil 5% 1% 0.5% 0.1	<i>Anisakis</i> L ₃	- 5 - -			228
<i>Xylopi</i> <i>aethiopica</i>	Leaves and Roots	Extract Ethanollic Water	<i>Anopheles</i> <i>gambiae</i> (Dip.)	LC50 3.57 4.50	(%) 125 (34.72%) 106 (29.44%)		229
<i>Xylopi</i> <i>aethiopica</i>	Uninformed	Extract (ppm) 0 10 100 1000	<i>Ostrinia</i> <i>nubilalis</i> (Lep.)	(mg) 3.78 3.69 3.87 3.84			221
<i>Xylopi</i> <i>aromatica</i>	Leaves/Barks and Roots	Extract	<i>Aedes aegypti</i> (Dip.)	(µg/ml) 384.37			167
<i>Xylopi</i> <i>caudata</i>	Leaves	Oil	<i>Aedes aegypti</i> (Dip.)	29.83 (21.87- 37.45)	60.33 (48.04- 82.47)	4.19±0.69	230
<i>Xylopi</i> <i>ferruginea</i>	Leaves	Oil	<i>Aedes aegypti</i> (Dip.)	74.51 (68.39- 86.52)	106.45 (90.23- 159.78)	8.27±1.88	230

3.3 Antimicrobial

Microbial diseases are infections caused by invading microorganisms or the individual's microbiota due to bodily imbalances such as low immunity, overpopulation of a certain microorganism or even because they are present in some tissue other than its natural habitat. The microbiota present in the human body is extremely important and bring many benefits, they coexist in a mutual relationship, protecting the host, they

compete for the nutrients where they are, but in an efficient and healthy way, different from the invading microorganisms. Microorganisms in the microbiota can produce important nutrients, such as the production of vitamin K and B, and contribute to the development of the immune system ^{231,232}.

Pathogenic bacteria, those that can cause disease in humans, can be classified into three different groups taking into account the composition of their cell wall: Gram-positive bacteria have a thick layer of peptideoglycan on their cell wall and after Gram stain it takes on a violet color; Gram-negative bacteria have a thin layer of peptideoglycan on their cell wall, but they have an outer portion of lipopolysaccharide and lipoproteins, when stained by the Gram method they assume a pink color; and, finally, acid-resistant bacteria, BAARS (acid-resistant bacilli), whose cell wall has complex lipids (mycolic acids) that obtain the primary dye even after subjected to acid-acid discoloration in the Ziehl Neelsen method ²³³⁻²³⁵.

There are also fungi that are also present in the human microbiota and there are those that can be inhaled, they do not cause damage to the organism as long as the natural defense barriers are intact. In this way, fungi have almost no infectious power until quite accentuated virulence ²³⁶⁻²³⁸.

Among the bacteria and fungi that cause most infections, the most frequent are *Staphylococcus aureus*, *Staphylococcus epidermides*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella Typhimarium*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Candida krusei*, *Candida albicans* and *Candida tropicalis* ^{234,235}.

There were many deaths from infections in mankind until antibiotics were discovered. In addition to being extremely important for the treatment and control of infectious diseases in humans and animals, antibiotics have made it possible to perform complex surgeries, intensive therapy, organ transplants, survival of immunosuppressed people, production of animals for consumption, etc ^{239,240}.

In the late 1930s, when antibiotics were introduced into therapy, mortality from pneumococcal pneumonia decreased by 20-40%, mortality from pneumococcal bacteremia by 50-80%, and mortality from infections by 60-75%. severe (bacterial meningitis and endocarditis). Antibiotics have sparked a major revolution in medicine and countless lives have been and are saved today since their discovery ^{239,241-246}.

With the use of antibiotics, microorganisms began to show resistance to them, thus increasing the spread of resistant bacteria and fungi as well as the number of deaths from infections ²³⁹. According to the World Health Organization (WHO), bacterial and

fungus resistance is characterized as a worldwide public health problem, whose simple strategies of combat will not be enough to interrupt this process completely, the emergence and spread of resistant infectious organisms^{239,247} Antimicrobial resistance (AMR) is a natural process for microorganisms, but it can be accelerated through the misuse of antibiotics such as the selective use of some antimicrobial agents^{231,239,248}.

AMR has become a major threat to the health of living beings, making treatment more difficult as well as increasing morbidity and mortality. Bacteria and fungi such as *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans* are examples of pathogenic microorganisms that show frequent microbial resistance. In 2020, WHO alerted the world to the shortage of new antimicrobial agents effective against super-resistant bacteria^{231,249,250}.

Clinical research focused on the discovery of new antimicrobial agents as well as the implementation of new antibiotics in therapy has been greatly reduced in recent years. But, natural sources have become rich sources of compounds with antimicrobial potential to be explored. Thus, the use of plants for studies in the search for new antimicrobial agents has become increasingly frequent^{231,239,248}.

In this perspective, some species of the Annonaceae have been studied and analyzed as to their antimicrobial profile, both for bacteria and fungi pathogenic for humans as well as for those that cause diseases in plants.

Table 3 describes some species of Annonaceae that have been studied for their antimicrobial profile with the strains of bacteria and fungi most prevalent in human pathologies: *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *S. typhimarium*, *E. faecalis*, *K. pneumoniae*, *C. krusei*, *C. albicans* e *C. tropicalis*.

A study by Pereira et al., 2016²⁵¹, verified the antimicrobial potential of the species *Cleistochlamys kirkii* (Benth) Oliv. Traditionally used as a medicinal plant in the treatment of infectious diseases. The authors isolated nine compounds from the methanolic extract of *C. kirkii* and tested them against six strains of *S. aureus* and against the bacterium *E. faecalis*. The compound dichamanetin was shown to be highly active against the tested strains and is often more potent than the control antibiotics used in the study. In addition to this substance, the compounds chamanetin, isochamanetin and cleistenolide also showed relevant antibacterial activity against the strains studied. Thus, the authors scientifically proved the antibacterial potential of the species *C. kirkii* as well as identified the compounds that generate this potent activity, being, therefore, valuable compounds to continue with studies and become a medicine in the control of infections caused by *S. aureus*.

Silva et al., 2015²⁵², conducted a study of antimicrobial activity with the polycarpol triterpene, a lanostane-type triterpene. Polycarpol has already been isolated from the species *Unonopsis duckei* R.E.Fr., *Unonopsis floribunda* Diels, *Unonopsis rufescens* (Baill.) R. E. Fr., *Unonopsis stipitata* Diels, *Onychopetalum amazonicum* R.E. Fr. e *Bocageopsis pleiosperma* Maas, species belonging to the Annonaceae. The antimicrobial potential of polycarpol was evaluated against *S. aureus*, *S. epidermides*, *E. coli*, *P. aeruginosa* and *C. albicans*. The results showed that polycarpol has significant activity against *S. aureus*, *S. epidermides* and *E. coli* with MIC (Minimum Inhibitory Concentration) varying between 25-50 µg / ml compared to the MIC of chloramphenicol (positive control used in the study).

Christopher et al., 2018²⁵³, conducted a study in order to evaluate the antimicrobial, antitrypanosomal and antiplamodial potential of some species of plants, including some species of Annonaceae. The methanolic extract of root bark from *Uvaria tanzaniae* proved to be quite potent against the species *S. aureus* (ATCC 25923), obtaining a MIC of 1.25 µg / ml, having better activity than the positive control used in the study, ciprofloxacin (MIC = 2.5 µg / ml). With this result, another species of Annonaceae has antimicrobial activity, which once again reveals the antimicrobial potential of Annonaceae.

Table 3: Summary of antimicrobial activity of species of the Annonaceae.

Plant	Used Material	Extract/ Substances	Microorganisms / MIC (µg/mL)										Ref.
			<i>S. aureus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhimarium</i>	<i>E. faecalis</i>	<i>K. pneumoniae</i>	<i>C. krusei</i>	<i>C. albicans</i>	<i>C. tropicalis</i>	
<i>Annona hypoglauca</i> Mart.	Bark		ATCC 29213		ATCC 25922			ATCC 299212					254
		FA5 (isoboldine, nornuciferine e anonaine)	70	-	-	-	-	70	-	-	-	-	
		FA6 (actinodaphnine)	70		90			80					
<i>Goniothalamus gracilipes</i>	Leaves		ATCC										255
		Gracilipin C	32	-	-	-	-	-	-	-	-	-	
		Streptomycin (Positive control)	32										

			ATCC 25923			ATCC 27853		ATCC 299212			ATCC 10231		
<i>Uvaria hamiltonii</i>	Leaves	Essential oil	20.34			12.34		7.99			32.57		256
		Nystatine	-			8.0		0.48			-		
		Streptomycin	1.07			-		-			-		
		Cycloheximide	-			-		-			3.2		
		(Positive controls)											
			ATCC 25923			ATCC 27853		ATCC 299212			ATCC 10231		
<i>Fissistigma kwangsiensis</i>	Leaves	Essential oil	55.67			3.45		33.62			16.45		256
		Nystatine	-			8.0		2.07			-		
		Streptomycin	3.2			-		-			-		
		Cycloheximide	-			-		-			3.2		
		(Positive controls)											
			ATCC 25923										255
<i>Goniiothalam us gracilipes</i>	Leaves												
		Gracilipins C	32										
					ATCC 25922								

<i>Xylopia staudtii</i>	Bark	Hydroethanolic extract			83.33								257
		Ciprofloxacin (Positive control)			0.97								
<i>Monodora myristica</i>	Fruits	Cyclohexane extract	25		-	25			-	12.5	6.3		258
		Ethyl acetate extract	25	-	-	50	-	-	50	12.5	12.5	-	
<i>Toussaintia orientalis</i> <i>Verdc.</i>	Seeds	Toussintine A	ATCC 25923		DSM 1103								259
		Toussintine B	-	-	10	-	-	-	-	-	-	-	
		Toussintine C	10		20								
		Toussintine D	-		10								
		Toussintine E	5		-								
		Ampicilin (Positive control)	-		-								
			2.5		2.5								
			NCIB 8586		NCIB 86 6.25	NCIB 950 25.0						NCCYC 6 6.25	

<i>Dennetia tripetala Baker f.</i>	Fresh fruits, dried seed, dried fruits and fresh leaves	Dried seeds essential oil	3.13		0.13	1.0							260
		Streptomycin (Positive control)	0.13									2.0	
		Acriflavin (Positive control)											
<i>Guatteriopsis hispida</i>			ATCC 6538	ATCC 12228	ATCC 11775	ATCC 133388					ATCC 10231		
		Essential oil	>1000	>1000	>1000	>1000					>1000		
		Oxide caryophyllene	-	-	-	-					600		
<i>Guatteriopsis blepharopylla</i>	seed	Essential oil	1000	>1000	>1000	>1000					700		261
		β-eudesmol	>1000	600	>1000	>1000					125		
		Y-eudesmol	600	700	>1000	300					500		
		α-eudesmol	250	700	-	200					125		

Guatterioopsis friesiana		Essential oil	125	100	900	900					500			
		β - pinene	-	-	-	-					100			
		α – pinene	-	-	-	-					250			
		(E)- caryophyllene	-	-	-	-								
		Chloramphenicol (Positive control)	20	40	40	850					-			
		Nystatin (Positive control)	-	-						50				
Cleistoichlamys kirkii (Benth) Oliv.	Root Bark		S. aureus						VRE					
			MSSA	MRSA			VISA							
			ATCC	ATCC	ATCC	FFHB	ATCC	CIP						
				6538	43866	9144	29593	700699	106760	HB164				
		Chamanetin	7.5	15	30	-	125	15	15					
		Isochamanetin	62	125	250	30	62	62	30					
		Dichamanetin	2	2	1	4	2	2	7.5					
		Cleistenolide	30	30	7.5	15	30	30	30					
		Acetylmelodorinol	>250	>250	125	>250	>250	>250	>250					
			Amoxicillin	0.2	62	250	>250	250	250	-				

		Oxacillin	0.2	125	125	250	250	250	-				
		Vancomycin	0.2	0.4	0.4	0.8	2	4	32				
		(Positive control)											
<i>Unonopsis</i> <i>duckei</i> R.E. Fr.	Stem Bark		ATCC 6538	ATCC 1228	ATCC 10538	ATCC 27853					ATCC 10231		
<i>Unonopsis</i> <i>floribunda</i> Diels		Polycarpol	25	50	50	-					250		252
<i>Unonopsis</i> <i>rufescens</i> (Baill.) R.E. Fr.		Chloramphenicol (Positive control)	25	50	50	>500					-		
<i>Unonopsis</i> <i>stipitala</i> Diels		Ketoconazole (Positive control)	-	-	-	-					12.5		
<i>Onychopetalu</i> <i>m</i> <i>amazonicum</i> R.E. Fr.													
<i>Bocageopsis</i> <i>pleiosperma</i> Maas													
			ATCC 25923		ATCC 8740								

<i>Greenwayode</i>	Root Bark	Methanol extracts	1000		-								253
<i>ndron</i>													
<i>suaveolens</i>	Roots	Pentacyclindole	4										262
<i>subs.</i>		Polyalthenol	4	-		-		-		-		-	262
<i>usambaricum</i>													
<i>Uvaria</i>	Root Bark	Methanol extracts	1.25		-								253
<i>tanzaniae</i>													
<i>Uvari dendro</i>	Leaves	Methanol extracts	8000		500								253
<i>n</i>	Stem Bark	(leaves)											253
<i>usambarens</i>		Methanol extracts	4000		-								
		(stem bark)											
		Ciprofloxacin	2.5		0.63								253
		(Positive control)											
									ATCC 11296				
	Root Bark	Crude extract							64				263

<i>Annonidium mannii</i>		Ciprofloxacin (Positive control)							2				
			ATCC 25923		ATCC 25922	ATCC 37853			ATCC 27853		ATCC 90028		
<i>Desmos chinensis</i>	Leaves	Essential oil	250		125	250			250		250		264
<i>Cyathocalyx zeylanicus</i>		Gentamycin (Positive control)	250		125	250			250		16		
		Muconazol (Positive control)	9.0		12.0	9.1			8.0		4.5		
			ATCC 25923		ATCC 10536		ATCC 51812						
<i>Polyalthia cinnamomea</i>	Leaves	Leaf extract	4000	4000	1000		2000						265
		Vancomycine	-	30	-		30						
		Streptomicyne	30	-	-		-						
		Kanamycine (Positive control)	-	-	30		-						

<i>Cananga odorata</i>	-	Essential oil									ATCC 48274		
		Essential oil of Thymus vulgaris (reference oil)									170		266
											60		
<i>Annona senegalensis</i>	Root bark	methanol-methylene chloride extract	8750			1080							
		kaur-16-en-19-oic acid	150			-							267
		F1 - (kaur-16-en-19- oic acid; 1- dodecanol; 1- naphthalenemethano l; 6,6-dimethyl- bicyclo[3.1.1]hept- 2-ene-2-ethanol; 3,3-dimethyl-2-(3- methylbuta-1,3- dienyl)cyclohexane-	-			40							

		1-methanol; 3-hydroxyandrostano-17-carboxylic acid)											
		Ciprofloxacin	1.18			3.6							
		Gentamicin	0.23			0.79							
		(Positive controls)											
			ATCC 6538		ATCC 8739	ATCC 9027		ATCC 29212			ATCC 10231		
<i>Bocageopsis multiflora</i> (Mart.) R.E. Fr.		Essential oil	190		1500	3000		90					268
<i>Ephedranthus amazonicus</i> R.E. Fr.	Leaves	Essential oil	90		1500	3000		190					268
<i>Guatteria blepharophylla</i> Mart.		Essential oil	50		1500	1500		50			-		268,269
		Isomoschatolin	-		-	-		-			50.81 µM L-1		
		Essential oil	1200		3000	3000		50					268

<i>Xylopia aromatica</i> (Lam.) Mart.		Chlorhexidine digluconate (Positive controls)	6		6	10		90					268
		Nistatin (Positive control)								54 µM L-1			15
<i>Polyalthia longifolia</i>	Stem bark	Butanol fraction	ATCC 29213/512 (MRSA)		ATCC 29212	ATCC 27853							
		3-o-methyl ellagic	320/320		320	160							270
			80/160		80	80							270
		Vancomycin	0.25/-		-	-							270
		Oxacillin	-/8.0		-	-							270
		Ciprofloxacin	-		0.015	0.25							
	Roots	Pendulamine A	0.2			2	0.02			2			271
		Pendulamine B	0.2			-	0.2			2			271
		Penduline	12.5			-	-			-			271

		Kanamycin sulfate (Control positive)	0.31			5	2.5		5				271
	Leaves	Methanol extract	125			125	500						272
		16(<i>R</i> and <i>S</i>)- hydroxy-cleroda- 3,13(14) <i>Z</i> -dien- 15,16-olide	7.8			250	125						272
		16-oxo-cleroda- 3,13(14) <i>E</i> -dien-15- oic acid	500			500	250						272
<i>Desmopsis bibracteata</i>	Leaves	Essential oil	ATCC 29213 625		ATCC 259222 2500								
<i>Desmopsis macrocarpa</i>	Leaves	Essential oil	1250		1250								273
<i>Guatteria costaricensis</i>	Leaves	Essential oil	1250		1250								

<i>Guatteria diospyroides</i>	Leaves	Essential oil	312		1250							
<i>Guatteria oliviformis</i>	Leaves	Essential oil	1250		625							
<i>Unonopsis costaricensis</i>	Leaves	Essential oil	625		1250							
<i>Annona muricata</i>	Leaves	Dried leaf powder:	ATCC 25923		ATCC 25922	ATCC 27853			ATCC 4352			
		AM0 (Size bands < 2000)	3120		1560	3120			3120			
		AM1 (Size bands between 0.500 and 0.350)	1560		1560	3120			3120			
		AM2 (Size bands between 0.250 and 0.149)	1560		1560	1560			1560			
		AM3							780			

		(Size bands between 0.149 and 0.074)	780		1560	1560							
		AM4 (Size bands <0.074)	780		780	1560			780				
<i>Guatteria citriodora</i>	Leaves and Stem bark	Crude hydroalcoholic extract of leaves	250	ATCC 4083 -									275
		Alkaloid fraction of leaves	-	125									
		Alkalidic fraction of stem bark	-	-									
		Imipenem (Positive control)	15.6	62.5									
<i>Goniothalam us longistipetes</i>		(+)-altholactone	eMRSA – 15		NCTC 10418	NCTC 10662			NCTC 10662				
									256				

	Bark	((2S,3R,3aS,7aS)-3-hydroxy-2-phenyl-2,3,3a,7a-tetrahydrobenzo-5(4H)-5-one)	64		256	500							
		(2S,3R,3aS,7aS)-3-hydroxy-2-phenyl-2,3,3a,7a-tetrahydrobenzo-5(4H)-5-one)	128		512	512			512				276
		2,6-dimethoxyisonicotin aldehyde	128		256	256			256				
		alkenyl-5-hydroxyl-phenyl benzoic acid	8-16		512	128			128				
					ATCC 11775								
<i>Guatteria selowiana</i>	Aerial parts	Essential oil			600								

<i>Guatteria latifolia</i>	Aerial parts	Essential oil			600								277
<i>Guatteria ferruginea</i>	Aerial parts	Essential oil			600								
<i>Guatteria australis</i>	Aerial parts	Essential oil			>1000								
		Chloramphenicol (Positive control)			40								
			ATCC 33591		CDC-EDL 933-171-0157:H3	ATCC 29336							
<i>Bocageopsis multiflora</i>	Aerial parts	Essential oil	4.68		4.68	4.68							
<i>Duquetia quitarensis</i>	Aerial parts	Essential oil	-		-	-							278

<i>Fusaea longifolia</i>	Aerial parts	Essential oil	37.5		-	37.5						
<i>Guatteria punctata</i>	Aerial parts	Essential oil	-		-	-						
		TIENAM (positive control)	4.68		1.17	2.34						
<i>Uvaria schefflera</i>	Leaves	5,7,8-trimetoxiflavona	NCTC 6571 -		NCTC 10418 125						HG 392 -	
		2',6'-dihydroxy-4'-methoxychalcone and 5,7-dihydroxyflavone (mixture)	125		-						31.2	279
		Ampicillin Ketoconazole (positive control)	0.01		0.25						0.125	
			ATCC 6538		ATCC 10536		ATCC 13311	ATCC 19433	ATCC 4552			

<i>Xylopiasericea</i>	Fruits	Essential oil	7.8		1000		500	1000	62.5				280
		Chloramphenicol	12.5		6.25		1.56	6.25	12.5				
		Ciprofloxacin	0.39		1.56		0.20	1.56	12.5				
		(positive control)											
<i>Mitrephoracelebica</i>	Stem bark		ATCC 43300			ATCC 27853							
	Leaves	Crude											
	Twigs	hydroalcoholic extract of leaves	>100			>100							
		Crude											
		hydroalcoholic extract of stem bark	12.5			>100							
		Crude											
		hydroalcoholic extract of twigs	>100			>100							281
		<i>Ent</i> -trachyloban-19- oic acid	6.25			>100							
		<i>ent</i> -kaur-16-en-19- oic acid	>100			>100							

		Gentamycin	>50			0.8							
		Oxacillin	6.2-12.5			-							
		Vancomycin	0.8			-							
		(Positive control)											
			ATCC 6538								ATCC 10231		
<i>Annona foetida</i>	Leaves	Essential oil	200								60		282
		Chloramphenicol	20								-		
		Nystatin	-								50		
		(Positive control)											
			MTCC 96			MTCC 741							
		11-hydroxy-16-hentriacontanona + 10-hydroxy-hentricontanona	25			25							
<i>Annona squamosa</i>	Leaves	Palmitone	12.5			6.25							
		Ciprofloxacin	0.78										
		(positive control)											

<i>Cleistopholis patens</i>	Leaves		ATCC 33591									
		Cleistetroside-8	8									284
		Cleistetroside-5	8									
		Cleistetroside-2	0.5									
		Vancomycin (positive control)	1									
<i>Duquetia lanceolata</i>	Stem bark		ATCC 6538								ATCC 10231	285
		Essential oil (T2)	60								60	
		Essential oil (T4)	125								100	
		Chloramphenicol (positive control)	2								15	
			ATCC 25923		ATCC 25922			ATCC 19433	ATCC 13883			

<i>Annona vepretorum</i>	Leaves	Crude ethanolic extract	3120		390			3120	3120				286
		Hexanic extract	780		390			3120	3120				
		Chloroform extract	1560		780			12500	6250				
		Gentamycin (positive control)	-		-			-	-				
<i>Enantia chlorantha</i>	Stem bark		ATCC 25923		E.C 136				K.L 128				287
		Palmitin	32		32				16				
		Chloramphenicol (positive control)	8		32				32				

3.4 Leishmanicidal

Leishmaniasis belongs to the Trypanosomatidae family and can be caused by any of the 20 species of the hemoflagellate protozoan of the genus *Leishmania*, which are most frequently transmitted to humans by the bite of one of the 30 species of arthropods of the genera *Lutzomyia* (New World Leishmaniasis) and *Phlebotomus*. (Old World Leishmaniasis) ^{288,289}.

It is a public health problem that affects 98 tropical and subtropical countries, a neglected disease that affects 15 million people around the world with approximately 1.7 million cases each year ^{290,291}. According to the World Health Organization (WHO), it is considered the second most important protozoan public disease, with extensive morbidity and mortality in most developing countries ^{253,292}.

Clinically, the disease can affect the skin (cutaneous leishmaniasis), mucosa (mucocutaneous leishmaniasis) and/or organs of the reticulo-endothelial system (visceral leishmaniasis). The forms of the disease are related to the type of parasite and differ in distribution, host and vector involved, incidence and mortality rate ^{289,293}.

Since the 1940s, pentavalent antimonial compounds (sodium stibogluconate and meglumine antimoniate) have been the first-line treatment for all forms of leishmaniasis, and in case of therapeutic resistance to these compounds, amphotericin B deoxycholate and liposomal amphotericin B (pentamidine and miltefosine) can also be used ²⁹¹. However, they are formulations that present high toxicity, high cost and development of parasitic resistance. Therefore, there is an urgent need for new therapies against leishmaniasis ²⁹⁰.

The lack of effective antiprotozoal drugs has aroused renewed interest in the study of medicinal plants as sources of new chemotherapeutic compounds with better activities and fewer side effects ^{253,294}. The literature describes several classes of natural substances with proven leishmanicidal activity in *in vitro* assays on promastigotes and/or amastigotes of *Leishmania*, such as: terpenes, chalcones, acetogenins, alkaloids, diterpenic acids, quinones and phenolic derivatives ^{290,292,293,295}.

The antiprotozoal activity generally reported for plants of the Annonaceae family is related to the importance of this botanical family in the traditional treatment of diseases such as malaria, Chagas disease, sleeping sickness and leishmaniasis, associated with metabolites found in various parts of the plants of this family, such as alkaloids, acetogenins, polyphenols, sterols and terpenes ^{296–298}.

The azaphenanthrene alkaloids: sampangine, imbiline 3, imbiline 1 and eupolaramine, isolated from the roots of *Anaxagorea dolichocarpa* showed promising activity against the promastigote forms of *L. donovani*, with an IC_{50} value $\leq 24.06 \mu M$ ²⁹². An aporphine (lysicamine) and a bis-aporphine (trivalvone) alkaloid isolated from *Annickia kummeriae* leaves showed increased activity against the amastigote form of *L. donovani*, with IC_{50} of 9.26 and 5.24 μM , respectively²⁹⁹. The alkaloids Duguetine β -N-oxide and Dicentrinone isolated from the bark of *Duguetia furfuracea*, presented IC_{50} of 0.11 and 0.01 μM against the promastigotes form of *L. braziliensis*³⁰⁰.

Some acetogenins have also shown leishmanicidal activity, such as annofolin and annotacin, isolated from *Annona cornifolia* seeds, IC_{50} of 6.4 and 7.2 μM , respectively, against the amastigote forms of *L. amazonensis*^{288,301}. A study with Annonacin A and Goniothalamycin isolated from *Annona glauca* seeds, showed activity against the promastigote form of *L. braziliensis*, *L. amazonensis* and *L. donovani*, with IC_{50} of 16.75 and 8.37 μM , respectively³⁰².

Some studies adopt the following criteria for the evaluation of in vitro antiprotozoal screening of extracts, fractions and isolated substances: $IC_{50} \leq 5 \mu g/mL$: pronounced activity; $5 < IC_{50} \leq 10 \mu g/mL$: good activity; $10 < IC_{50} \leq 20 \mu g/mL$: moderate activity; $20 < IC_{50} \leq 40 \mu g/mL$: low activity; $IC_{50} > 40 \mu g/mL$: inactive^{296,303} (Table 4).

Table 4: Summary of leishmanicidal activity of species of the Annonaceae

Plant	Used material	Extracts/ Compounds	<i>Leishmania spp.</i>	Parasite form	IC ₅₀		Ref.
					µM	µg/ml	
<i>Anaxagorea dolichocarpa</i>	root	Sampangine	<i>L. donovani</i>	Promastigote	24.06	5.59	
		Imbiline 3			16.91	5.45	292
		Imbiline 1			18.20	5.32	
		EupolaUramine			19.90	5.26	
<i>Annickia kummeriae</i>	leave	Methanolic extract	<i>L. donovani</i>	Amastigote		9.25	299
		Lysicamine			9.26	2.7	
		Trivalvone			5.24	2.9	
		Palmatine			22.13	7.8	
		Jatrorrhizine			60.28	20.4	
		Jatrorrhizinne/ Columbamine			19.35	13.1	
		Palmatine/ Tetrahydro-palmetine			9.88	7.0	
<i>Annona crassiflora</i>	Stem bark, stem wood, root bark and root wood	Ethanolic extract	<i>L. donovani</i>	Promastigote		12.4, 8.3, 3.7 and 8.7	288,304
	leave	Total alkaloids				24.9	
<i>Annona coriaceae</i>	leave	Essential oil	<i>L. chagasi</i>	Promastigote		39.93	305
<i>Annona cornifolia</i>	seed	Annofolin	<i>L. amazonensis</i>	Amastigote	6.4		288,301

		Annotacin			7.2	
		Extract			175.0	
<i>Annona foetida</i>	bark	Hexane extract	<i>L. braziliensis</i> and <i>L. guyanensis</i>	Promastigote	>160.0 and 42.7	282,288
		Dichloromethane extract			23.0 and 2.7	
		Alkaloid fraction (Dichloromethane extract)			23.0 and 2.7	
		Methanolic extract			40.4 and 23.6	
		Alkaloid fraction (Methanolic extract)			24.3 and 9.1	
		N-hydroxyannonomontine			911.35 and 1577.82	
		O-methylmoschatolin			998.35 and 322.72	
		Liriodenine			212.52 and 78.10	
		Annonomontine			>2346.15	
	leave	Essential oil	<i>L. amazonensis</i> , <i>L. braziliensis</i> , <i>L. chagasi</i> and <i>L. guyanensis</i>		16.2, 9.9, 27.2 and 4.1	
<i>Annona glabra</i>	leave	Hydroalcoholic extract	<i>L. amazonensis</i>	Promastigote	37.8	288
<i>Annona glauca</i>					IC₁₀₀	
	seed	Dichloromethane extract	<i>L. braziliensis</i> , <i>L. amazonensis</i> and <i>L. donovani</i>	Promastigote	25.0	302
		Hexane extract			>100.0	
		Annonacin A			16.75	
		Goniothalamycin			8.37	

		Glaucanisin			40.13	25.0	
		Rolliniastatin-2			40.13	25.0	
		Squamocin			40.13	25.0	
		Glaucafilin			41.88	25.0	
		Molvizarin			>168.09	>100.0	
		Parviflorin			>168.09	>100.0	
		Annonacin			21.61	12.5	
<i>Annona haematantha</i>	root	Argentilactone	<i>L. donovani</i> , <i>L. major</i> and <i>L. amazonensis</i>	Promastigote	51.47	10.0	306
<i>Annona mucosa</i>	Leave	Hexane extract	<i>L. amazonensis</i> and <i>L. braziliensis</i>	Promastigote		24.24 and 65.17	288,298
		Dichloromethane extrac				9.32 and 27.42	
		Methanol extract				28.32 and 44.74	
	seed	Hexane extract				44.22 and 170.15	
		Methanol extract				46.54 and 133.17	
		Liriodenine			5.19 and 203.15	1.43 and 55.92	
<i>Annona muricata</i>	leave	Ethil acetate extract	<i>L. amazonensis</i> , <i>L. braziliensis</i> and <i>L. donovani</i>	Promastigote		25.0	288,294,297
		Hexane extract		Promastigote		>100.0	
		Methanolic extrac		Promastigote		>100.0	
	stem	Hexane extract		Promastigote		98.6, 76.3 and 83.1	
		Ethil acetate extract		Promastigote		63.2	
		Methanolic extrac		Promastigote		98.6	

	seeds	Annonacinone	<i>L. chagasi</i>	Promastigote and Amastigote	63.20 and 22.69	37.6 and 13.5	
			<i>L. donovani, L. mexicana and L. major</i>	Promastigote	12.87, 13.44 and 11.29	7.66, 8.00 and 6.72	
		Corossolone	<i>L. chagasi</i>	Promastigote and Amastigote	44.74 and 49.57	25.9 and 28.7	
			<i>L. donovani, L. mexicana and L. major</i>	Promastigote	32.35, 32.19 and 27.88	18.73, 18.64 and 16.14	
		Scoparone	<i>L. donovani, L. mexicana and L. major</i>	Promastigote	133.42, 44.18 and 69.69	27.51, 9.11 and 14.37	
<i>Annona purpurea</i>	Bark and seed	Methanolic extract	<i>L. donovani</i>	Promastigote		113.24 and 28.57	288
		Aqueous extract				289.0 and 179.9	
	leave	Hydroalcoholic fraction	<i>L. panamensis</i>	Promastigote		0.961	
<i>Annona spinescens</i>						IC₁₀₀	
	Bark	Anonaine	<i>L. braziliensis, L. amazonensis and L. donovani</i>	Promastigote	188.45, 94.22 and 376.91	50.0, 25.0 and 100.0	307
	Root	Liriodenine			363.29,	100.0	
<i>Annona squamosa</i>	leave	O-methylarmepavine	<i>L. chagasi</i>	Promastigote and Amastigote	71.16 and 77.58	23.3 and 25.4	297

		C ₃₇ trihydroxy adjacent bistetrahydrofuran acetogenin			42.44 and 40.67	26.4 and 25.3	
<i>Annona senegalensis</i>	leave	Ethanolic extract	<i>L. donovani</i>	Promastigote		10.8	288,308
	Stem	Ethanolic extract				27.8	
<i>Bocageopsis multiflora</i>	leave	Essential oil	<i>L. amazonensis</i>	Promastigote		14.6	309
<i>Duguetia furfuracea</i>	bark	Alkaloid extract	<i>L. braziliensis</i>	Promastigote	16.32		300
		Duguetine			4.32		
		Duguetine β-N-oxide			0.11		
		Dicentrinone			0.01		
		N- methylnetetrahydropalmatine			17.03		
		N-methylglaucine			4.88		
<i>Duguetia lanceolata</i>	leave	Glaucine	<i>L. infatum</i>	Amastigote and Promastigote	21.10 and >281.37	7.5 and >100.0	310
<i>Enantia chlorantha</i>	stem bark	Aqueous extract	<i>L. infatum</i>	Promastigote		10.08	311
<i>Guatteria amplifolia</i>	leave	Xylopine	<i>L. mexicana</i> and <i>L. panamensis</i>	Promastigote	3.0 and 6.0		312
		Nornuciferine			14.0 and 28.0		
<i>Guatteria australis</i>	leave	Essential oil	<i>L. infatum</i>	Promastigote		30.71	313
<i>Guatteria boliviana</i>	bark	Ethanolic extract	<i>L. amazonensis</i> , <i>L. braziliensis</i> and <i>L. donovani</i>	Promastigote		100.0	314
		Puertogaline A			177.74	100.0	
		Puertogaline B			177.74	100.0	
		Sepeerine			168.15	100.0	
<i>Guatteria dumetorum</i>	leave	Cryptodrine	<i>L. mexicana</i> and <i>L. panamensis</i>	Promastigote	3.0 and 6.0		312

		Nornantenine		24.0 and 15.0	
<i>Guatteria latifolia</i>	branch	Crude extract	<i>L. amazonensis</i>	Promastigote	51.7 and 30.5 ²⁹⁰
		Buthanolic fraction 1		and Amastigote	25.6 and 10.4
		Buthanolic fraction 2			16.0 and 7.4
<i>Greenwayodendron suaveolens</i>	Fruit, leave, root bark and stem bark	Dichlorometahne fraction rich in alkaloids	<i>L. infatum</i>	Promastigote	24.05, 34.56, 0.63 ³⁰³
		Petroleum ether fraction rich in lipids and waxes			and 20.32
		Methanolic fraction rich in steroids and terpenes			24.05, 8.0, 27.27 and 5.04
	Leave, root bark and stem bark	Crude ethanolic extract			40.32, 32.46, 7.51 and 6.82
	Stem bark	Polycarpol			43.07, 8.11 and 24.05
		Dihydropolycarpol		3.2	
		Polyathanol		8.0	
				8.1	
<i>Isolona hexaloba</i>	leave	Aqueous extract	<i>L. infatum</i>	Promastigote	2.0 ²⁹⁶
		Methanolic extract			6.35
	root bark	Dichloromethane fraction			6.96
	stem bark	Dichloromethane fraction			8.0
<i>Polyathia macropoda</i>	Stem bark	(4S,9R,10R) methyl 18- carboxy-labda-8, 13(E)- diene-15-oate	<i>L. donovani</i>	Promastigote	0.75 ³¹⁵
<i>Polyathia suaveolens</i>	stem bark	Methanolic extract	<i>L. infatum</i>	Promastigote	1.8 ²⁹¹

<i>Porcelia macrocarpa</i>	Seeds	Docos-13-yn-21-enoic acid	<i>L. infantum</i>	Amastigotes	48.5	316
		3-hydroxy-4-methylene-2-(eicos-11'-yn-19'-enyl)but-2-enolide			9.2	
		3-hydroxy-4-methylene-2-(octadec-9'-yn-17'-enyl)but-2-enolide			10.4	
		3-hydroxy-4-methylene-2-(hexadec-7'-yn-15'-enyl)but-2-enolide			11.0	
		(2 <i>S</i> ,3 <i>R</i> ,4 <i>R</i>)-3-hydroxy-4-methyl-2-(eicos-11'-yn-19'-enyl)butanolide			29.9	
		Miltefosine (positive control)			17.8	
<i>Porcelia macrocarpa</i>	Seeds	(2 <i>S</i> ,3 <i>R</i> ,4 <i>R</i>)-3-hydroxy-4-methyl-2-(<i>n</i> -eicos-11'-yn-19'-enyl)butanolide (1)	<i>L. infantum</i>	Amastigotes	29.9	317

		(2S,3R,4R)-3-hydroxy-4-methyl-2-(<i>n</i> -eicos-11'-ynyl)butanolide (2)			Non active		
		Mixture of 1 and 2					
		2:1			8.4		
		1:1			13.6		
		1:2			19.4		
		(2S,3R,4R)-3-hydroxy-4-methyl-2-(<i>n</i> -eicosyl)butanolide (3)			Non active		
<i>Raimondia monoica</i>	leave	(-)-argentilactone	<i>L. panamensis</i>	Promastigote	51.47	10.0	318
		(6S)-(50-oxohepten-10E,30Edienyl)-5,6-dihydro-2H-pyran-2-one			9.2	1.9	
		(6R)-(50-oxohepten-10Z,30Edienyl)-5,6-dihydro-2H-pyran-2-one			2.03	0.42	
<i>Rollinia emarginata</i>						IC₁₀₀	319
	stem bark	Hexanic extract	<i>L. braziliensis</i> , <i>L. amazonensis</i> and	Promastigote		>100.0	
		Dichloromethane extract	<i>L. donovani</i>			100.0	
		Methanolic extract				>100.0	

		Rollidecin B		78.25	50.0	
		Rolliniastatin-1		8.02	5.0	
		Lirioresinol B		>239.0	>100.0	
		Squamocin		8.02	5.0	
		Liriodenine		18.16	5.0	
		Sylvaticin		15.65	10.0	
<i>Rollinia exsucca</i>	stem	Hexane extract	<i>L. amazonensis, L. braziliensis and L. donovani</i>	Promastigote	20.8	294
<i>Rollinia pittieri</i>	leave	Hexane extract	<i>L. amazonensis, L. braziliensis and L. donovani</i>	Promastigote	12.6, 10.7 and 10.7	294
		Ethyl acetate extract			20.8	
		Metanolic extract			19.7, 31.4 and 43.8	
	stem	Hexane extract			13.5, 15.1 and 15.1	
		Ethyl acetate extract			20.8, 25.0 and 19.7	
<i>Unonopsis buchtienii</i>					IC₁₀₀	320
	stem bark	Petroleum ether extract	<i>L. major and L. donovani</i>	Promastigote	50.0	
		Dichloromethane extract			100.0	
		O-methylmoschatoline		155.61	50.0	
		Lysicamine		85.82	25.0	
		Fraction containing			25.0	
		Unonopsine				
		β-Sitosterol		>241.13	>100.0	
		Stigmasterol		>242.30	>100.0	
		Liriodenine		11.33	3.12	

<i>Unonopsis duckei</i>	Twigs, barks and leaves	Alkaloidal fraction	<i>L. amazonensis</i>	Promastigote	155.61, 32.16 and 4.0	295
<i>Unonopsis guatteriodes</i>	Twigs, barks and leaves	Alkaloidal fraction	<i>L. amazonensis</i>	Promastigote	1.07, 1.90 and 2.79	295
<i>Uvaria afzelii</i>	root	Bigervone	<i>L. donovani</i> and <i>L. major</i>	Promastigote	38.9 and 44.4	321
<i>Uvaria klaineana</i>	stem	Klaivanolide	<i>L. donovani</i>	Promastigote	1.75	322
<i>Xylopi aromatic</i>	leave	Methanolic extract	<i>L. amazonensis</i> , <i>L. braziliensis</i> and <i>L. donovani</i>	Promastigote	20.8	294
<i>Xylopi discret</i>	Leave	Ethanol extract	<i>L. panamensis</i>	Promastigote	25.0	289
		Ether petroleum extract			50.0	
		Acetate extract			50.0	
		Methanol extract			37.5	
		Essential oil			6.25	
	seed	Ethanol extract			6.25	
<i>Xylopi excellens</i>	leave	7β-O-β-D-glucopyranoside-ent-kaur-16-ene	<i>L. amazonensis</i>	Promastigote	33.74	253
<i>Xylopi parviflora</i>	roots	Dichloromethane extract	<i>L. donovani</i>	Amastigote	5.01	323

3.5 Cytotoxic

Cytotoxicity comprises a harmful property of cells ³²⁴. It is said that a cell can be classified as cytotoxic, when it has the ability to release harmful substances that culminate in the destruction of other cells ³²⁵. Cytotoxic compounds can induce cell death through mechanisms such as: necrosis, which comprises the loss of cell membrane integrity, leading to cell death due to membrane lysis or rupture ³²⁶; as well as apoptosis, better known as programmed cell death that implies eradicating cell growth and uncontrolled disruption of cells that can be harmful to the body ^{327,328}.

The cytotoxicity tests consist of placing the biological material under study directly or indirectly in contact with a culture of mammalian cells, checking cellular changes by different mechanisms, including the incorporation of vital dyes or the inhibition of formation cell colonies ³²⁹. The most used parameter to assess toxicity is cell viability, which can be evidenced with the help of vital dyes, such as neutral red, which is a water-soluble dye that passes through the cell membrane, settling on the lysosomes ³³⁰. The distinction of cell viability is made by measuring the color intensity of the cell culture ³³¹.

In this perspective, some authors have carried out research on some species of the Annonaceae in search of new cytotoxic agents. Huong and collaborators (2005)³³², evaluated the cytotoxic potential of a new flavone called Miliufavol [8-(2-hydroxybenzyl)-5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,7-dimethoxy-4H-chromen-4-one or 8-C-(o)-hydroxybenzylpachypodol] in addition to four other compounds already known: Ombuína, Crisosplenol B, Paquipodol e Crisosplenol C, isolated in the species *Miliusa balansae* and elucidated by spectroscopic means. The cytotoxic assays were performed at the Institute of Natural Product Chemistry-VAST, Hanoi, Viet Nam. Cell lines (KB - human epidemic carcinoma, Hep-G2 - Hepatoma-G2 and RD - Rhabdomyosarcoma) were grown in Dulbecco's modified essential medium. (DMEM) (GIBCO) supplemented with 10% wild bovine serum (FBS) and 100 units / ml of penicillin G, 100 mg / ml of streptomycin sulfate, 0.25 mg / ml of amphotericin B (Fungizone) (PSF) (GIBCO). IC₅₀ values were calculated using a table curve program with logarithm values based on different sample concentration scales and OD values. The results showed that all four compounds were found active against three tested cell lines. Among them, paquipodol (4) has strong activities against two cell lines (KB: 0.7 mg / ml, Hep-G2: 0.55 mg/ml).

Majekodunmi and Colaborators (1996)³³³ evaluated the cytotoxic activity of four ent-kaurenoids derivatives isolated from the stem bark of the species *Annona senegalensis*. The brine shrimp lethality test (BST) was performed according to standard protocols. LC₅₀ values, in µg/mL, were determined for partitioned fractions, pooled chromatographic fractions, and the isolated compounds. The fractions of the compounds under study were evaluated for cytotoxic activity against strains of human solid tumor cell lines for: A-549 (lung carcinoma), MCF-7 (breast carcinoma), HT-29 colon (adenocarcinoma), A-498 (kidney carcinoma), PC-3 (prostate adenocarcinoma), and PACA-2 (pancreatic carcinoma) with Adriamycin as a positive control. The fraction partitioned in 10% aqueous MeOH from the stem bark CHCl₃ extract showed lethality (LC₅₀ <1.0 µg / mL) in a lethality test (BST) brine, was also cytotoxic to several solid tumor cell lines ED₅₀ values of <10-2 µg / mL. Compound 2 was active in the artemia test (BST LC₅₀ 16 µg / mL), and was significantly selective for MCF-7 cells (breast cancer, ED₅₀ 1.0 µg / mL) among six human solid tumor cell lines. Thus, it is concluded that diterpene 2, demonstrated a significant cytotoxic selectivity that would serve to justify future investigations on the structure-activity relationships between this class of compounds.

The authors Silva and collaborators (2016)⁷⁵ evaluated the cytotoxic activity of compounds belonging to the classes of carotenoids, flavonoids and tannins obtained from extracts from the leaves and fruits of the species of *Annona squamosa* L. Vero cell lines, (Vero, ATCC-CL 81) kindly provided by the Butantan Institute (São Paulo, Brazil), were used to perform cytotoxic assays. To verify the cytotoxicity of the hexane, ethanolic and aqueous extracts, 3-(4,5-dimethylazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) mitochondrial reduction assay was used. In addition, the cells were stained with May-Grünwald-Giemsa to analyze cellular morphological changes. The cytotoxic concentration was calculated for 50% of the cell culture (CC₅₀). The cytotoxic morphological findings confirmed the potential of the compounds of *A. squamosa* in causing apoptotic effects on tumor cells. Cells treated with hexane and aqueous extracts showed signs of apoptosis characterized by reduced cell volume, nuclear fragmentation and the presence of apoptotic bodies. It was also observed that the aqueous extract of the leaves was more toxic than the aqueous extract of the seeds, which is in accordance with its tannin content. In general, cytotoxicity was higher in hydrophobic extract than in hydroalcoholic extracts.

Suedee and collaborators (2007)³³⁴ selected the species *Polyalthia jucunda* to assess cytotoxic activity. The extract from the stem bark of the dry plant provided four compounds, which corresponded to: 4-Hydroxy-4,7-dimethyl-a-tetralone (1), 4,5-dihydroblumenol A (2), N-trans-feruloiltyramine (3) and 24-methylenelanosta-7,9 (11)-dien-3-b, 15a-diol (4). To perform the cytotoxicity evaluation, four human cell lines were used, corresponding to: the estrogen-dependent ER (p) MCF-7, the estrogen-independent (ER), MDA-MD-231 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer). The non-tumor cell line MRC-5 (human fetal lung) was also used. For each tested compound, a dose-response curve was generated and the growth inhibition was 50% (GI₅₀), corresponding to the concentration of the compound that inhibits 50% of the net cell growth. The results showed that compounds 1, 2 and 3 were ineffective as inhibitors of cell growth, even when tested in concentrations of 150 mM. On the contrary, compound 4 exhibited, after continuous exposure for 48 h, an interesting dose-dependent growth inhibitory effect not only against tumor cell lines, but also against non-tumor MRC-5 cells. However, a more pronounced effect was observed against tumor cells, suggesting a high sensitivity of these cells in relation to this compound. In general, this study showed that compound 4 can exert its growth inhibitory effect on NCI-H460 cells through the involvement of apoptotic events.

The evaluation of the cytotoxicity of the components of *Xylopia laevigata* was the objective of the study developed by Menezes and collaborators (2016)³³⁵. In this study, the authors isolated 19 alkaloids using the plant stem extract, respectively: (‘) -roemerina, (+) - anonaine, lanuginosine, (+) - glaucine, (+) - xylopine, oxoglaucine, (+) - norglaucine, asimilobin, (‘) -xylopinin, (+) - norpurpurein, (+) - N-methylurotetanine, (+) - norpredicentrine, (+) - discretin, (+) - kallikinin, (+) - laurotetanine, (+) - reticulín, (‘) - cotenquin, (+) - discretamine and (+) - flavinantine. Cytotoxic activity was evaluated against tumor cells B16-F10 (mouse melanoma), HepG2 (human hepatocellular carcinoma), K562 (human chronic myelocytic leukemia) and HL-60 (human promyelocytic leukemia) and non-tumoral peripheral blood mononuclear cells (PBMCs) were tested using the Alamar Blue assay. In conclusion, 19 alkaloids were isolated from the stem of *X. laevigata*, of which lanuginosine, (+) - xylopine and (+) - norglaucine had the most potent cytotoxic activity. Lanuginosine and (+) - xylopine were able to induce apoptosis. In addition, treatment with (+) - xylopine caused G2 / M cycle to stop in HepG2 cells. These data suggest that *X. laevigata* is a potential source of cytotoxic alkaloids.

Through these studies, the cytotoxic activity of species of the Annonaceae is proven, in which studies have identified this activity in the most diverse classes of secondary metabolites from this family, the most significant effects being caused by alkaloids, terpenoids and flavonoids. Table 5 shows the substances tested in each study as well as their respective cytotoxic potential.

Table 5: Summary of cytotoxic activity of species of the Annonaceae.

Plant	Used material	Substances	Methodology	IC ₅₀ (µg/mL)			Ref
				KB	Hep-G2	RD	
<i>Miliusa balansae</i> (specie)	Leaves and branches	Flavonoids	Cell culture:				332
		Ombuine	IC₅₀ (µg/mL)	> 5	1.5	> 5	
		Crysosplenol B		4.6	0.93	> 5	
		Pachypodol	- KB (Human Epidermoide Carcinoma);	0.7	0.55	3.01	
		Crysosplenol C	- Hep-G2 (Hepatoma G2);	4.3	0.57	2.09	
		Control	- RD (Rhabdosarcoma).				
		Eliptin (Sigma)		0.002	0.001	0.001	
<i>Xylopi</i> <i>aethiopica</i>	Fruits	Cell culture:	IC₅₀ (µg/mL)	MCF-7	MDA-MB-231	Caco-2	336
		Aqueous extract		171.3	94.5	199.8	
		Aqueous extrat in gold nanoparticles	- MCF-7 (Bresat cancer);	>200	141.4	>200	
			- MDA-MB-231;				
			- Caco-2 cells.	>120 nM	<120 nM	-	
		Fulvestrant		-	-	>100	
		5-Fluororacil					
		(Positive control)					

Uvaria pandensis	Leaves	Pandensenol D	Cell culture: MCF-7 (breast cancer cells)	EC50 (µM)		337	
		Pandensone A		>523.4			
		(8’α,9’β-dihydroxy)-3-farnesyldindole		349.8			
		(6’,7’-dihydro-8’α,9’β-dihydroxy)-3-farnesyldindole		117.1			
Annona Mucosa	Leaves and Fruits	Fraction	Cytotoxicity was studied after 96 h incubation of peritoneal macrophages with concentrations ranging from 6 to 100 µg.mL-1 of each extract and liriodenine. Results are expressed as 50% lethal concentrations (LC50)	Cytotoxicity LC50 (µg.mL-1) + SEMc	LC50/ IC50		298
		Hexane Extrat (L); Dichloromethane extract (L); Methanol extract (L); Hexane extrat (S); Methanol extrat (S);			PH8	M2903	
					24.07 ± 4.02	2.58	
					29.41 ± 0.89	1.03	
					262.33 ± 5.81	5.93	
					139.00 ± 3.13	2.98	
		Compounds Liriodenine Pentamiddine			19.11 ± 1.6	13.36	
		Fractions		ED50 (µg/mL)			

<i>Annona senegalensis</i> Pers.	The bark	MeOH Extract				333
				MeOH Extract	Adriamicina	
				LC ₅₀ <1,0	-	
		Compounds Adriamicina	BST (brine shrimp lethality test)			
			Human solid tumor cell lines:			
			A-549 (lung carcinoma);	<10 ⁻²	-	
			MCF-7 (breast carcinoma);	<10 ⁻²	1,85 × 10 ⁻¹	
			HT-29 colon (adenocarcinoma);	1,0	4,37 × 10 ⁻²	
			A-498 (kidney carcinoma);	<10 ⁻²	1 × 10 ⁻²	
			PC-3 (prostate adenocarcinoma);	<10 ⁻²	2,23 × 10 ⁻²	
			PACA-2 (pancreatic carcinoma)	<10 ⁻²	2,05 × 10 ⁻²	
				GI ₅₀ - lg/mL		

<i>Annona cacans</i> Warm	The fruits and leaves	PHME-AC (pulp extract)	MCF-7 (breast)	PHME-AC		PEAF-AC		Acetogenin -PAC-1	111
		PEAF-AC (ethyl acetate fraction)				11.3		6.4	
		Acetogenin – PAC-1		8.8		5.68			
		Positive control Doxorubicin chloride (0.025–25 lg/mL).		6.10		7.84			
<i>Annona crassiflora</i>	Leaves	Crassiflorine Xylopine Stephalagine Doxorubicin (Positive control)	Cells line: HCT-116 – Colon carcinoma MCF-7 – Breast	IC ₅₀ µM					338
				HCT-116		MCF-7			
				143.4		Not determined			
				30.2		32.9			
				48.5		Not determined			
				0.07		0.15			
		Compounds	Cytotoxic activities towards tumor and non-tumor cells lines were investigated for compounds 1–6 with Tumor cells:	IC ₅₀ in µg/mL (µM)					
				B16-F10	Hep-G2	HL-60	K562	PBMC	
				21.02	15.50	9.92	2.49	7.20	

<i>Annona vepretorum</i>	Stem bark	ent-3β-hydroxy-kaur-16-en-19-al	B16-F10, Hep-G2, HL-60, K562 and Non-tumor cells: PBMC	>25	>25	>25	>25	8.93	339
		ent-3β,19-dihydroxy-kaur-16-eno		19.12	19.38	9.86	2.94	6.49	
		ent-3β-hydroxy-kaur-16-eno		>25	>25	>25	>25	>25	
		ent-3β-acetoxy-kaur-16-eno		>25	>25	24.21	20.21	>25	
		ent-3β-hydroxy-kaurenoic acid		16.56	15.33	13.33	21.92	24.41	
		Kaurenoic acid							
		Positive Control Doxorubicin		2.30	0.23	0.83	0.68	5.09	
<i>Diclinanona calycina</i>	Barks	Compounds	Cancer cells	HL-60	MCF-7	HCT116	HepG2	MRC-5	340
		Thalifoline	HL-60 – Human promyelocytic leukemia	μM Not determined	μM Not determined	μM >25	μM 20.08	μM >25	
		(S)-(+)-Reticuline	MCF-7 – Breast adenocarcinoma	Not determined	Not determined	>25	22.54	>25	
		1S,2R-Reticuline N _β -oxide	HepG2 – hepatocellular carcinoma	Not determined	Not determined	>25	23.11	>25	
		1S,2S-Reticuline N _α -oxide	Non-cancerous cell:	Not determined	Not determined	>25	>25	>25	

		Bisnorargemonine	MRC-5 – Human lung fibroblast	Not determined	Not determined	>25	>25	>25		
		Isochamanetin		Not determined	Not determined	>25	19.79	24.69		
		Dichamanetin		15.78	23.59	18.99	>25	>25		
		Uvarinol + Isouvarinol		9.74	>25	17.31	>25	>25		
		Doxorubicin (Positive control)		0.04	3.08	0.85	2.05	3.19		
<i>Neouvaria acuminatissima</i>	Stem bark	Compounds	Screened for cytotoxicity against a panel of human cancer cell lines and murine P388 cells, according to established protocols. 14 ED ₅₀ values of >4 ~tg/ml were regarded as negative. Among the cell lines represented, a human lung cancer cell line (Lul) was used to guide the fractionation against the HT-29 human colorectal and KB human epidermoid carcinoma models, Doxorubicin was run as a positive control.	ED₅₀ Values: tg/ml					341	
		Acuminolide (1) 17-O-Acetylacuminolide (2) Spiroacuminolide (3) Positive Control Doxorubicin		Compounds 1 and 2 were broadly cytotoxic, exhibiting ED ₅₀ values, ranging from 10 ~ to 10 ° ttg/ml in several cell lines. With the human cell lines, the most potent activity was observed with melanoma (Mel2) (ED ₅₀ : 0.7 ttg/ml) and prostate (LNCaP) (ED ₅₀ : 0.8 ~tg/ml) cells for compounds 1 and 2, respectively. Compound 3 was not significantly active for any of the cancer cell lines tested. Acuminolide (1) was inactive when tested in vivo against a HT-29 human colorectal xenograft model in nude mice at 40-60 mg/kg (maximum tolerated dose 70 mg/kg). 17-O-Acetylacuminolide (2) showed no significant activity when tested in vivo against a KB human epidermoid carcinoma murine model at 110 mg/kg.						
		Compounds		Cell line (ED₅₀ µg/mL)						
				P-388	KB	Col-2	BCA-1	Lu-1	ASK	

<i>Polyalthia crassa</i>	Leaves and twigs	(+)-Crassalactone A	Cytotoxicity assays of compounds 1-4, 10, and 11 were performed employing the colorimetric method.	0.18	1.7	1.9	0.92	1.9	1.6	342
		(+)-Crassalactone B		3.8	>5	>5	>5	>5	>5	
		(+)-Crassalactone C		>5	>5	>5	>5	>5	>5	
		(+)-Crassalactone D		1.1	3.3	4.0	3.2	>5	3.1	
		Aristolactam AII		2.7	>5	>5	>5	>5	>5	
		(+)-tricinnamate		3.1	>5	>5	>5	>5	>5	
		Ellipticine		0.52	0.65	0.53	0.53	0.56	0.60	
		Positive Control								
<i>Polyalthia jucunda</i>	Dried and powdered stem bark.	Compound	The effects of compounds on the growth of the human tumor and non–tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (USA) for the in vitro anticancer drug discovery	GI ₅₀ (μM)						334
				MCF-7	MDA-MB-231	SF-268	NCL-H460	MRC-5		
				>150	>150	>150	>150	>150		
				>150	>150	>150	>150	>150		
				>150	>150	>150	>150	>150		
		24–Methy- lenelanosta-7,9(11)-dien-3-β,15α-diol		19.3±1.2	18.8±2.0	21.8±0.6	23.0±1.7	40.3±3.4		
			Cell culture	ED50 values (μg/ml)						

<i>Dasymaschalon blumei</i>	The combined leaves and twigs	Extract:	P-388 (mouse lymphoid neoplasma)	P-388	KB	Col-2	MCF-7	Lu-1	ASK	Hek 293	343
		Acetate de ethyl extract	KB (human epidermoid carcinoma in the mouth)								
			Col-2 (human colon cancer)	<4	<4	17.38	-	16.46	-	-	
			MCF-7 (human breast cancer)								
			Lu-1 (human lung cancer)								
			ASK (rat glioma)	<4	4.12	-	-	14.04	-	-	
	Stems	Compounds:	Hek 293 (noncancerous human embryonic kidney cell).	2.13	2.97	-	-	-	3.04	14.75	
		3,5-diidroxy-2,4-dimethoxyaristolactam	The Hek 293 cell assay was used as a primary assay for assessing the specificity of an anticancer agent toward cancer cell lines in comparison with the normal mammalian cell.	11.18	-	-	3.60	-	-	-	
		Aristolactam BI		2.59	1.98	-	9.45	-	12.7	17.97	
		Goniopedaline		13.82	-	-	-	-	6	-	
		Griffithinam		0.60	2.30	0.91	2.74	0.76	-	1.6	
		Oxodiscoguttine		10.28	4.56	7.34	9.05	4.0	2.11	3.76	
		Dicentrinone		9.43	-	-	19.11	-	10.2	-	
		Duguevalline							0		
									-		
		Positive control		0.65	0.62	0.65	0.69	0.17		0.56	
		Eliphticine							0.61		
		Essential oil		IC₅₀ values μL/mL							

<i>Xylopia aethiopica</i> (Dunal) A. Rich; <i>Xylopia parviflora</i> A. Rich (Benth.); <i>Monodora myristica</i> (Gaertn).	Fruits	Composition: Monoterpenes hydrocarbons; Oxygenated monoterpenes; Sesquiterpene hydrocarbons; Oxygenated sesquiterpenes.	The cytotoxic activity of all essential oils was evaluated on human breast cancer (MCF-7) and normal epithelial (ARPE-19) cell lines using the MTT assay based on cell viability. Cells were exposed to the oils at concentrations ranging from 0.1 to 2 µL/mL.	The six essential oils exerted cytotoxic activity against cancer (MCF-7) and normal cell lines (ARPE-19), with more pronounced effect on neoplastic cells in most cases. The highest selectivity was obtained with the essential oils of <i>X. parviflora</i> from Chad and Cameroon (5.87 and 5.54) which were more cytotoxic against MCF-7 than against normal cell line (ARPE-19) with IC ₅₀ values of 0.155 µL/mL and 0.166 µL/mL respectively					344
<i>Annona squamosa</i>	Mature leaves and fruits	Leaves aqueous extract (LAq) Seeds aqueous extract (SAq) Seeds hexane extract (SHex)	Vero cell lines, (Vero, ATCC-CL 81) kindly provided by Instituto Butantan (São Paulo, Brazil), were used to perform cytotoxic.	Citotoxicity Assays					75
				Tree Age		CC ₅₀ (mg.mL ⁻¹)			
				4		0.32			
				14		0.42			
				4		1.43			
				14		1.51			
				4		0.49			
				14		0.40			
		Compounds	B16-F10, HepG2, K562 and HL-60 tumor cell lines were kindly donated by	IC ₅₀ µg/mL (µM)					
				B16-F10	HepG2	HL60	K562	PBMC	
				NA	NA	NA	NA	NA	

<i>Xylopi laevigata</i>	Leaves	(-)-Roemerine	Hospital A.C. Camargo, São Paulo, Brazil. Cell viability was quantified using the Alamar Blue assay.	18.80	14.04	10.09	10.62	NA	335
		(+)-Anonaine		8.46	3.89	7.81	6.61	24.53	
		Lanuginosine		NA	NA	NA	NA	NA	
		(+)-Glaucine		3.77	1.87	1.87	3.12	4.08	
		(+)-Xylopine		19.14	NA	5.90	12.48	10.25	
		Oxoglaucine		8.48	3.78	6.84	7.84	6.70	
		(+)-Norglaucine		NA	NA	NA	NA	NA	
		(-)-Xylopinine		21.08	NA	10.11	16.72	17.94	
		(+)-Norpurpureine		NA	NA	NA	NA	NA	
		(+)-N-Methyllaurotetanine		NA	NA	NA	NA	NA	
		(+)-Norpredicentrine		16.15	7.89	12.97	14.85	NA	
		(+)-Discretine		22.17	NA	18.59	NA	NA	

		(+)-Calycinine		NA	NA	NA	NA	NA	
		(+)-Laurotetanine		NA	15.35	23.81	NA	NA	
		(+)-Reticuline		NA	NA	NA	NA	NA	
		(-)-Corytenchine		18.80	14.04	10.09	10.62	NA	
		(+)-Discretamine		NA	NA	NA	NA	NA	
		(+)-Flavinantine		0.08	0.08	0.09	0.15	2.47	
		Positive Control Doxorubicin							
		Extract	Human tumor cell lines were plated in 96-well plates: HCT-116, SF-295, HL-60, Sarcoma-180.	Cell proliferation inhibition (%)					
				HCT-116	SF-295	HL-60	Sarcoma-180	IC₅₀ (μM)	
		Av-MeOH	The concentration that caused 50 % cell growth inhibition (IC ₅₀) was determined from the concentration-response curves by non-linear	98.16 ±0.92	63.98 ±4.84	82.23 ±4.84	82.34 ±1.36	2.81 ±0.41	
		Av-HexC		29.96	86.54	17.11	79.43	4.87	

<i>Annona vepretorum</i>	Leaves	Av-Hex	regression with a confidence interval of 95 %.	±1.60	±3.31	±7.34	±4.39	±0.83	345
				56.04	65.43	55.85	86.24	45.82	
				±21.0	±6.52	±3.56	±1.09	±9.07	
		Av-CHCl ₃		74.28	82.05	29.79	81.32	2.88	
				±0.25	±24.67	±1.82	±6.79	±1.39	
		AV-AcOEt		17.8	27.98	68.72	63.15	22.82	
				7±6.45	±5.16	±38.28	±6.57	±3.76	
		Av-H ₂ O		9.52	6.72	-8.20	78.57	71.18	
				±11.68	±1.25	±3.33	±5.91	±1.69	
		<i>Asimina triloba</i>		Seeds	Acetogenins of <i>Asimin triloba</i>	Cytotoxicity tests against human tumor cell lines: A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma) and MIA PaCa-2 (human pancreatic carcinoma).	Human cancer cell line (ED ₅₀ µg/ml)		
1	2		Adriamycin				Cells		
1.31x10 ⁻¹	4.29x10 ⁻³		Not Tested		BST LC ₅₀				
4.39x10 ⁻³	1.65x10 ⁻³		1.74x10 ⁻²		A-549				
2	2.11x10 ⁻³		1.69x10 ⁻³		4.40x10 ⁻¹		MCF-7		
Adramicyn	2.09		4.40x10 ⁻¹		1.16x10 ⁻²		HT-29		

				2.78	2.19	1.16x10 ⁻²	A-498	
				2.28	1.06	4.61x10 ⁻²	PC-3	
				3.99x10 ⁻⁵	2.88x10 ⁻⁴	7.81x10 ⁻³	MIA PaCa-2	
<i>Artabotrys zeylanicus</i>	Not specified	Compounds N-methoxynorcepharadione Atherospennidine Positive control Camptotecin	Cytotoxicity assessed by IC50 by assay with cells: RS 322YK (rad52Y), RS 321N, RS 188N (rad+), P-388 (wild-type), P-388 (Camptothecin resistant.	IC₅₀ (µg/ml)				347
				1	2	Camptotecin	Cell lines	
				2.16	16	0.6	RS 322YK (rad52Y)	
				1.20	27	-	RS 321N	
				>200	>50	100	RS 188N (rad+)	
				159	Not tested	0.012	P-388 (wild-type)	
				1.12	Not tested	>20	P-388 (Camptot	

							hecin resistant	
<i>Guatteria megalophylla Diels</i>	Leaves	Essential oil (EO) Positive Control Doxorubicin (DOX) 5-Fluorouracil (5-FU)	Toxicity Assays with Human cancer cell lines: HL-60 Promyelocytic leukemia; MCF-7 Breast adenocarcinoma; Cal27 Oral squamous cell carcinoma; HSC-3 Oral squamous cell carcinoma; HepG2 Hepatocellular carcinoma; HCT116 Colon carcinoma and Human non-cancer cell line: MRC- 5 Lung fibroblast.	IC ₅₀ (µg/mL)				348
				EO	DOX	5-FU	Human Cancer Cells	
				12.51	0.02	1.85	HL-60	
				35.45	6.16	10.13	MCF-7	
				7.58	1.09	2.56	CAL-27	
				14.90	0.86	1.01	HSC-3	
				21.62	0.02	13.71	Hep-g2	
				30.27	0.02	0.53	HCT116	
				29.80	3.32	5.96	MRC-5	
<i>Xylopi langsдорffiana</i>	Stems	Compounds		IC ₅₀ (µM)				
				V79		K562		

		ent-7r-Acetoxytrachyloban-18-oic acid (1)	Cytotoxic activity of compound 1 was evaluated against V79 cells and rat hepatocytes using the MTT method.	224 and 231 μM	200 μM		349	
<i>Polyalthia longifolia</i> var. <i>pendula</i>	Leaves	Compounds 16α-hydroxycleroda3,13-dien-15,16-olide 5-hydroxy-6-Methoxyonychine (-)-anonnaine	Tested against four human cancer cell lines: AGS (gastric cancer cells), DLD1 (colon cancer cells), HepG2 (hepatoma cells), and HA59T (hepatoma cells).	IC₅₀ (μM)				350
				AGS	DLD	HA597	HepG2	
				26.9	>30	23.6	>30	
				>30	>30	21.7	>30	
				8.6	28.9	16.4	20.8	
				% Kill at 100 μg/mL				
				MDA-MB-231		Hs 578T		

<i>Desmopsis bibracteata</i>	Leaves	Essential oil	Human MDA-MB-231 breast adenocarcinoma cells and Human Hs 578T breast ductal carcinoma cells.	99.3 (0.7)		100		273
<i>Desmopsis macrocarpa</i>				53.0 (9.6)		8.2 (14.0)		
<i>Guatteria costaricensis</i>				54.6 (5.7)		0		
<i>Guatteria diospyroides</i>				98.8 (1.2)		21.1 (8.2)		
<i>Guatteria oliviformis</i>				100		35.6 (1.9)		
<i>Unonopsis costariensis</i>				100		17.0 (10.3)		
				IC ₅₀				
				HepG2		MDA-MB231		
		Compounds		Mean	SD	Mean	SD	

<i>Pseuduvaria trimera</i> (Craib)	Leaves And twigs	8-hydroxyartabonatine C	The human hepatocellular carcinoma HepG2 and breast cancer MDA-MB231 cells.	26.36	±5.18	64.75	±4.45	351
		Ouregidione		12.88	±2.49	67.06	±3.5	
		Positive control Doxorubicin		2.21	±1.72	1.83	±0.09	
<i>Polyalthia longifolia</i> var. <i>pendula</i>	The bark samples	Compounds 16(<i>R</i> & <i>S</i>)- 3,13 <i>Z</i> -kolavadien-15,16-olide-2-one 16-hydroxycleroda- 3,13-dien-15,16-olide 16-hydroxycleroda-4(18),13-dien-15,16-olide 16-oxocleroda-3,13(14) <i>E</i> -dien-15-oic acid methyl ester Solidagonal acid (4→2)- <i>abeo</i> - 16(<i>R</i> & <i>S</i>)-2,13 <i>Z</i> -clerodadien-15,16-olide-3-al labd-13 <i>E</i> -en-8-ol-15-oic acid Polylongine Liriodenine Lysicamine	Tested against four human cancer cell lines: MCF-7, MDA-MB-231, Hep-G2, Hep 38.	IC₅₀ (μM)				131
				MCF-7	MDA-MB-231	Hep-G2	Hep38	
				18.28	4.50	2.88	2.96	
				14.42	8.29	4.42	2.83	
				10.43	3.22	3.35	1.97	
				14.34	13.22	-	-	
				18.12	14.67	-	-	
				11.89	11.65	2.36	8.94	
				-	-	18.33	15.40	
				10.41	9.94	-	-	
				4.46	10.28	-	-	
				8.94	16.75	-	-	

		(+)-Stepharine (–)-Stepholidine <i>N-trans</i> -feruloyltyramine <i>N-trans</i> -p-coumaroyltyramine Positive control Doxorubicin		9.40 16.56 25.53 17.35 0.04	9.90 - 25.54 - 0.32	- - 21.17 - 0.18	- - 24.98 - 0.23	
<i>Anaxagorea dolichocarpa</i> and <i>Duguetia chrysocarpa</i>	Stem barks	Extract	Human tumor cell lines, including OVCAR-8 (ovarian), SF-295 (brain) and HCT-116 (colon).	Cell proliferation inhibition (%)				352
		<i>A. dolichocarpa</i>		OVCAR-8		SF-295	HCT-116	
		Ad-EtOH (Ethanolic)		53.94		65.49	50.19	
		Ad-Hex (Hexanic)		44.31		62.68	89.02	
		Ad-CHCl ₃ (Chloroform)		52.51		58.82	67.15	
		Ad-AcOEt (Ethyl acetate)		-5.38		0.09	3.40	
		<i>D. chrysocarpa</i>		49.65		43.95	60.16	
		Dc-EtOH (Ethanolic)						

		Dc-Hex (Hexanic)		18.39	34.84	15.61	
		Dc-CHCl ₃ (Chloroform)		42.24	63.17	59.35	
		Dc-AcOEt (Ethyl acetate)		13.01	30.99	9.76	

3.6 Antitumor

Cancer comprises a heterogeneous group of disorders, which is caused by a series of “genetic” alterations selected clonally in tumor suppressor genes and oncogenes ³⁵³. However, evidence accumulated in recent years indicates that the heterogeneity of tumor cells is partly due to the contributions of “epigenetic” changes in cancer cells ³⁵⁴. Thus, it is believed that cancer is the manifestation of genetic and epigenetic changes ^{355,356}. However, despite a small contribution from the inheritance of tumors, it is believed that most cancers can also result from changes that accumulate throughout life due to exposure to various endogenous factors such as nutrients, infections, physical activity, social behavior and other environmental factors ³⁵⁷.

In Brazil, according to data from the National Cancer Institute (INCA, 2019) the disease is already emerging as the second leading cause of mortality in the country ³⁵⁸. In this way, innumerable anticancer drugs have been introduced in therapy in the last decades, these being obtained by plant screening programs. From this perspective, considering the high incidence of cancer and the toxic effects that antineoplastic drugs have on normal cells, it is important to highlight the role that natural products have played in the search for efficient alternatives for antineoplastic therapy ^{359–361}.

Thus, some authors conducted research on some species of the Annonaceae in search of new antitumor agents. Rinaldi and collaborators (2017) ²⁵⁴ evaluated the antitumor activity of alkaloids isolated from the stem extract of species of *Annona hypoglauca* Mart., collected in the Igapó Forest belonging to the Amazon Rainforest. The isolated alkaloids corresponded to respectively: Isoboldine (compound 1), Nornuciferine (compound 2), Anonaine (compound 3), Actinodaphnine (compound 4). The cytotoxicity of the compounds was evaluated against human cell lines (MCF-7, breast adenocarcinoma; KM-12, colon adenocarcinoma; RPMI-8226, multiple myeloma; PC-3, prostate carcinoma; SF-268 glioblastoma and NCI- H460, non-small cell lung cancer), obtained from the National Cancer Institute. The total alkaloid fraction showed significant cytotoxicity against MCF-7 (–8.90% lethality) and also showed cytotoxicity against SF-268 (26.7% growth inhibition), against NCIH461 (29.0% growth inhibition) and against KM-12 (67.6% growth inhibition). This may indicate that alkaloids are responsible for cytotoxicity against breast cancer cell lines.

The study on the antitumor activity of alkaloids of Annonaceae species was also the objective of Suassuna and collaborators (2011) ³⁶². In this study, the authors evaluated

the activity of the alkaloids Eupolauramine (1), Sampangine (2) and Imbiline 1 (3), both isolated from the stem bark of *Anaxagorea dolichocarpa* collected in the city of Santa Rita, State of Paraíba, Brazil, in February 2006. The evaluation of cytotoxicity was performed against the cell line the human leukemic strain K562 (Chronic Myeloid Leukemia), inhibition being determined through the 50% inhibition concentration for cell growth (IC_{50}). The alkaloids eupolauramine and sampangine demonstrated antitumor activity against human cells strain K562 showing IC_{50} values of 18.97 (17.06–21.10) $\mu\text{g/mL}$ and 10.95 (10.15–11.80) $\mu\text{g/mL}$, respectively. The interesting antitumoral activities of these alkaloids suggests great potential in the ongoing search for molecules with activity against cancer.

Unlike previous studies, Chen and colleagues (2011)³⁶³ evaluated the antitumor activity of acetogenins isolated from leaves of the species *Annona squamosa* Linn. The compounds under study comprised respectively: Squamostatin A (compound 1), Squamostatin E (compound 2), 4-deoxyannorecticin (compound 3), desacetylurvaricin (compound 4) e bullatacin (compound 5). Acetogenins had their antitumor activity investigated using S180 and HepS xenografts bearing mice simultaneously. The results revealed that select ACGs suppressed tumor growth in a dose-dependent fashion. Tested ACGs showed more selective antitumor activity against HepS. Furthermore, adjacent bis-THF ACGs were more active than mono-THF and nonadjacent bis-THF ACGs against HepS and S180; nonadjacent bis-THF ACGs were more active than mono-THF ACGs against S180, but mono-THF ACGs were more potent than nonadjacent bis-THF ACGs against HepS.

Meng and collaborators (2011)³⁶⁴ chose to evaluate the antitumor activity of clerodane-type diterpenes, the compounds being: 6a,16,18-trihydroxycleroda-3(4),13(14)-dien-15,16-olide (compound 1) and 16-hydroxycleroda-3(4),13(14)-dien-15,16-olide (compound 2) isolated from the aerial parts of *Mitrephora thorelii* were collected in the suburb of Guangzhou, Guangdong province, China, in December 2003. Tumor inhibition was performed against exhibited inhibitory activity against the proliferation of human hepatoma BEL-7402 cells *in vitro* through studies with rats. Cyclophosphamide was used as a positive control. The authors concluded that compounds 1 and 2 inhibited the proliferation of BEL-7402 cells *in vitro* with the IC_{50} values 44.6 and 20.1 mM, respectively. The compound 2 significantly inhibited the growth of hepatoma H22 with the percentage inhibition of 30.7% ($P, 0.05$ vs control). The above

results revealed compound 2 as a potential anti-tumour natural product, and further in vivo anti-tumor evaluation is warranted.

Kim and collaborators (2005)³⁶⁵ evaluated the antitumor activity of acetogenins obtained from the leaves of *Asimina triloba* Possessing. The isolated compounds comprised the Asimitrin (compound 1) and the 4-Hydroxytrilobin (compound 2). The biological activity was performed using protocols for A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma), and MIA PaCa-2 (human pancreatic carcinoma), with adriamycin as a positive control. The results revealed that the Compound 1 was selectively cytotoxic against prostate adenocarcinoma (PC3) at about 10 000 times and against colon adenocarcinoma (HT-29) at about 100 times the potency of adriamycin, respectively. In turn, compound 2 was especially active against the prostate adenocarcinoma (PC-3) at about 100 times and against colon adenocarcinoma (HT-29) cell line at about 10 000 times the potency of adriamycin. The acetogenins exert their cytotoxic and their in vivo antitumor effects, in part, by inhibiting complex I of the electron transport system in the mitochondria and by blocking the NADH oxidase enzyme peculiar to the plasma membranes of cancerous cells.

Through these studies, the pharmacological potential of compounds derived from the Annonaceae is evident in the face of tumor inhibition, where the top five studies addressed the action of compounds belonging to the classes of acetogenins, terpenoids and especially alkaloids. Table 6 shows the substances tested in each study as well as their respective anti-tumor potential.

Table 6: Summary of antitumor activity of species of the Annonaceae.

Plant	Used material	Substances	Methodology	Inhibition						Ref.
				MCF-7	PC-3	NCI-H460	KM-12	SF-268	RPMI-8226	
<i>Annona hypoglauca</i>	Stem	Crude extract	Human tumor cell lines (MCF-7, breast adenocarcinoma; KM-12, colon adenocarcinoma; RPMI-8226, multiple myeloma; PC-3, prostate carcinoma; SF-268 glioblastoma and NCI-H460, non-small lung-cell carcinoma).	-32.80	11.4	1.3	-2.5	58.5	17.3	254
		Fraction hexane		NI	77.4	48.1	NI	NI	53.5	
		Dichloromethane/ Methanol		2.90	59.6	44.9	NI	93.7	53.4	
		Fraction ethyl acetate		Not tested	67.1	38.6	NI	77.4	NI	
		Fraction butanol		11.90	46.6	65.6	NI	NI	83.8	
		Total alkaloid fraction		-8.90	17.04	71.7	32.4	73.3	NI	
		Fraction alkaloid 4.4		Not tested	-3.8	-26.0	11.7	45.4	29.7	
		Fraction alkaloid 5		-11.60	Not tested	Not tested	-14.4	Not tested	Not tested	

		Fraction alkaloid 6		-3.10	Not tested	Not tested	-8.4	Not tested	Not tested	
		Doxorubicin (Positive control)		-16.31	-50.0	-46.2	-5.1	-34.0	-14.3	
<i>Annona vepretorum</i>	Leaves	Essential Oil	The in vivo antitumour effect was evaluated in C57BL/6 mice inoculated with B16- F10 melanoma. Tumour cells (2 9 106 cells per 500 IL) were implanted subcutaneously into the left hind groin of mice. Animals were euthanized by cervical dislocation, and tumours were excised and weighed. Drug effects are expressed as the per cent inhibition of control.	IC₅₀ (µg/mL)						366
				B16-F10	HepG2	K562	HL-60	PBMCs		
				9.90	10.60	8.43	6.14	22.82		
				7.81-12.55	8.55-13.26	5.48-12.97	4.15-9.12	19.18-27.15		
				11.67	11.19	3.79	11.38	15.59		
				9.76-13.96	9.58-13.07	1.48-9.70	8.46-15.31	13.12-18.53		
				15.44	17.30	>25	20.18	>25		
				6.54-36.42	13.89-21.55		16.91-24.08			
				>25	>25	>25	>25	>25		
				11.46	13.05	14.00	14.96	>25		
		5-Fluorouracil		0.68	0.04	0.15	0.29	14.00		
				0.21-1.45	0.01-1.22	0.01-1.86	0.21-0.38	9.83-23.31		

		(Positive control)								
<i>Xylopi frutescens</i> Aubl.	Leaves	Essential oil Doxorubicin Positive Control	The in vivo antitumour effect was evaluated using Sarcoma 180 ascites tumour cells. Ten-day-old Sarcoma 180 ascites tumour cells (2 106 cells per 500 ll) were implanted subcutaneously into the left hind groin of mice.	IC ₅₀ (µg/mL)						367
				OVCAR-8		NCI-H358M		PC-3M		
				33.9 24.9-46.3		24.6 14.9-40.7		40.0 31.3-51.2		
				1.2 0.9-1.6		0.9 0.6-1.3		1.6 1.1-2.4		
<i>Polyalthia evecta</i>	Leaves	Hexane fraction (F1) 500 (µg/mL) Chloroform fraction (F2) 500 (µg/mL) Ethylacetate fraction (F3) 500 (µg/mL)	Determination the cytotoxicity of the samples in the cell model, neutral red (NR) uptake assay was used for identification of vital cells. The cytotoxicity assays were performed with HepG2 and Vero cells.	% Cytotoxicity				% Apoptotic cells in HepG2	62	
				HepG2		Vero				
				74.6 ± 1.6		54.2 ± 15.4		46.4 ± 2.6		
				24.3 ± 9.3		2.2 ± 3.8		9.8 ± 4.7		
				29.6 ± 8.8		32.5 ± 8.1		3.2 ± 1.6		

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Dichloromethane fraction (F4) 500 (µg/mL)	24.0 ± 7.4	25.5 ± 8.1	2.9 ± 1.1
Ethanol fraction (F5) 500 (µg/mL)	22.7 ± 8.8	7.6 ± 8.3	2.4 ± 1.9
Methanol fraction (F6) 500 (µg/mL)	9.9 ± 2.5	12.3 ± 1.3	4.6 ± 1.6
Water fraction (F7) 500 (µg/mL)	7.8 ± 2.5	10.8 ± 0.8	9.2 ± 2.1
Hexane: water (500:100)	98.9 ± 1.8	4.0 ± 2.9	n.d.
Hexane: water (500:250)	81.8 ± 10.6	43.9 ± 3.3	n.d.
Hexane: water (500:500)	100.0 ± 1.0	45.1 ± 4.1	72.7 ± 13.6
Hexane: methanol (500: 100)			

	Hexane: methanol (500: 250)	91.0 ± 6.2	26.9 ± 5.6	n.d.
	Hexane: methanol (500:500)	100.0 ± 5.7	36.1 ± 8.0	n.d.
	<i>P. evecia</i> crude extract (EW-L) 140 (µg/mL) <i>P.</i> <i>evecia</i> crude extract (EW-L) 500 (µg/mL)	100.0 ± 8.9	39.9 ± 3.2	54.9 ± 10.8
		60.0 ± 4.8	30.2 ± 9.3	46.4 ± 2.6
	Melphalan 76 (µg/mL)	100.0 ± 4.1	37.2 ± 4.2	92.8 ± 10.8
		67.2 ± 3.1	70.3 ± 3.1	41.6 ± 2.1
		(%) of citotoxicity		

<i>Annona squamosa</i>	Fruits	Compounds	Cytotoxic activity using in vitro cultures of Dalton’s lymphoma cells as well as HeLa cells. Cytotoxicity was detected by the Trypan blue exclusion test and induction of apoptosis was evaluated by [3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide] assay (MTS assay) and DNA ladder assay. The inhibitory concentration required for 50% cytotoxicity (IC ₅₀) was also determined.	There was an increase in the percentage cytotoxicity with increasing concentrations of the fraction containing the compounds. However a slight decrease in activity with increasing incubation time was noted, which implies that the compound has very high cytotoxicity even after 24 h of incubation. Even at a concentration of 1.65 lg/ml, the compounds exhibited more than 50% cytotoxicity after 24 h. The cytotoxic activity for different time intervals is compared. The highest activity was noted after 24 h, which implies that the efficacy of secondary metabolites as cytotoxic agents at a low dose and a short duration.						368
		(-)-entkaur-16-en-19-oic acid (1)	16- α ,17-dihydroxy-ent-kauran-19-oic acid (2)							
<i>Annona squamosa</i>	Seeds	Compounds:	The antitumor activities of 1–5 and standard control taxol against the growth of S180 and HepS in mice were	Treatment effects of annonaceous acetogenins in the Heps and S180 xenograft tumor model						
					HepS		S180			
		Treatment group		Dose (µg/kg)	Tumor weight (g: mean±SD)	Inhibition ratio (%)	Tumor weight (g: mean±SD)	Inhibition ratio (%)		
		Control			1.13±0.46	-	0.75±0.16	-		
		Taxol		40	0.43±0.15	62.2	0.38±0.12	48.9		

		Positive control	measured by methods reported previously.	Squamostatin A	15 60	0.96±0.19 0.79±0.12	15.0 31.2	0.36±0.09 0.34±0.10	52.7 54.2	363
				Squamostatin E	15 60	0.75±0.16 0.55±0.18	34.2 51.3	0.55±0.10 0.37±0.08	27.2 51.1	
				4-deoxyannoreti- cucin	15 60	0.43±0.12 0.37±0.14	61.5 67.3	0.42±0.10 0.39±0.11	43.9 48.3	
				Desacetylurari- cin	15 60	0.77±0.18 0.33±0.22	32.5 70.9	0.43±0.12 0.27±0.08	42.3 63.9	
				Bullatacin	15 60	0.38±0.18 -	63.4 -	0.26±0.06 -	65.8 -	
<i>Annona muricata</i>	Leaf, Seed, Fruit, Pericarp, Twing,		This current review demonstrates <i>Annona muricata</i> 's anticancer potential and other health-related benefits by providing insights into its bioactive chemical constituents	Review Article Acetogenins or other <i>A. muricata</i> -derived compounds could be tested as monotherapy or as sensitizers in combination with standard cancer treatments for cancer patients.						369

	Root	Acetogenins, extracts and fractions	as well as the in vitro and in vivo studies that have been carried out in order to elucidate the molecular mechanisms of action of these constituents.						
<i>Xylopi sericea</i>	Dried roots	Compounds kauren-19-oic acid (KA); (–)-kauran-19-oic acid (KAH).	The human cancer cell-lines used in this work were HL60 and K562 (leukemias), MDA-MB435 (melanoma) and SF295 (glioblastoma). The growth of tumour cells and PBLs was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiozoly)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product.	Mitotic index, frequency of chromosomal aberrations and numeric changes in human lymphocytes in culture after kaurenoic acid (KA) and their hydrogenated derivative (KAH) treatments.					
						Mitotic index		Aberrant cells	
				Substance	Treatment	%	Mean±S.E.M	%	Mean±S.E.M
				MMS (Positive control)	4x10 ⁻⁵ M	2.8	2.46±0.3	5.3	6.2±1.8
						2.1		8.3	
						2.5		5.0	
				DMSO (Vehicle)	0.1%	4.1	3.86±0.3	0.7	0.46±0.4
						4.0		0.0	
						3.5		0.7	
				KA	2.5µg/mL	4.3	4.23±0.1	0.0	0.0
						4.1		0.0	
						4.3		0.0	
					5.0 µg/mL	4.0	4.0±0.2	0.0	0.23±0.4
						3.8		0.0	

						4.2		0.0	
						10.0 µg/mL	3.3	3.13±0.1	0.7
							3.0		0.0
							3.1		0.7
						30.0 µg/mL	2.3	2.23±0.4	5.3
							1.8		4.7
							2.6		5.3
						60.0 µg/mL	1.3	1.36±0.3	4.3
							1.7		5.0
							1.1		6.2
				KAH		2.5 µg/mL	4.4	4.13±0.2	0.0
							4.0		0.0
							4.0		0.0
						5.0 µg/mL	4.2	4.0±0.2	0.0
							4.1		0.0
							3.7		0.0
						10.0 µg/mL	3.5	3.5±0.4	0.0
							3.9		0.0

					30.0 µg/mL	3.1 3.2 3.7 3.4	3.43±0.2	0.0 0.0 0.7 0.0	0.23±0.4	
					60.0 µg/mL	3.0 3.1 3.6	3.43±0.3	0.0 0.7 0.7	0.43±0.4	
<i>Guatteria australis</i> <i>Guatteria ferruginea</i> <i>Guatteria latifolia</i>	The aerial parts	Essential Oils Doxorubicin Positive control	The antiproliferative activity of the materials tested was evaluated using eight human tumor cell lines: U251 (central nervous system, CNS, glioma), MCF-7 (breast cancer) NCI-ADR/RES (ovarian tumor with multidrug resistance phenotype), 786-0 (kidney cancer), NCI-H460 (non-small-cell lung cancer), OVCAR-03 (ovarian carcinoma), HT-29 (colorectal cancer), and K562	Total growth inhibition (TGI) [µg/ml]						277
				Cell lines	<i>G. australis</i>	<i>G. ferruginea</i>	<i>G. latifolia</i>	<i>G. sellowiana</i>	Doxorubicin	
				U251	40.4	36.3	36.6	89.1	2.7	
				MCF-7	37.8	37.6	47.2	95.5	0.88	
				NCI-ADR/RES	15.2	34.6	10.0	250	>25	
				786-0	45.6	50.9	63.9	107.8	3.1	
				NCI-H460	49.5	69.4	44.9	82.8	>25	

<i>Guatteria sellowiana</i>			(leukemia). Using the concentration-response curve for each cell line.	OVCAR-3	3.2	1.8	1.1	4.1	11.7	
				HT-29	38.6	52.7	39.7	143.1	3.6	
				K562	86.2	18.6	15.6	1.1	0.031	
				HaCat	48.0	63.4	41.0	75.6	1.0	
<i>Anaxagorea dolichocarpa</i> <i>Sprague & Sandwith</i>	The stem bark	Alkaloids	The cytotoxicity was evaluated through the MTT reduction assay, which determines the number of living cells able to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to formazan. The determination of the 50% inhibition concentration for cell growth (IC ₅₀) of The human leukemic strain K562.	IC ₅₀ µg/mL						362
		Compounds		The in vitro effects of eupolauramine (1) and sampangine (2) against the K562 cell line were determined in three experiments and in quadruplicate. Both compounds exhibited concentration-dependent inhibitory effect on the proliferation of K562 cells. The IC ₅₀ values were 18.97 (17.06–21.10) µg/mL and 10.95 (10.15–11.80) µg/mL respectively.						
		Eupolauramine (1)								
		Sampangine (2)								
		Compound		IC ₅₀ mM						

<i>Mitrephora thorelii</i>	The aerial parts	6a,16,18-Trihydroxyclerod a-3(4),13(14)-dien-15,16-olide (1)	<i>In vivo</i> evaluation female mice (5–6 weeks old) of Kunming strain were purchased from Shanghai SLAC Laboratory Animal Co. (Shanghai, China). Murine hepatoma H22 is maintained by serial intraperitoneal passage in Kunming mice. H22 cells were subcutaneously implanted into Kunming mice at Diterpenes from <i>M. thorelii</i> 683 1 £ 106 cells/mouse.	Compounds 1 and 2 inhibited the proliferation of BEL-7402 cells in vitro with the IC₅₀ values 44.6 and 20.1 mM, respectively. In vivo anti-tumor effect of compound 2 was further evaluated in murine hepatoma H22 model. It was found that compound 2 significantly inhibited the growth of hepatoma H22 with the percentage inhibition of 30.7% (P, 0.05 vs control). The alkylation agent cyclophosphamide served as a positive control (63.8%, P, 0.01 vs control). The mice were well tolerated towards compound 2, and no significant lose of body weight was observed compared with control group (P. 0.05, data not shown).				364
		16-Hydroxycleroda-3(4),13(14)-dien-15,16-olide (2) Cyclophosphamide Positive control						
<i>Annona crassiflora</i>	The leaves	Control	To evaluate the antimutagenic/chemopreventive activity through the <i>Allium cepa</i> test, we used 5, 10, and 15 mg/L of extract, and for the micronucleus test in the peripheral blood, we used the dose of 15 mg/kg.	Chromosomal aberrations				113
		MMS		Treatment	Damage Reduction Percentages of mitotic index	Total	Damage Reduction Percentages	
		<i>A. crassiflora</i> (AC)		Mutagenicity				
				Control	-	40	-	
				MMS	3.51	238	-	
				AC - 5mg/L	-1.62	31	-	
				AC - 10mg/L	-11.35	27	-	

				AC - 15 mg/L	-29.46	34	-	
Asimina triloba	Seeds	Compounds	In vitro cytotoxicity tests against human tumor cell lines s for A-549 (human lung carcinoma),26 MCF-7 (human breast carcinoma),27 HT-29 (human colon adenocarcinoma),28 A-498 (human kidney carcinoma),26 PC-3 (human prostate adenocarcinoma),29 and MIA PaCa-2 (human pancreatic carcinoma)	Human cancer cell line ED50 (µg/mL)				365
				Compounds				
		Cell lines		1	2	Adriamicyn (Control)		
		BST		2.07x10 ⁻²	7.00x10 ⁻²	NT		
		A-549		1.19	1.54	6.22x10 ⁻⁴		
		MCF-7		2.12	3.79	9.53x10 ⁻¹		
		HT-29		1.19x10 ⁻⁴	1,54x10 ⁻⁶	2.87x10 ⁻²		
		A-498		7.50x10 ⁻¹	3.62x10 ⁻²	2.86x10 ⁻³		
		PC-3		1.72x10 ⁻⁶	2.01x10 ⁻⁴	5.77x10 ⁻²		
		MIA PaCa-2		2.11x10 ⁻⁴	2.01x10 ⁻⁴	6.10x10 ⁻³		
			The antitumor against the normal cell line derived from	IC50 ± SE (µg/mL)				
				MCF-7		PC-3		

<i>Guatteria elliptica</i> R. E. Fries	Leaves	Essential oil	mouse fibroblasts (BALB/c 3T3, ATCC CCL163) was	7.01 ± 0.23	5.32 ± 0.35	371
		Spathulenol	tested using the MTS method. The IC50 values were used to determine the median lethal dose (LD50) for cell lines MCF-7 (human breast cancer) and PC-3 (human prostate cancer.	5,38 ± 0.20	2.25 ± 0.28	
<i>Polyalthia longifolia</i> var. <i>pendula</i>	Stems	15 of the compounds isolated were evaluated for cytotoxicity against MCF-7 (human breast carcinoma) and A549 (non-small cell lung cancer) cells with cell viabilities assessed using a MTT assay.	Cytotoxicity assessment was performed with Human breast carcinoma (MCF-7) cells and non-small cell lung cancer (A549) cells.	IC50 µM		372
				Compounds tested, only 16-oxo-cleroda3,13-dien-15-oic acid (8) was cytotoxic against both MCF-7 and A549 cell lines, with IC50 values of 3.7 (0.2 and 3.1 (0.3 µM, respectively. Under the same conditions, the IC50 values of the corresponding positive controls, paclitaxel and doxorubicin, were 0.0020 (0.0001 and 0.837 (0.034 µM, respectively.		
		Compounds		IC50 µM		

<i>Mitrephora glabra</i>	Stem Bark	4- <i>epi</i> -kaurenoic acid (1) Mitrekaurenone (2) Methylmitrekaurenate (3) Oropheic acid (4) Methyloropheate (5) Octadeca-9,11,13-triynoic acid (6) Oropheolide (7) 9,10-Dehydrooropheolide (8) Liriodenine (9)	The cytotoxicity measurements against the KB human oral epidermoid carcinoma; MCF-7 human breast carcinoma, NCI-H460 human large cell lung carcinoma, and SF-268 human astrocytoma.	The ent-kaurane diterpenoids (1-3), all were inactive (IC ₅₀ values >10 µM); the diterpenoids of the ent-trachylobane class, reported previously from the same plant, were more potent in this regard. The polyacetylenes (4 and 6-8) gave IC ₅₀ values ranging from 10 to 40 µM. However, compound 5 was completely inactive, suggesting that the methyl ester diminishes cytotoxicity. The known alkaloid liriodenine (9) was the most cytotoxic against all four of the cell lines in which it was evaluated (IC ₅₀ value ~5 µM).		
		Extract	The tumor cell lines used were: U251 – glioma, MCF-7 – breast, NCI/ADR-RES – multidrug-resistant ovarian, 786-0 – kidney, NCI-H460 – non-small cell lung cancer, PC-3 – prostate, OVCAR –	Total inhibition of cancer cells proliferation (µg/ml)		
				Cell Lines	DOX	ALO
				Glioma (U251)	0.06	47.23
				Breast (MCF-7)	0.21	49.91
					1.35	>250

Annona leptopetala	Leaves	Annona leptopetala leaves (ALO)	ovarian, HT29 – colon and K562 – leukemia, and HaCaT human keratinocytes served as the normal cell line. Sarcoma 180 tumor cells were maintained in the peritoneal cavity of Swiss mice.	Ovary Multidrug Resistance Phenotype (NCI-ADR/RES)	0.04	101.52	374			
		Doxurubicin (DOX)		Kidney (786-O)				0.01	75.53	
		Positive control		Lung (NCI-H460)				0.27	45.12	
				Prostate (PC-3)				0.26	>250	
				Ovary (OVCAR)				0.22	75.26	
				Colon (HT-29)				0.40	0.64	
				Leukemia (K-562)				0.23	>250	
				Skin (line of non-tumor cells) (HaCat)						
				Antitumor activity sarcoma 180 ascites tumor cells				375		
				Dose (mg/kg/day)				Tumor (g)	Inhibition (%)	
-	1.97±0.14	-								
25	0.63±0.16	67.8								
Xylopi laevigata	Leaves	5% DMSO	The in vivo antitumor effect was evaluated using sarcoma 180 ascites tumor cells. Ten-day-old sarcoma 180 ascites tumor cells (2 × 106 cells per 500 µL) were implanted							
		5-Fluororacil								
		Positive control								

		Essential oil	subcutaneously into the left hind groin of mice.	50 100	1.24±0.09 1.13±0.27	37.3 42.5	
<i>Duguetia furfuracea</i>	Bark	Compounds	The cytotoxic potential of alkaloids was evaluated by the MTT assay. Against three human tumor cell lines: SF-295 (glioblastoma), HCT-8 (colon cancer) and MDA/MB-435 (melanoma).	Percentage of inhibition (%)			300
				HCT-8 (%)	SF-295 (%)	MDA/MB-435 (%)	
		Duguetine (1)		91.1±0.8	86.0±1.1	98.1±0.1	
		Duguetine β- <i>N</i> -oxide (2)		92.0±1.0	87.1±0.7	84.5±1.6	
		Dicentrinone (3)		68.0±0.7	50.3±1.5	37.5±4.0	
		<i>N</i> -methyltetrahydrop almatine (4)		57.6±0.7	39.8±0.7	31.1±0.8	
		<i>N</i> -methylglaucine (5)		45.2±2.1	25.1±2.3	15.1±6.4	
		Alkaloid extract		90.2±1.1	85.1±0.6	99.6±0.4	
		Extract					
		Doxorubicin		93.3±1.7	92.3±2.2	97.0±1.1	

		(Positive control)					
<i>Fissistigma cavaleriei</i>	The dried roots	5-methoxy-2-methylisoindolin-1-yl, 4-methoxyphenyl Doxorubicin Positive control	Evaluation of the antiproliferative effect of compound 1 under study against cells: K562, S-180, A549.	IC₅₀ (mol/L)			376
				K562	S-180	A549	
				2.54±0.22x10 ⁻⁵ 1.16±0.14x10 ⁻⁶	7.26±0.24x10 ⁻⁵ 1.61±0.11x10 ⁻⁷	8.76±0.18x10 ⁻⁵ 8.12±0.12x10 ⁻⁷	
<i>Miliusa balansae</i>		Flavonoids Ombuine Crysosplenol B	Cell culture: - KB (Human Epidermoide Carcinom); - Hep-G2 (Hepatoma G2); - RD (Rhabdosarcoma).	IC₅₀ (µg/mL)			332
				KB	Hep-G2	RD	
				> 5 4.6	1.5 0.93	> 5 > 5	

Leaves and branches	Pachypodol	0.7	0.55	3.01
	Crysosplenol C	4.3	0.57	2.09
	Control Eliptin (Sigma)	0.002	0.001	0.001

3.7 Trypanocidal

Chagas disease (CD) is a Neglected Tropical Disease, that is, a group of diseases that suffer from a lack of financial support for research and development of new drugs, which mostly affect the population of the tropics ³⁷⁷⁻³⁸⁰.

CD was discovered in 1909 by Carlos Ribeiro Justina das Chagas, a Brazilian scientist, who found that the entry of the monoflagellated protozoan into the bloodstream of human beings occurs through the feces of triatomids, which led to the development of the disease. The protozoan was named *Trypanosoma cruzi*, honoring Oswaldo Cruz, and the disease came to be called and known as Chagas disease. Carlos Chagas was able to identify and characterize a new vector, a new parasite and a new disease ^{377,381,382}.

T. cruzi has a complex biological cycle, of the heteroxenic type, that is, its intermediate host is triatomids insects and vertebrate animals such as humans, armadillos, bats, paca, porcupine, monkey, dog, cats, among others. Where, in turn, they can assume the role of definitive host, completing the parasite's evolutionary cycle ^{380,383,384}.

T. cruzi has different names and morphologies that vary according to the stage they are in. The infecting morphology of the disease is trypomastigotes. It is transmitted through the excreta of the triatomids, this hematophagous insect that, when it bites to feed, generates a gateway for the disease-causing agent, and after feeding, defecates on the skin. Once trypomastigotes are found in the host's blood, it characterizes the acute phase of CD ^{377,385-387}.

Amastigotes are the morphological form found in the tissues of the hosts, characterizing the chronic form of the disease, whose main infected tissue is the heart, but can also infect the tissues of the colon and esophagus. They have the ability to multiply by binary fission and have a spherical shape and absence of flagellum ^{377,378,384}.

CD can also be transmitted through other routes such as blood transfusion or organ transplants, from mother to child during pregnancy or childbirth, contaminated drinking, laboratory accidents, among others ^{377,378,384}.

As for symptoms, CD has two phases: an acute phase that is characterized by being short and mainly oligosymptomatic followed by the chronic phase, a long-lasting phase that can trigger cardiac and/or digestive disorders and also the death of the patient ³⁷⁷⁻³⁸⁰.

One of the main causes of heart disease in endemic areas is CD, it is estimated that more than six million people are infected with *T. cruzi*, mainly in the poorest regions

of 21 Latin American countries, and it is still estimated that a million women are infected at reproductive age ³⁷⁷.

According to the World Health Organization (Who), 2022 ³⁸⁸, and the Drug for Neglected Diseases initiative (DNDi), 2022 ³⁸⁹, it is estimated that more than 75 million people are at risk of acquiring CD, and about 173,000 new cases occur annually with more than 75,000 deaths per year, yet less than 10% of infected people are diagnosed and less than 1% are treated. Compared to other parasitic diseases, CD is the disease that kills more people each year, and even with this unfortunate scenario, its treatment still represents a great challenge ^{377,388,389}.

The treatment of CD is based on only two drugs that have been in clinical use for about 50 years, nifurtimox and benznidazole. Both drugs have low efficacy in the treatment of the chronic phase, are contraindicated during pregnancy and cause serious side effects, which leads to a high rate of treatment abandonment by patients, in addition to the fact that these two drugs are ineffective in the treatment of strains of parasites naturally resistant to both nitroderivatives ^{377,378,390–392}.

These deficiencies in the treatment of CD justify the search for new therapeutic options, one of the strategies being the search for new molecules from natural products ^{98,377,378}.

In this context, the Annonaceae family presents a great structural diversity of secondary metabolites present in its species, a fact resulting from its biogenetic diversity, in addition to presenting a great variety of biological activity. Therefore, it is an excellent family to search for new active substances against *T. cruzi*.

Table 7 describes some species of Annonaceae that have been studied for their trypanocidal activity against different parasitic forms of *T. cruzi* (epimastigote, trypomastigote and amastigote).

Several studies report the trypanocidal activity of extracts, essential oils and substances isolated from Annonaceae species. A study by Lima et al., 2014 ³⁰¹, evaluated the trypanocidal profile of acetogenins isolated from *Annona cornifolia* seeds. Isolated acetogenins (folianin A, squamocin M, annofolin and annotacin) as well as mixtures of two acetogenins (Glaucanisin + Parviflorin; and Glaucanisin + glaucanetin) show activity against amastigote and trypomastigote forms of *T. cruzi* more than 10 times more potent than positive control Benznidazole. However, despite the high trypanocidal activity, the authors observed that acetogenins also have high toxicity, with no selective toxicity to the parasites being observed.

Costa et al., 2011³⁹³, investigated the trypanocidal activity of oxoaporphine and pyrimidine β -carboline alkaloids that they isolated from branches of *Annona foetida* Mart. The alkaloids liriodenine, *O*-methylnoschatoline, atherospermidine and anomontine were evaluated for their trypanocidal activity against the epimastigote and trypomastigote forms of *T. cruzi*. The alkaloids are active against both parasitic forms, but were more active against the epimastigote form, with *O*-methylnoschatoline being the most active among them ($IC_{50} = 11.82 \mu M$). All alkaloids were more active against epimastigote when compared to the positive control Crystal Violet ($IC_{50} = 31.37 \mu M$), and a dose-dependent effect was also observed.

Alkaloids isolated from stem bark of *Duguetia furfuraceae* and also the alkaloid extract were investigated for trypanocidal activity by Silva et al., 2009³⁰⁰, against the trypomastigote forms of *T. cruzi*. The three alkaloids (Duguetine, Duguetine β -*N*-oxide and dicentrinone) and the alkaloid extract are shown to be active, with the duguetine alkaloid being the most active ($IC_{50} = 11.82 \mu M$) among them and stronger than the positive control Gentian violet ($IC_{50} = 31 \mu M$).

Bay et al., 2019 carried out a study to evaluate the chemical composition and trypanocidal and antimicrobial activity of four species of Annonaceae: *Bocageopsis multiflora* (MART.) REFR., *Duguetia quitarensis* BENTH., *Fusaea longifolia* (AUBL.) SAFF., and *Guatteria punctata* (AUBL.) RAHOWARD. The methodology used by the authors in the trypanocidal activity simultaneously evaluates the activity against trypomastigote and amastigote. The four essential oils showed trypanocidal activity, being the essential oil of *G. punctata* the most active ($IC_{50} = 0.029 \mu M/ml$) and selectivity index (SI) = 32, 34 times more active than the control drug used, benznidazole. The authors also report that the strong activity in the essential oil of *G. punctata* can be attributed to the presence of (*E*)-caryophyllene and germacrene D. Published studies show that EOs with high concentrations of these compounds have antiprotozoal properties, especially against *T. cruzi*^{392,394}. But one must also consider the synergistic action of the substances present in the essential oil.

With this result, another species of Annonaceae has trypanocidal activity, which once again reveals the potential of Annonaceae against *T. cruzi*.

Table 7: Summary of trypanocidal activity of species of the Annonaceae.

Plant	Used material	Extracts/ Compounds	Parasite form	<i>T. cruzi</i>		Ref.
				IC ₅₀ μM	IC ₅₀ μg/ml	SI
<i>Annona cornifolia</i>	Seed	4-Desoxylongimicin B + Folianin A	Amastigote and Trypomastigote	0.12		1
		Squamocin M		0.1		1
		Annofolin		0.11		1
		Annotacin		1.7		1
		Glaucanisin + Parviflorin		0.13		1
		Glaucanisin + Glaucanetin		1.7		1
		Benznidazole (Positive Control)		3.8		625
<i>Isolona hexaloba</i>		Aqueous Extract			30.05	>2.13
		Dried crude extract			33.71	>1.90
		80% crude ethanol extract			8.33	3.92

	Dichloromethane fraction	Not determined		
	rich in alkaloids		32.79	>1.95
	Alkaline aqueous fraction			
	rich in salts and			
	hydrophilic substances			
Leaves	Petroleum ether fraction		>64.00	-
	rich in lipids and waxes			
	90% methanol fraction			
	rich in steroids and		8.50	2.52
	terpenes			
	Aqueous Extract		16.54	>3.87
	Dried crude extract			
	80% crude ethanol extract			
	Dichloromethane fraction			296
	rich in alkaloids		33.07	>1.94
	Alkaline aqueous fraction		21.30	>3.00
	rich in salts and		34.56	>1.85
	hydrophilic substances			
	Petroleum ether fraction		10.34	4.11
	rich in lipids and waxes			
Root bark	90% methanol fraction		>64.00	-
	rich in steroids and			
	terpenes			

		21.71	1.59
	Aqueous Extract		
	Dried crude extract		
	80% crude ethanol extract	20.71	>3.09
	Dichloromethane fraction		
	rich in alkaloids		
	Alkaline aqueous fraction		
	rich in salts and	-	-
	hydrophilic substances	15.06	>4.25
	Petroleum ether fraction	34.27	>1.87
	rich in lipids and waxes		
	90% methanol fraction		
	rich in steroids and	17.55	>3.65
	terpenes		
		>64.00	-
	Benznidazole		
	(Positive control)		
Stem bark	Suramine	>64.00	-
	(Positive control)		
		45.02	>1.42

				3.19		
				-		
<i>Annona muricata</i> L.	Leaves	Hexane extract		100.0	294	
		Ethyl acetate extract		25.0		
				40.2 ±11	395	
		Methanol extract	epimastigote	>100.0	294	
	Stems	Hexane extract		74.9		
		Ethyl acetate extract		63.2		
		Methanol extract		98.6		
		Benznidazole		2.0		
		(Positive control)				
	<i>Desmos panamensis</i> (B.L. Rob) Saff.	Leaves	Hexane extract		61.4	294
				10.7 ±3.5	1.77	395
Ethyl acetate extract				85.6		294
				26 ±13.6	1.02	395
Methanol extract				98.6		294
			Epimastigote	11.9 ±5.2	3.36	395
Stems		Hexane extract		98.6		294
				17.7 ±6.4	1.35	395
		Ethyl acetate extract		>100.0		294

<i>Pseudomalmea boyacana</i> (J.F. Macbr.) L. W. Chatrou.	Leaves	Methanol extract Benznidazole (Positive control)	Epimastigote	14.5 ±2.7	2.4	395
				87.5		294
				18.2 ±3.6	2.86	395
				2.0		
	Stems	Hexane extract Ethyl acetate extract Methanol extract Benznidazole (Positive control)	Epimastigote	74.0		294
				89.2		
				100.0		
				15.2 ±3.6	1.19	395
				50.4		294
<i>Rollinia exsucca</i> (DC. Ex Dunal) A. DC.	Leaves	Hexane extract Ethyl acetate extract Methanol extract Benznidazole (Positive control)	Epimastigote	100.0		
				12.8 ±1.3		395
				2.0		
	Stems	Hexane extract Ethyl acetate extract Methanol extract Benznidazole (Positive control)	Epimastigote	74.4		294
				98.6		
				61.4		
				26.1		
				18.1 ±7.2		395

		Ethyl acetate extract		58.3	294	
				41.7 ±12	395	
		Methanol extract		>100.0	294	
		Benznidazole (Positive control)		2.0		
<i>Rollinia pittieri</i> Saff	Leaves	Hexane extract		16.5	294	
				46.4 ±3.1	395	
		Ethyl acetate extract		20.8	294	
			Methanol extract	Epimastigote	39.8	
	Stems	Hexane extract		16.4		
		Ethyl acetate extract		20.8		
		Methanol extract		>100.0		
		Benznidazole (Positive control)		2.0		
	<i>Xylopia aromatica</i> (Lam.) Mart.	Leaves	Hexane extract		99.2	
Ethyl acetate extract				66.0		
Methanol extract				26.1		
				Epimastigote		294
Stems		Hexane extract		>100.0		
		Ethyl acetate extract		58.3		
		Methanol extract		>100.0		

		Benznidazole		2.0	
		(Positive control)			
<i>Guatteria boliviana</i>	Stem bark	Lanuginosine		>818.8	396
		Pangkorimine		203.32	
		Funiferine		47.69	
		Tiliageine	Trypomastigote	287.66	
		Antioquine		77.87	
		Puertogaline A		242.26	
		Puertogaline B		76.13	
		Sepeerine		131.32	
		Guatteboline		100.05	
<i>Bocageopsis multiflora</i> (MART.)	Aerial parts	Essential oil	Trypomastigote and Amastigote	0.46 ± 0.07	2.9
REFR.					278
		Benznidazole		1	625
		(Positive control)			
<i>Duquetia quitarensis</i>	Aerial parts	Essential oil	Trypomastigote and Amastigote	0.26 ± 0.06	2.1
BENTH.					278
		Benznidazole		1	625
		(Positive control)			
<i>Fusaea longifolia</i> (AUBL.)	Aerial parts	Essential oil	Trypomastigote and Amastigote	0.3 ± 0.11	3.1
SAFF					

						278
Benznidazole (Positive control)				1	625	
<i>Guatteria punctata</i> (AUBL.) RAHOWARD.	Aerial parts	Essential oil	Trypomastigote and Amastigote	0.029 ± 0.014	32	278
Benznidazole (Positive control)				1	625	
<i>Annona amazonica</i>	Stem bark	Acanthoic acid	Epimastigote	59	5.9	397
Benznidazole (Positive control)				7		
<i>Guatteria xf.</i> <i>tonduzii</i>	Leaves Stem	Hexane extract Hexane extract	Epimastigote	34.0 ±17 25.2 ±5.2	0.77 1.09	395
<i>Annona coriaceae</i>	Leaves	Essential oil	Trypomastigotes	168.5		305
Benznidazole (Positive control)				45.02		
<i>Annona foetida</i>	Stem	Liriodenine O-methylmoschatoline Annomontine	Epimastigote and Trypomastigote	645.2 / 14.53 286.3 / 11.82 757.8 / 16.07		
Benznidazole (Positive control)				7.6 / -		393

		Crystal violet			- / 31.37	
		(Positive control)				
<i>Piptostigma preussi</i>	-	Polycarpol	Trypomastigotas	5.114		398
	Branches	Ethanollic extract		250.2	0.21	399
	Leaves	Ethanollic extract	Epimastigote	157.9	2.11	
		Glaucine		129.4 / 80.4		
		Oxoglaucine + liriodenine		132.4 / not active		
<i>Duquetia lanceolata</i>		Oxoglaucine + lanuginosine + dehydroglaucine	Trypomastigote / Amastigote	>99.0 / not active		310
		Norglaucine + Isocorydine + N-methylaurotetanine		>97.6 / not active		
		Benznidazole		17.67 / 4.99	11.77	
		(Positive control)			(epimastigote)	
<i>Guatteria elliptica</i>		Ethanollic extract	Epimastigote	345.1	0.30	399
	Leaves	Benznidazole		11.77		
		(Positive control)				

<i>Xylopi</i>	Fruits	Ethanol	Epimastigote	253.1	0.39	399
		Benznidazole		11.77		
		(Positive control)				
	Root wood	Hexanic extract	Amastigote	21.6 ±6.0		304
	Root bark	Hexanic extract		23.5 ±4.7		
		Benznidazole		1.0 ±0.1		
		(Positive control)				
<i>Xylopi frutescens</i>	Leaves	Essential oil	Epimastigote / Trypomastigote	20.2 / 11.9		394
		Benznidazole		2.8 / 2.8		
		(Positive control)				
<i>Xylopi laevigata</i>	Leaves	Essential oil	Epimastigote / Trypomastigote	22.2 / 12.7		394
		Benznidazole		2.8 / 2.8		
		(Positive control)				
<i>Annona crassiflora</i>	Stem bark	Ethanol		14.9 ±2.3		304
	Stem wood	Ethanol		20.5 ±1.1		
		Hexanic extract		45.9 ±3.1		
	Root Bark	Ethanol	Amastigote	5.9 ±1.3		

	Root wood	Hexanic extract		18.6 ±6.8	
		Ethanollic extract		9.9 ±0.5	
		Benznidazole (Positive control)		1.0 ±0.1	
<i>Cardiopetalum calophyllum</i>	Stem bark	Hexanic extract	Amastigote	60.4 ±0.1	304
		Benznidazole (Positive control)		1.0 ±0.1	
<i>Duguetia furfuracea</i>	Stem	Hexanic extract		50.0 ±1.6	304
	Root bark	Ethanollic extract		30.4 ±1.3	
		Hexanic extract	Amastigote	6.6 ±0.6	
	Root wood	Ethanollic extract		25.6 ±1.5	
		Benznidazole (Positive control)		1.0 ±0.1	
		Duguetine	Trypomastigote	9.32	300
	Stem bark	Duguetine β-N-oxide		30.79	
		Dicentrinone		18.83	

		N-methyltetrahydropalmitine		9072	
		N-methylglucine Alkaloid extract		4957	22.44
		Gentian violet (Positive control)		31	
<i>Xylopi</i>	Leaves	Hexanic extract	Amastigote	57.6 ±2.4	304
		Benznidazole (Positive control)		1.0 ±0.1	
<i>Guatteria</i>	Leaves	Essential oil	Epimastigote / Trypomastigote	11.9	400
		Benznidazole (Positive control)		2.7	
<i>Guatteria</i>	Leaves	Essential oil	Epimastigote / Trypomastigote	28.0	400
<i>pogonopus</i>		Benznidazole (Positive control)		2.7	
<i>Porcelia</i>		Stearolic acid		27.6	>7.2
<i>macrocarpa</i>		Santalbic acid		59.9	>3.3
	Flours	8-hydroxyoctadec-9,11-diynoic		57.3	>3.5
					401

		Isanolic acid	Trypomastigote	Not active	-	
	Seed	12,14-octadecadiynoic acid/macrocarpic acid		38,77	4.1	402
		Benznidazole		16.4	>12.2	401
		(Positive control)		534.2	0.9	402
		Crude ethanol extract		34.27	>1.87	
		Dichloromethane fraction rich in alkaloids		14.79	2.34	
<i>Greenwayodendron suaveolens</i> (Engl. & Diels)	Fruits	Alkaline aqueous rich in salts e hydrophilic substances		>64.0	-	
Verdc. (sin. <i>Polyalthia suaveolens</i> Engl. & Diels)		Petroleum ether fraction rich in lipids and waxes		7.49	4.07	
		90% methanol fraction rich in steroids and terpenes		3.58	>17.88	
		Crude ethanol extract		27.86	>2.30	
		Dichloromethane fraction rich in alkaloids		31.17	>2.05	
				>64.0	-	
	Leaves		Not determined			303

	Alkaline aqueous rich in salts e hydrophilic substances	8.33	3.84
	Petroleum ether fraction rich in lipids and waxes	8.06	4.62
	90% methanol fraction rich in steroids and terpenes	7.38	1.04
		0.25	>256.0
	Crude ethanol extract		
Root bark	Dichloromethane fraction rich in alkaloids	>64.0	-
	Alkaline aqueous rich in salts e hydrophilic substances	7.88	2.90
	Petroleum ether fraction rich in lipids and waxes	2.11	13.92
	90% methanol fraction rich in steroids and terpenes	2.0	17.94
		28.28	>2.26
	Crude ethanol extract		
Stem bark	Dichloromethane fraction rich in alkaloids	>64.0	-

		Alkaline aqueous rich in salts e hydrophilic substances	2.0	8.66	
		Petroleum ether fraction rich in lipids and waxes	2.05	3.64	
		90% methanol fraction rich in steroids and terpenes			
<i>Oxandra sessiflora</i>	Stem	4 α ,10 β -aromadendronediol	23.7 $\pm$ 3.8		
		4 β ,10 α -aromadendronediol	31.7 $\pm$ 6.9		
		4 α ,10 α -aromadendranediol	29.8 $\pm$ 8.3		
		1 β ,6 α -dihydroxy-4(15)-eudesmeno	47.5 $\pm$ 5.8		378
	Trypomastigote	4 β ,10 α -dihydroxy-guai-6-eno	16.3 $\pm$ 4.7		
		4 β ,6 β ,7 β ,10 α -tetrahydroxy-guaiane	17.6 $\pm$ 2.3		
		Benznidazole (Positive control)	16.4 $\pm$ 0.8		

<i>Annona vepretorum</i>	Leaves	Essential oil	Trypomastigote	11.2	403
			Epimastigote	16.2	
		Benznidazole		2.7	
		(Positive control)			
<i>Annona squamosa</i>	Leaves	Essential oil	Trypomastigote	12.7	403
			Epimastigote	14.9	
		Benznidazole		2.7	
		(Positive control)			

3.8 Antioxidant

Antioxidant compounds have the ability to interfere with oxidation chain reactions that result in the production of toxic compounds, both in the initiation or propagation of these reactions, slowing, neutralizing or preventing actions of oxidizable substrates, and can also inhibit lipid peroxidation and lipoxygenase ^{404–406}.

The action of antioxidant compounds occurs through one or more mechanisms, such as the inhibition of free radicals and metal complexation ^{407,408}. Free radicals can be acquired exogenously and are also produced endogenously, and when in excess they can cause oxidative stress, cell damage that can culminate in the appearance of cardiovascular and neurological diseases, some cancers, cataracts, diabetes, rheumatism, aging of the body, etc ^{409–412}. Thus, antioxidants bring great benefits to human health, increasing their quality of life, since they protect the body from damage caused by free radicals. ⁴¹³.

Antioxidants can be synthetic or natural. The synthetic ones have greater stability and good efficiency when compared to the natural ones, but the natural ones have less toxicity, being therefore the most sought after in addition to having a greater effect on the oxidation processes than the synthetic ones ^{406,408,411}.

In the search for natural antioxidants, the Annonaceae can be a great source, due to the great chemical diversity of this family. Thus, some researchers have analyzed the antioxidant potential of Annonaceae species.

Karioti et al., 2004 ⁴¹⁴, conducted a study with essential oils from leaves, stem bark, roots and fruits of *Xylopia aethiopica*, belonging to the Annonaceae, in order to analyze the antioxidant potential of these oils as well as the major components of each of the essential oils of *X. aethiopica*. The essential oil of the leaves was the one that obtained the best antioxidant potential in the elimination of free radicals with a percentage of activity greater than 86%.

Konan et al., 2009 ⁴¹⁵, also analyzed the essential oils of dried fruits of *X. aethiopica* leaves for antioxidant activity as well as the chemical composition obtained. The authors observed that the nut oil was more potent in terms of antioxidant activity than the essential oil of the leaves, with an IC₅₀ of 4.1 and 4.9 mg/mL, respectively. Unlike Karioti et al., 2004 ⁴¹⁴, Konan and collaborators ⁴¹⁵ observed that the essential oil of the fruits had the highest antioxidant activity.

Silva et al., 2009 ⁴¹⁶, developed a phytochemical investigation of the leaves and stem bark of *Xylopia langsdorffiana*, Annonaceae. The authors were able to isolate seven

compounds, four alkaloids, corytenchine, xylopinine, xylopine and discretamine, a diterpene, ent-atisan-16 α -hydroxy-18-oic acid, a phaephorbide, 132(S)hydroxy-173-ethoxyphaephorbide, and a flavonoid. After isolation, the authors submitted the substances to the antioxidant activity test using the 1,2-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay and observed that the alkaloid discretamine obtained excellent results with a percentage of activity greater than 90%, besides that they could conclude that the antioxidant effect depends on the concentration.

A study by Sacchetti e colaboradores, 2004 ²⁶⁶, analyzes eleven essential oils and among them the oil of *Cananga odorata*, a species belonging to the Annonaceae, regarding the properties related to the functional ingredients of foods, especially for the antioxidant potential of these oils in order to find possible substitutes for synthetic preservatives. Among the essential oils analyzed in the DPPH test, *C. odorata* oil proved to be one of the most effective with an activity percentage greater than 75%.

Silva et al., 2019 ¹²², analyzed the biological potential of the species *Annona nutans*, Annonaceae. One of the activities analyzed was the antioxidant potential, assessed by eliminating the DPPH radical. The hydromethanolic fraction of *A. nutans* extract showed dose-dependent antioxidant activity, with an IC₅₀ of 4.89 μ g/mL that was comparable to the commercial antioxidant BHT.

Another study on the antioxidant potential of species of Annonaceae, Jain et al., 2014 ²⁷⁰, analyzed the species *Polyalthia longifolia*. The authors isolated the 3-O-methyl Ellagic Acid compound and then evaluated its antioxidant and antibacterial potential. The antioxidant activity was evaluated by the DPPH method and obtained an IC₅₀ de 24.28 μ g/ml.

Xavier et al., 2016 ⁴¹⁷, carried out a study in order to characterize the chemical composition of the essential oil of the leaves of *Cardiopetalum calophyllum*, Annonaceae, as well as to evaluate the antioxidant, antimicrobial and antifungal potential of this oil. The authors obtained an IC₅₀ of 9.66 μ g/ml for antioxidant activity by the DPPH method, and when compared to the quercetin standard (IC₅₀ = 3.13 μ g/ml), the low antioxidant potential of the oil of *C. calophyllum* leaves was proven.

Santos et al., 2018 ¹²⁶, analyzed the methanolic extract of *Duguetia furfuracea*, Annonaceae, and its fractions to evaluate the antioxidant and anti-inflammatory potential of this species. Since tea and syrups of this kind are used in folk medicine to treat rheumatism and back pain, and rheumatic diseases are known to be anti-inflammatory. In

the DPPH antioxidant assay, the methanolic extract exhibited the greatest potential with an IC₅₀ de 22.46 µg/mL.

These studies showed that the species of Annonaceae have good antioxidant potential and can be analyzed in the search for new antioxidants from natural sources. Table 8 summarizes the results presented in that section.

Table 8: Summary of antioxidant activity of species of the Annonaceae.

Plant	Used material	Extracts / Compound	IC ₅₀	%Interaction	DPPH			Ref.
					%Interaction 20 min	%Interaction 60 min	%Free radical scavenging	
<i>Xylopia aethiopica</i>	Essential oil of leaf, stem bark, roots and fruits	Leaf oil	0.048 g/mL	-	43.8	75.9	86.8	414
		Leaf oil	4.9 mg/mL	50.0				415
		Root bark oil	0.033 g/mL	-	36.5	43.2	68.4	414
		Stem bark oil	0.037 g/mL	-	32.4	40.3	73.9	414
		Fresh fruit oil	0.045 g/mL	-	85.6	85.8	66.5	414
		Dried fruit oil	0.042 g/mL	-	21.2	54.8	57.2	414
		Dried fruit oil	4.1 mg/mL	50.0				415
<i>Xylopia langsdorffiana</i>	Leaf and stem bark	Concentrations Tested						
			240 µg/mL	94.25				
		Discretamine	120 µg/mL	93.93	-	-	-	416
			60 µg/mL	91.51				
			30 µg/mL	90.05				
<i>Cananga Odorata</i>	Essential oil	Essential oil		75.50	-	-	63.80	266
<i>Annona nutans</i>	Leaves	Hydromethanolic fraction	4.89 µg/mL				50.0	122
<i>Polyalthia longifolia</i>	Stem bark	3-O-methyl Ellagic Acid	2.5 µg/mL				10.36	
			5 µg/mL				28.17	
			10 µg/mL				34.83	
			20 µg/mL	-	-	-	45.80	270
			40 µg/mL				69.81	
			24.28 µg / ml				50.00	
	Seed	Extract	0.36					

<i>Annona squamosa</i>	Pulp	Extract	0.83						57
		Vitamin C (positive control)	0.011	-	-	-	-		
<i>Sphaerocoryne affinis</i>	Fruit	Rumdul fruit water extract	85.62 µg/mL	-	-	-			418
<i>Cardiopetalum calophyllum</i>	Leaf	Essential oil	9.66 µg/mL	-	-	-	-		417
		Quercetin (standard compound)	3.13 µg/mL	-	-	-	-		
<i>Duguetia furfuracea</i>	Leaves	Methanolic extract	22.46 µg/mL	50.0					126
		Chloroform fraction	176.88 µg/mL	50.0					
		Ethyl acetate fraction	60.56 µg/mL	50.0					
		Hydromethanol fraction	28.21 µg/mL	50.0					

3.9 Antimalarials

Malaria is an endemic disease in tropical and subtropical areas, occurring mainly in Southeast Asia, the Amazon and Africa ^{419,420}. Despite several attempts to control and eradicate the disease, it is estimated that in 2018 there were 228 million cases, and in 2019 the global mortality rate from malaria can vary by up to 30% in the severe form of the disease. Therefore, malaria is considered a public health problem ^{420–423}.

The etiological agent of malaria is a protozoan belonging to the genus *Plasmodium* and some species have the ability to cause disease in humans ^{421,424,425}. This protozoan is an intracellular parasite and can infect red blood cells and some tissues, depending on the species. There are five species that can infect humans, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malarie* e *P. knowlesi* ^{420–422,426}.

The mosquito and parasite developed resistance to some drugs used for their control and eradication, which ended up not making it possible to eradicate this disease from the world, in addition to administrative problems ^{421,427}. Thus, research for new drugs and insecticides is still important, as well as studies with the vaccine.

Plants are always an excellent source of new drugs, so the Annonaceae, due to its chemical diversity, can be interesting in the study of new antimalarial drugs. In this sense, several researchers have already analyzed the antimalarial potential of several species of Annonaceae, the results are described below.

Asase et al., 2010⁴²⁷, studied 30 species of plants, belonging to 28 genera and 20 families, used by indigenous communities in the western district of Dangme, Ghana, in the treatment of malaria. Five species were described for the first time in the treatment of malaria, among them the species *Greenwayodendron* sp. of the Annonaceae. The authors report that the use of the leaves of *Greenwayodendron* sp. it must be made from the boiling of the leaves and indicate one cup three times a day for adults and half a cup for children until complete recovery.

Another study by Kanokmedhakul et al., 2003⁴²⁸, evaluated the phytochemical and pharmacological profile of roots of the species *Polyalthia debilis*, of the Annonaceae. The authors isolated four dimeric aporphinoids alkaloids called bidebiline A-D (1-4). Then, they performed pharmacological analysis of the extract and alkaloids for antimalarial activity. The dichloromethane extract from the roots showed activity against *P. falciparum*, IC₅₀ 1.35 µg / mL. Among the four isolated alkaloids, and alkaloids 3 and 4 (bidebiline C and D - bis-7,7'-dehydro-8,8'-dimethoxyanonaine and 7,7'-dehydro-10,10'-dimethoxyanonaine, respectively) showed moderate activity against *P. falciparum* with an IC₅₀ of 5.4 and 4.1 µg / mL respectively.

Boyom et al., 2003⁴²⁹, evaluated the antimalarial potential of five species of Cameroon, four species belonging to the Annonaceae: *Xylopia phloiodara*, *Pachypodanthium confine*, *Xylopia aethiopica* e *Hexalobus crispiflorus*. The study was carried out using essential oil obtained from stem bark. The four species of Annonaceae obtained some degree of activity against *P. falciparum*, with *Hexalobus crispiflorus* being the most promising with an IC₅₀ of 2.0 µg/mL. *Xylopia aethiopica*, *X. phloiodora* and *P. confine* obtained IC₅₀ of 17.8, 17.9 and 16.6 µg/mL, respectively.

In a more recent study, Boyom et al., 2011⁴³⁰, evaluated the antiplasmodial activity of essential oils of two species of Annonaceae, *Cleistopholis patens* and *Uvariastrum pierreanum*. The oils were collected from stem bark and leaves and evaluated in vitro against *P. falciparum*. For the species *C. patens*, the essential oil of the leaves showed activity with IC₅₀ 15.19 µg / mL and 9.19 for the oil of the leaves. The species *U. pierreanum* also showed a moderate activity for *P. falciparum* with an IC₅₀ of 6.08 and 13.96 µg / mL, for stem bark and leaf oils. These results indicate that essential

oils may offer a promising alternative for the development of new antimalarials. The authors also carried out the determination of the chemical profile of the essential oils of both species and for *C. patens*, the main components were α -copaene, δ -cadinene and germacrene D for shell oil and β -karyophyllene, germacrene D and germacrene B for leaf oil. *U. pierreanum* stem bark oil was found to contain mainly β -bisabolene and α -bisabolol, while α and β -pinenes were more abundant in leaf oil.

Osorio et al., 2006⁴³¹, analyzed alkaloids isolated from two species of the Annonaceae, *Rollinia pittieri* and *Pseudomalmea boycana*, and investigated the antiplasmodial effect of these molecules as well as the ability to inhibit β -hematin, a synthetic substance and identical to hemozine, a pigment⁴³¹. From the alkaloid fraction of *R. pittieri*, the alkaloids O-methylmoschatoline and melosmin were isolated, and from the alkaloid fraction of *P. boycana* lyriodenine, atherospermidine and isomoschatoline were isolated. The authors found that among the alkaloids investigated, lyriodenine exhibited the highest activity both against strains sensitive to chloroquine and resistant strains of *P. falciparum* with $IC_{50} = 8.0-10.0 \mu\text{g} / \text{mL}$. And in the assessment of β -hematin inhibition, the alkaloids O-methylmoschatoline and melosmin showed inhibition greater than 96% at a concentration of 2 mg/mL.

In a study by Kouam et al., 2014⁴³², six substances isolated from the stem bark of *Polyalthia oliveri*, 8a-polyvinolinone, N-acetyl-8a-polyvinolinone, N-acetyl-polyveoline, dehydro-O-methylisopiline, were investigated. N-methylurabaine and polycarpol. The authors found that the indolosesquiterpene alkaloids, N-acetyl-8a-polyvinolinone and N-acetyl-polyveoline exhibited moderate activity against erythrocyte stages of the *P. falciparum* strain NF54 (sensitive to chloroquine).

Gbedema et al., 2015⁴³³, carried out a biomonitoring study of the ethanolic extract of the shells of *Polyalthia longifolia* against multi-resistant strains (k1) of *P. falciparum*. The extract was found to be active and was then subjected to chromatographic analysis for the isolation of active compounds. The authors isolated three clerodane diterpenes, 16-hydroxycleroda-3,13-dien-16,15-olide (1), 16-oxocleroda-3,13E-dien-15-oic acid (2) and 3,16-dihydroxycleroda-4(18), 13(14)Z-dien-15,16-olide (3), a steroid, β -stigmaterol (4) and two alkaloids, darienin (5) and stefolidin (6). The authors subjected the isolated compounds to antiplasmodial tests against the k1 strain and found that while compounds 4, 5 and 6 had weak antiplasmodic activity (IC_{50} ranging from 22 to 105 $\mu\text{g}/\text{mL}$), the clerodane diterpenes were quite potent in antiplasmodic activity with IC_{50} between 3-6 $\mu\text{g}/\text{mL}$. The authors also carried out a study combining the isolated molecules with

chloroquine for analysis of synergistic or antagonistic activity and found that compounds 1, 2, 3 and 5 antagonized the antiplasmodic activity of chloroquine and compounds 4 and 6 had synergistic activity with chloroquine.

Malebo et al., 2013⁴³⁴, performed a biomonitored study of the methanolic extract of the leaves of *Annikia kummeriae*. The authors initially evaluated the antiplasmodial potential of the methanolic extract and observed a strong activity against the multi-resistant strain of *P. falciparum* (k1) with an $IC_{50} = 0.12 \mu\text{g/mL}$. Then the authors subjected the extract to chromatography processes to isolate substances and thus obtained four pure alkaloids: lysicamine, trivalvone, palmatine and jatrorizine. The alkaloids were then subjected to an in vitro test to analyze antiplasmodial activity against the *P. falciparum* k1 strain. At the end of the study, the authors concluded that alkaloids isolated from *A. kummeriae* have strong to moderate activity against *P. falciparum* with IC_{50} ranging from 0.08 ± 0.001 - $2.4 \pm 0.642 \mu\text{g/mL}$, and thus show good structures for the development of new antiplasmodial drugs.

The results presented so far regarding the antiplasmodial activity of substances and extracts of species of the Annonaceae are summarized in table 9.

Table 9: Antiplasmodial activity of substances and extracts from species of the Annonaceae.

Plant	Used material	Extracts/ Compounds	<i>P. falciparum</i> (strain) (IC_{50})				Iff-h (%) 2mg/ml	Ref.
			F32 ($\mu\text{g/ml}$)	W2 ($\mu\text{g/ml}$)	K1 ($\mu\text{g/ml}$)	NF54 (μM)		
<i>Polyalthia debilis</i>	Roots	dichloromethane extract from the roots	-	-	1.35	-	-	435
		Bidebiline C			5.4			
		Bidebiline D			4.1			
<i>Cleistopholis patens</i>	Steam bark			9.19 (SB) 15.19 (L)				
<i>Uvariastrum pierreanum</i>	(SB) and leaves (L)	Essential oil		6.08 (SB) 13.96 (L)				430
			-		-	-	-	

		(-)-(7R,8S)- mitrephentosin C			13.3 µM			
<i>Mitrephora tomentosa</i>	Leaves and twigs	(-)-(7R,8S)- mitrephentosin F			18.7 µM			436
		Cyclogunil (Positive control)			5.87 µM			
<i>Xylopiaphloiodara</i>				17.9				
<i>Pachypodanthium confine</i>	Steam bark	Essential oil		16.6				
<i>Xylopiathaiopica</i>			-	17.8	-	-	-	429
<i>Hexalobus crispiflorus</i>				2.0				
<i>Rollinia pittieri</i>		O- metilmoschatolina	-	-			98.3	
		Melosmina	-	-			96.4	
<i>Pseudomalmea boyacana</i>		Liriodenina	8 – 10	8.0		-	11.4	431
		Atherospermidina	-	-			5.12	
		Isomoschatolina	-	-			2.40	
		Chloroquine	0.01	0.90			97.0	
<i>Polyalthia oliveri</i>	Steam bark	N-acetil-8α- polivolinona				7.6		
		N-acetil-polveolina	-	-	-	29.1	-	432
		Chloroquine				0.006		
		16-hydroxycleroda- 3,13-dien-16,15- olide	-	-	5.33	-	-	
		Acid 16-oxocleroda- 3,13E-dien-15-óico						
					3.05			

<i>Polyalthia longifolia</i>	Steam bark	3,16-dihydroxycleroda-4						
		(18),13 (14) Z-dien-15,16-olida						433
					6.15			
		β -Stigmasterol	-	-		-	-	
		Darienine			63.3			
		L-Stepholidine			22.05			
					104.33			
<i>Annickia kummeriae</i>	Leaves	Extract metanolic			0.12			
		Lysicamine			2.4			
		Trivalvone	-	-	1.6	-	-	434
		Palmatine			0.08			
		Jatrorrhizine			0.24			

K1 - multidrug resistant strain, F32 - chloroquine sensitive strain, W2 - chloroquine resistant strain, If β -h - inhibition of β -hematin formation

3.10 Gastroprotective

Gastric ulcer (GU) is a very common disease of the digestive system, with a high morbidity and has become a major problem for public health ^{437,438}. The cause for the development of a GU can be related to several factors, such as, for example, the indiscriminate use of non-steroidal anti-inflammatory drugs, protective factors of the gastric mucosa (mainly related to the levels of prostaglandins and antioxidant activity), which results rupture of the defensive barrier of the gastric mucosa, culminating then in the appearance of gastric ulcer ⁴³⁸⁻⁴⁴⁰. Another factor that has been proven to cause GU is oxidative stress induced by ethanol, which generates highly cytotoxic free radicals ^{438,441-443}. Other factors that cause GU are stress and infection by *Helicobacter pylori* bacteria ^{444,445}.

Although there are many medications on the market for the treatment of GU, such as antacids, proton pump inhibitors, anticholinergics, cytoprotective agents, etc., the majority in prolonged use cause a series of side effects, such as hypersensitivity, arrhythmia, impotence, gynecomastia, hematopoietic changes, hyper gastrinemia ^{437,444,446}.

Thus, it is necessary to search for new drugs that are less toxic and more effective in the treatment of GU. In this context, plants are an excellent source of new drugs and the Annonaceae stands out for having numerous pharmacological activities, as previously mentioned.

In this sense, some authors have carried out research on some species of Annonaceae in search of new gastroprotective agents. Olate et al., 2012⁴⁴⁷, evaluated the gastroprotective potential of 11 amides derived from the diterpene (4S, 9R, 10R) methyl 18-carboxy-labda-8,13 (E) -dien-15-oate (PMD) and its 8 (9) -en isomer [PMD 8 (9) -en], isolated in the species *Polyalthia macropoda*. The gastric lesion model was induced by ethanol/HCl in mice. In the single oral dose of 0.1 mg/kg, compounds 1, 10 and 11 showed a strong gastroprotective effect, at least comparable to that of the reference lansoprazole by 1 mg/kg, reducing gastric lesions by 76.7, 67.7 and 77.2%, respectively. Compounds 10 and 11 are the most promising because they do not have cytotoxic effects.

Vendramini-Costa et al., 2014⁴⁴⁸, evaluated the gastroprotective activity of the racemic mixture of goniothalamine (GNT), a sterile lactone found naturally in its enantiomeric form in plants of the genus *Goniothalamus* (Annonaceae). Gastroprotective activity was also assessed using the ethanol-induced gastric lesion model. Vendramini-Costa et al., 2014⁴⁴⁸, observed a potent gastroprotective effect of GNT at concentrations of 30 and 60 mg/kg, with ED₅₀ = 18 mg/kg, and that this activity depends on the generation of sulfhydryl and prostaglandin compounds, but is independent of nitric oxide (NO), gastric secretion and mucus production. This hypothesis that GNT acts as a mild irritant inducing the production of sulfhydryl and prostaglandin compounds is a process known as adaptive cytoprotection. This hypothesis of mechanism of action suggested by the authors was supported by the fact that Michael acceptors (GNT is a Michael acceptor) are more potent inducers of antioxidant responses through the generation of mild oxidative stress and saw that the gastroprotective activity of GNT was inhibited after pretreatment with N-ethylmaleimide and NSAID, thus highlighting the importance of sulfhydryl and prostaglandin compounds in the activity of GNT.

Moghadamtousi et al., 2014⁴⁴⁹, analyzed the gastroprotective effects of ethyl acetate extract from *Anona muricata* leaves (EEAM) using the ethanol-induced gastric lesion model in rats. The study was carried out using two doses of EMEA, one of 200 mg/kg and the other of 400 mg/kg, and the anti-ulcer medication omeprazole (20mg/kg) was used as a control. There was a significant suppression in the ulcer injury rate of rats pretreated with oral administration of EEAM, which was comparable to the effect of

omeprazole in the control group, with the dose of 400 mg/kg of EEAM being the one that obtained the best results. The authors also concluded that the potential and promising gastroprotective effect of EEAM can be attributed to its suppressive effect against oxidative damage and conservative effects on mucus in the gastric wall.

Montenegro et al., 2014 ⁴⁴⁴, selected the species *Xylopi langsdorffiana* A. St.-Hil. & Tul., to investigate the capacity of gastroprotective effect of the ethanolic extract (EtOHE) and the hexane phase (HexPh) obtained from the leaves of *X. langsdorffiana* (XL). They carried out a dense research in order not only to define the gastroprotective activity of EtOHE and HexPh, but also to elucidate the mechanism of action. The authors started the study by analyzing the gastroprotective activity in the ethanol-induced gastric lesion model and observed that the XL-EtOHE extract and the XL-HexPh phase significantly inhibited the ulcers when compared to the negative controls, suggesting high gastroprotective activity with doses of 500 mg/kg for XL-EtOH and 250 mg/kg for XL-HexPh being the most effective. After this result, the authors carried out several experiments to identify the mechanism of action of the extract and the phase of *X. langsdorffiana*. At the end of the study, the authors were able to conclude that gastroprotective activity is performed involving the action of sulfhydryl and NO groups, and the anti-secretory mechanism and increased mucus production are not involved. The authors also performed a phytochemical analysis of the extract and hexane phase of *X. langsdorffiana* and identified four diterpenes as major components, which suggests that these substances may be responsible for the gastroprotective effect of *X. langsdorffiana*.

Through these studies the gastroprotective activity of species of the Annonaceae is proven, where two of the studies identified this activity being responsible for the class of secondary metabolites the diterpenes, and a study carried out with the GNT a sterile lactone. Table 10 shows the substances tested in each study as well as their respective gastroprotective potential.

Table 10: Summary of the gastroprotective activity of Annonaceae species.

Plant	Used material	Substances	Methodology	Dose	Ulcer lesion index	%Lesion reduction (gastroprotection)	Ref.
<i>Polyalthia macropoda</i>	Stem bark	PMD	Ethanol induced ulcer model	1 mg/kg	20.4	68.3	447
				5 mg/kg	17.7	72.5	
		Amides from PMD		10 mg/kg	9.4	85.4	

		1		0.1 mg/kg	10.4	76.7	
		10		0.1 mg/kg	20.8	67.7	
		11		0.1 mg/kg	14.7	77.2	
		Control					
		Lansoprazole		1 mg/kg	13.8	78.6	
<i>Goniothalamus</i> <i>sp.</i>	-	Rac-GNT	Ethanol induced ulcer model	15 mg/kg		41.7	448
				30 mg/kg		70.5	
				60 mg/kg	-	86.7	
				18 mg/kg (ED ₅₀)		50.0	
		Control Carbenoxolone 200		200mg/kg		93.5	
<i>Annona muricata</i>	Leaves	EEAM	Ethanol induced ulcer model	200 mg/kg	39.0		449
				400 mg/kg	23.0	-	
		Control Omeprazole		20 mg/kg	9.0		
<i>Xylopi</i> <i>langsдорffiana</i>	Leaves	EtOHE	Ethanol induced ulcer model	500 mg/kg		83	444
		HexPh		250 mg/kg	-	81	
				500 mg/kg		84	
		Control Carbenoxolone		100 mg/kg		40	

Perspectives

Studies related to natural products are extremely important because they are sources of secondary metabolites that have different biological activities and applications for humans. In this review, the exciting potential of the Annonaceae family in terms of biological activities was highlighted, being, therefore, extremely promising. There are several biological activities that Annonaceae perform, but it stands out in the anti-inflammatory, insecticidal, antimicrobial, leishmanicidal, cytotoxic, antitumor, trypanocidal, antioxidant, gastroprotective, antimalarial activities, among others. Unfortunately, most studies focus on the activities of different plant extracts and essential oils, thus having a deficit of studies testing isolated molecules and elucidating the

mechanism of action. The identification of biological activity in isolated compounds is of fundamental importance for the identification of new drugs and pharmaceutical and agricultural production. For studies that have *in vitro* tests with isolated molecules, evidencing the biological potential of these substances, we encourage researchers to delve deeper into *in vivo* and clinical experiments, for the production and commercialization of new drugs. This review also compiles a lot of vital information for the pharmaceutical and agricultural industry.

Acknowledgments

We would like to thank the CNPq and Capes for financial Support.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This research was funded by National Council for Scientific and Technological Development (CNPq) and Coordination of Superior Level Sta Improvement (CAPES).

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The background of the page is white. At the bottom, there is a decorative horizontal band. This band features a network of interconnected nodes and lines, resembling a molecular or network structure, in shades of blue and purple. Interspersed within this band are faint, light gray icons: a speech bubble, a camera, and a magnifying glass. The title 'CAPÍTULO II' is centered within this decorative band.

CAPÍTULO II

Neste capítulo foi realizada uma revisão sobre aprendizado de máquina aplicado a QSAR com intuito de oferecer um artigo científico escrito em português com esse tema que é tão atual e necessário.

A área da descoberta de medicamentos, em especial o QSAR, está constantemente evoluindo e aderindo a novas tecnologias, marcado pelo aumento do uso de mineração de dados, aprendizado de máquina e automação. Neste contexto, é importante que os químicos e farmacêuticos busquem se familiarizar com essas novas abordagens computacionais.

Foi observado que há uma escassez de artigos científicos explicando as técnicas de ML aplicado a QSAR, abordando como construir modelos, os tipos de modelos, algoritmos, para a comunidade científica brasileira.

Desta forma, o objetivo com essa revisão é abordar o assunto de forma simples e didática para estudantes e pesquisadores que estão iniciando nesta área tão promissora e importante. Descreveremos a teoria detalhadamente explicada de aprendizado de máquina aplicado a QSAR abstraindo a complexidade, e bem ilustrada.

Aprendizado de Máquina Aplicado a QSAR

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Artigo em fase de correção

Será submetido na revista Química Nova

Fator de Impacto: 0.961

QUALIS: A4

Abstract: Over the years the study of the quantitative structure-activity relationship (QSAR) has transformed from a simple regression analysis to the implementation of machine learning (ML) with multiple statistics. Today ML-based QSAR models are quite important and play a notable role in drug design and screening, property prediction, biological activity, etc. ML methods applied to QSAR build classification or regression models to describe/predict the complex relationships between the chemical structure of molecules and biological activity. Even with the increase in scientific publications addressing this topic written in Portuguese, there is still a shortage of scientific articles explaining ML techniques applied to QSAR, how to build models, the types of models, algorithms, for the Brazilian scientific community. And to fill this need, we intend to approach the subject in a simple and didactic way for students and researchers who are starting in this very promising and important area. We will describe the fully explained theory of machine learning by applying QSAR, abstracting the complexity, and well-illustrated.

Keywords: Machine Learning; QSAR; Predictive Models; Data Mining; Virtual Screening.

Introdução

A quimioinformática surgiu por volta dos anos 90 e tem se desenvolvido e aperfeiçoado com o passar dos anos e com a evolução da tecnologia. Tem se estabelecido cada vez mais no campo da descoberta de novas drogas, inclusive, uma das principais razões para o seu surgimento foi a necessidade de trabalhar com a imensa quantidade de dados gerados por abordagens para a descoberta de novos medicamentos, como Alta Triagem de Rendimento (High-throughput screening – HTS) e Química Combinatória.^{1,2}

Um dos fundadores da quimioinformática, Frank Brown, a definiu em 1998 como:^{3,4}

“A mistura de recursos de informação para transformar dados em informação e informação em conhecimento, no intuito de tomar decisões melhores e mais rápidas na área de identificação e otimização de compostos líderes.”

Outros pesquisadores, tidos como pilares da quimioinformática, também a definiram ao longo dos anos. J. Gasteiger, a definiu em 2004 como sendo a aplicação de métodos de informática para resolver problemas químicos. E em 2007 Alexander Varneck definiu a quimioinformática como um campo que lida com objetos moleculares (gráficos, vetores) no espaço químico multidimensional.^{1,5,6}

O aperfeiçoamento da quimioinformática tornou possível a exploração de grandes bases de dados químicos na busca e descoberta de novos compostos com potenciais elevados de atividade biológica ou propriedade química desejadas, ou seja, tem sido implementada para a Triagem Virtual (Virtual Screening – VS), acelerando a descoberta de medicamentos.^{1,3,7,8}

Essa ciência tem papel fundamental no desenvolvimento racional de novas drogas antes de iniciar as análises de fase clínica, tornando possível a seleção racional de compostos promissores para seguir os estudos biológicos e clínicos poupando tempo de pesquisa e custos.^{7,9,10}

Uma das técnicas da quimioinformática mais conhecida, utilizada e estudada é a Relação Quantitativa Estrutura-Atividade (Quantitative Structure-Activity Relationship – QSAR), definida como um conjunto de métodos e procedimentos computacionais bastante estabelecida para análise de dados químicos.¹¹ O objetivo principal do QSAR é estabelecer a relação entre a atividade/propriedade/toxicidade molecular entre outros e seus parâmetros estruturais.^{12,13}

Relação Quantitativa Estrutura-Atividade (QSAR)

O QSAR é constantemente definido como a geração de modelos matemáticos estabelecendo relações empíricas, estatisticamente significativas, lineares ou não lineares entre valores de descritores químicos calculados a partir da estrutura molecular e propriedades ou bioatividade dessas moléculas medidas experimentalmente, seguidas da aplicação desses modelos para prever ou projetar novos produtos químicos com propriedades desejadas.^{3,8,11,14-16}

Apesar do relato mais antigo da origem do QSAR nos levar a um estudo sobre a relação estrutura-atividade de compostos orgânicos no século 19^{17,18}, o método proposto por Hansch e Fujita no começo dos anos 60 marcou o início do 2D-QSAR seguido pelo estudo de Free-Wilson.^{17,19-21} O QSAR continuou evoluindo com o tempo, seguindo com o método representativo 3D-QSAR de análise comparativa de campo molecular (CoMFA), proposto e amplamente utilizado no projeto e previsão de novas moléculas.^{17,22} Com a evolução da tecnologia o QSAR 4D, 5D, 6D e 7D foram desenvolvidos no decorrer dos anos.²³⁻²⁵ Até os dias atuais, os métodos de QSAR tem experimentado um progresso enorme, em uma ampla área de pesquisa se estendendo até fora dos limites tradicionais (desenvolvimento de novas drogas), como planejamento de rotas sintéticas, nanotecnologia, ciência de materiais, biomateriais, na indústria de alimentos, clínica informática, entre outros.^{11,17,26}

O método de QSAR tem facilitado e muito a descoberta e desenvolvimento de novas droga sendo bastante eficiente na redução do custo e tempo nesse processo além de aumento na taxa de sucesso e retorno do investimento.²⁷⁻³¹ Uma vez que nenhum composto precisa ser sintetizado ou testado antes da avaliação computacional.

Podemos afirmar que no decorrer dos anos o QSAR se transformou de uma análise de regressão simples, ou seja, que só podia trabalhar com séries de compostos estruturalmente semelhantes, para o implemento da técnica de Aprendizado de Máquina (Machine learning – ML) com estatísticas múltiplas, sendo possível analisar grandes conjuntos de moléculas estruturalmente diversas.³⁰ Sendo amplamente utilizado em indústrias, universidades e centros de pesquisas em todo o mundo.^{14,32}

Atualmente, os modelos de QSAR baseados em ML são bastante importantes e tem desempenhado um papel notável no desenho e triagem de drogas, previsão de propriedade, atividade biológica etc. A aplicação de técnicas de ML em QSAR teve início desde a década de 1990, com o uso de algoritmos como máquina de vetor de suporte (Support Vector

Machine – SVM) e florestas aleatórias (Random Forest – RF) para a descoberta ou projeção de novos medicamentos.³⁰

O ML pode ser definido como o uso de algoritmos de inteligência artificial que podem aprender do seu ambiente.³³ Os métodos de ML aplicado a QSAR constroem modelos de classificação ou de regressão para descrever/prever as relações complexas entre a estrutura química das moléculas e a atividade biológica.³⁴

O ML tem a capacidade de aprender com os dados quais recursos são mais importantes para determinar a relação estrutura-atividade e realizar a predição. Então, na construção dos modelos preditivos é imprescindível ter um banco de dados com todos os recursos/informações possíveis relacionados a finalidade desejada do QSAR e quando aplicado o algoritmo ele irá aprender e extrair aquelas informações mais importantes.

Independente da natureza dos dados, as abordagens de ML podem ser usadas universalmente para analisar e processar dados em qualquer domínio. É importante lembrar que mesmo a ideia geral do ML aplicado ao QSAR seja a mesma, as características de entrada, isto é, os dados utilizados, podem ser diferentes assim como os alvos. Em suma, quando o ML é aplicado ao QSAR gera uma ampla aplicabilidade, que só comprova o QSAR como uma ferramenta de modelagem e análise de dados preditiva e robusta.^{11,30}

Triagem Virtual

A triagem virtual (Virtual Screening – VS), foi um termo criado na década de 1990 com o intuito de descrever a utilização de modelos computacionais e algoritmos para analisar grandes bancos de moléculas de forma automatizada. Seu objetivo é triar e selecionar compostos potencialmente ativos, além de ser possível identificar e remover compostos tóxicos, ou que apresentem propriedades farmacodinâmicas e farmacocinéticas desfavoráveis.^{3,35–37}

Podemos compreender a VS como um método que utiliza uma cascata de filtros sequenciais que irão restringir o espaço químico e selecionar as moléculas mais semelhantes aos compostos líderes e assim apresentarem uma maior probabilidade de potencial ativo.^{7,38}

Teve seu surgimento na mesma década que o QSAR, ambas estão intimamente relacionados e foram implementadas para solucionar alguns problemas do HTS, como a grande quantidade de dados gerados, diminuir o tempo e os gastos da pesquisa. Enquanto no HTS a busca por compostos potencialmente ativos é uma espécie de busca aleatória, na VS o processo além de ser computacional, *in silico*, ele é orientado pelo conhecimento.⁷

A disponibilidade de informação é imprescindível para a realização da VS, e a depender do tipo de informação a VS pode ser realizada através de duas técnicas: Triagem Virtual Baseada na Estrutura do Receptor (Structure-Based Virtual Screening – SBVS) e a Triagem Virtual Baseada na Estrutura do Ligante (Ligand-Based Virtual Screening – LBVS).^{9,39}

Na técnica de SBVS, é necessário o conhecimento tridimensional do alvo biológico, ou seja, da proteína alvo. A partir dessa informação, inicia-se a busca por ligantes que possam interagir no sítio ativo dessa proteína, sendo, portanto, o Docking Molecular, a principal estratégia desta técnica. Essa abordagem permite que o pesquisador aprofunde seus conhecimentos da estrutura 3D do alvo e seu sítio ativo possibilitando estudos de otimização dos compostos para as interações moleculares com a proteína alvo além de otimização de propriedades farmacocinéticas.^{7,9,40}

Na técnica de LBVS não é preciso a informação da estrutura da proteína alvo, o mais importante é ter conhecimento de moléculas que já foram avaliadas experimentalmente quanto a atividade biológica desejada. Explorando o princípio de que moléculas semelhantes apresentam perfil de atividade/propriedade semelhante, a técnica LBVS busca a partir de parâmetros, propriedades físico-químicas, impressões digitais (fingerprint) das moléculas, obter compostos com potencial atividade biológica através de uma análise de QSAR.^{7,9,38,40} Por não precisar do conhecimento da estrutura 3D da proteína alvo, a abordagem LBVS acaba ficando restrita ao espaço químico dos compostos que já foram avaliados experimentalmente utilizados como referência.^{36,41,42}

Neste artigo, focaremos nos modelos computacionais utilizando o ML na técnica de LBVS em QSAR. O processo de criação do modelo computacional com abordagem LBVS em QSAR é um ciclo que pode ser resumido da seguinte maneira:³⁴

- 1- A coleta de dados
- 2- Cálculo de descritores moleculares, descritores de impressão digital, ou outras informações moleculares
- 3- Construção dos modelos
- 4- Verificação e Validação dos modelos
- 5- Aplicação *in vitro*

Esse ciclo tem início com a coleta de dados a partir de fontes externas, no qual serão coletadas bancos de moléculas que já tiveram sua atividade alvo avaliada, um exemplo de fonte é o ChEMBL⁴³ uma base de dados online que disponibiliza informações de ensaios

biológicos (*in vitro*, *in vivo*) e resultados de ensaios de HTS. Em seguida, é necessário o cálculo de descritores moleculares, descritores de impressão digital, ou obtenção de outras informações (dados de RMN, espectros de massas etc.) que descrevam as moléculas que foram coletadas para que os modelos computacionais possam ser criados. Os modelos então serão desenvolvidos, verificados e validados para serem utilizados na predição de compostos potencialmente ativos. Uma vez identificados esses compostos, eles serão encaminhados para a avaliação *in vitro*, gerando novas informações que podem ser coletadas e dar início novamente ao ciclo. Isto é, a cada estudo realizado, novas informações são geradas que ficarão disponíveis para novos estudos.

A figura 1 resume essas etapas. Cada uma dessas etapas engloba vários outros passos que precisam ser seguidos para que a qualidade e sucesso do modelo QSAR seja atingido. A seguir cada um desses passos será abordado e explicado a fim de que o leitor tenha uma boa compreensão sobre a criação de modelos preditivos de QSAR com o ML.

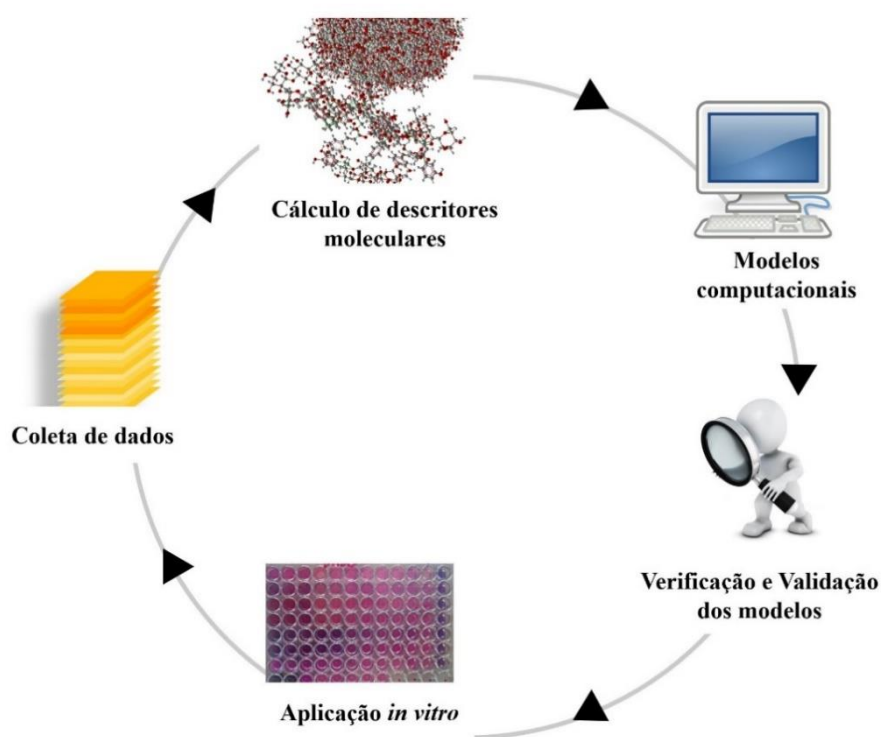


Figura 1: Ciclo do processo de criação do modelo computacional com abordagem LBVS em QSAR

Mineração De Dados

Há disponível hoje, uma grande quantidade de informação de moléculas disponíveis em vários repositórios de domínio público e privado, e para processar essas informações é necessário ferramentas computacionais. Por exemplo, no PubChem, um repositório público de estruturas químicas e suas propriedades biológicas há mais de 97,3 milhões de compostos e 1,1 milhão de bioensaios.⁴⁴⁻⁴⁶ É uma quantidade de dados absurda que são atualizados diariamente, possuindo uma variedade enorme de informação de resposta alvo.

Semelhante ao PubChem, há também o ChEMBL que já foi mencionado acima. Comparado ao PubChem, no ChEMBL há uma grande quantidade de informações de dados selecionados manualmente da literatura. Estima-se que no ChEMBL contenha mais de 2,2 milhões de compostos testados contra mais de doze mil alvos biológicos.^{44,47} Há outras fontes projetadas especificamente para medicamentos e candidatos a fármacos, como o DrugBank que é um banco de dados público que contém todos os medicamentos aprovados e informações como seus mecanismos de ação, interações e alvos relevantes.^{44,48}

Esses dados disponíveis podem e devem ser trabalhados para gerarem informações, e no nosso caso, esses dados devem ser trabalhos para obtenção de informação de novos possíveis medicamentos, ou seja, na descoberta de novas drogas. Assim, após a obtenção de dados precisamos minerá-lo para extrair as informações e o conhecimento que buscamos.

A mineração de dados são processos e etapas que serão realizadas para explorar e analisar uma grande quantidade de dados em busca de padrões, previsões, erros, associações etc.⁴⁹ A mineração de dados está geralmente associada ao aprendizado de máquina. Mas para minerar dados é importante que esses dados sejam de qualidade. Dados com problemas, como erros em estruturas químicas e resultados experimentais, dificultam e se tornam um grande obstáculo para a construção de modelos preditivos.^{32,50,51}

Por essa razão, é preciso avaliar a qualidade dos dados, o que Fourches, Muratov e Tropsha (2015)⁵² denominaram de “cinco Is”, ou seja, avaliar se os dados podem estar incompletos, imprecisos, incorretos, incompatíveis e/ou irreprodutíveis. Os autores, reforçam a necessidade de tratar os dados como o primeiro passo crítico em qualquer estudo de análise de dados, com o intuito de garantir estabilidade e confiabilidade dos resultados obtidos. Fourches, Muratov e Tropsha (2015)⁵² propuseram ainda um fluxo geral de tratamento de dados químicos e biológicos, visando estudos de QSAR. O fluxo está representado na figura 2 o qual apresenta 8 etapas que serão explicadas a seguir:

Etapa 1: Aplicação de algumas abordagens quimioinformática para sinalizar e, em alguns casos, corrigir entradas possivelmente errôneas em grandes conjuntos de dados. É nesta etapa que será feita a remoção de misturas, de compostos inorgânicos e organometálicos, verificação da falta de estruturas químicas, conversão estrutural, limpeza/remoção de sal, padronização de quimiotipos específicos como por exemplo a representação canônica personalizada, ou seja, alterar a forma de escrever o anel aromático de modo a incluir todas as formas ressonantes. Tratamento de formas tautoméricas, e esta etapa inclui também uma inspeção manual para verificação das estruturas químicas, se há erros em suas estruturas.^{3,52}

Etapa 2: Análise de duplicatas. Pode acontecer do banco de dados apresentar moléculas repetidas, caso positivo é preciso avaliar a atividade experimental dessas moléculas. Se apresentarem valores de atividades contraditórios, essas moléculas devem ser investigadas, e apresentando valores semelhantes, retira-se apenas uma delas. Moléculas repetidas no conjunto de teste geram modelos superestimados. Moléculas repetidas no conjunto de treino podem afetar o modelo de várias formas, como na sua confiabilidade e super ajuste.

Etapa 3 e 4: Análise da variabilidade intra- e inter-laboratorial e exclusão de fontes não confiáveis. Nessas etapas, os dados experimentais serão analisados e aqueles de fontes não confiáveis devem ser excluídos. Com esses passos, a qualidade dos dados é aumentada e consequentemente, aumenta-se a confiabilidade do modelo.

Etapa 5: Detecção e análise dos “cliffs” de atividade. Um “cliff” de atividade pode ser explicado como sendo estruturas semelhantes, mas com atividade muito diferentes.^{3,52,53} É nesse passo também, que os “outliers” serão detectados e analisados, ou seja, aquelas amostras com valores de atividade atípicos. A análise de “cliffs” e “outliers” devem ser rigorosas e a remoção dessas amostras devem ser justificadas. Uma vez que a remoção delas pode levar a melhora das estatísticas dos modelos, assim, se não houver uma justificativa, esse ato pode ser considerado manipulação dos dados.

Etapa 6: Cálculo do índice de modelabilidade do conjunto de dados. Esse índice foi criado por Golbraikh et al., (2014)⁵⁴ que estima a viabilidade de obter modelos QSAR preditivos de bancos de dados binário de compostos bioativos. Através do cálculo desse índice, é possível identificar conjuntos de dados modeláveis ou não modeláveis. Sendo, portanto, um indicador da qualidade dos dados.

Etapa 7: Predição consensual por múltiplos modelos de QSAR. Durante esta etapa, serão gerados diversos modelos de QSAR independentes, variando tipos de descritores

moleculares e/ou algoritmos. Em seguida, será realizado o consenso dos modelos, o qual será calculado a média das predições dos modelos independentes.^{3,52}

Etapa 8: Identificação e correção de compostos erroneamente anotados. Através do passo 7, é possível realizar o último passo do fluxo de curadoria de dados, uma vez que, dados biológicos incorretos podem ser identificados investigando-se e analisando aqueles compostos que apresentaram atividade biológica predita muito diferente do valor experimental.^{3,52}



Figura 2: Esquema geral de tratamento de dados (adaptado de ref. ⁵²)

Estrutura dos Dados

Após obter o banco de dados tratado com o conjunto de moléculas e informações biológicas confiáveis, o próximo passo é obter informações das moléculas. Essas informações são os descritores moleculares.

De acordo com os pesquisadores quimiométricos Todeschini e Consonni (2000)⁵⁵, descritores moleculares são um resultado final de um procedimento lógico e matemático que transforma a informação química codificada dentro de uma representação simbólica de uma molécula em um número útil, ou o resultado de algum experimento padronizado.

Em outras palavras, um descritor molecular é uma representação matemática da informação química de uma molécula, ou seja, de alguma característica da estrutura da molécula, oferecendo uma visualização da molécula com uma perspectiva diferente.^{12,36} É através dos descritores moleculares que é possível encontrar a relação entre a estrutura molecular e suas propriedades biológicas.

Os descritores químicos calculados podem ser agrupados nas seguintes categorias de acordo sua natureza:^{12,36,56}

- Descritores constitucionais: possuem informações como número de ligações, peso molecular etc.
- Descritores topológicos: informações de número de polaridade, índice de Zagreb etc.
- Descritores quânticos: HOMO (orbital molecular ocupado mais alto) e LUMO (orbital molecular não ocupado mais baixo)
- Parâmetros estéricos e eletrônicos: constante de Taft, constante de Hammett, por exemplo.
- Descritores geométricos: índice de aromaticidade, excentricidade molecular etc.

Os descritores moleculares, podem ainda ser classificados quanto a sua dimensionalidade. Descritores unidimensionais (1D), os mais simples, descritores bidimensionais (2D), tridimensionais (3D), descritores 4D proposto por Hopfinger et al., (1997)^{3,57} que utiliza simulação de dinâmica molecular, descritores 5D propostos por Vedani e Dobler (2002)^{3,58}, que são na verdade uma extensão dos descritores 4D, o qual adiciona liberdade conformacional ao modelo de dinâmica o que permite uma representação múltipla da topologia das moléculas. E posteriormente o mesmo grupo também propôs os descritores 6D que consideram vários modelos de solvatação ao mesmo tempo.^{3,59}

Há disponível, atualmente, uma grande diversidade de programas, softwares e web tool que calculam descritores moleculares. Como por exemplo o Dragon®⁶⁰, CDK® (<http://www.rguha.net/code/java/cdkdesc.html>), Volsurf®⁶¹, RDKit (<http://www.rdkit.org/>), entre outros. Uma vez calculado os descritores moleculares, é gerada uma tabela na qual as linhas representam as moléculas e as colunas são os descritores moleculares.

Alguns programas são capazes de calcular milhares de descritores moleculares, como o Dragon que pode calcular mais de cinco mil descritores.⁶⁰ E pode nos levar a pensar que quanto mais descritores moleculares melhor será o modelo que será gerado. Mas o efeito pode ser justamente o oposto, uma vez que, muitos descritores podem causar super ajuste no modelo, tornando-o ineficaz.

Para evitar esse problema, é preciso realizar a seleção de variáveis/descriptores. Seleccionamos aqueles descritores mais importantes, que dão informações importantes na relação estrutura atividade. Rotineiramente, são excluídos aqueles descritores que apresentam valores iguais para todas as moléculas, aqueles que o valor muda apenas para uma ou duas moléculas, e aqueles descritores altamente correlacionados ($r > 0.9$) que geram “ruídos” e prejudicam a qualidade do modelo.^{12,36,62,63}

Com a tabela já finalizada, acrescentamos por fim, a coluna com a informação da atividade biológica/ou propriedade que desejamos encontrar a relação com a estrutura química das moléculas. Essa informação é também chamada de classe (Y). A depender de como essa informação for fornecida um tipo específico de aprendizado de máquina será empregado. Se essa informação, for dada por valores numéricos, é chamado de dados contínuos e caso seja nominal, é chamado então de dados categóricos.^{44,49,64}

Aprendizado de Máquina (ML)

O aprendizado de máquina como já mencionado, é a utilização de algoritmos matemáticos de inteligência artificial, que podem aprender com os dados de entrada e construir com essas informações modelos computacionais a fim de obter previsões, decisões guiadas, agrupamentos.

O ML, pode ser classificado quanto a saída desejada em três grandes grupos: classificação, regressão e agrupamentos.^{49,65–67} Na classificação, as entradas da classe Y, são divididas em duas ou mais classes, e o modelo será treinado, a partir dos dados de entrada, a classificar as amostras nas classes corretas e assim ficar apto a analisar novas amostras e a partir de suas características (descriptores moleculares) atribuir uma classe. A regressão, pode ser dita que, é um tipo de classificação, enquanto na classificação nós vamos ter a classe Y nominal ou categórico, na regressão, a classe Y, é um valor numérico. A figura 3 representa o modelo de classificação.^{49,65–67}

ML do tipo agrupamentos não existe classe. O objetivo é que o algoritmo observe os dados de entrada, e crie grupos a partir de suas características. Ou seja, o algoritmo irá buscar por semelhanças entre as características/descriptores moleculares das moléculas dos dados de

entrada e atribuir um grupo a elas. A depender do tipo de agrupamento que for utilizado, uma molécula pode pertencer a mais de um grupo ou mesmo não ser agrupado, sendo considerado um ruído.^{49,65–67} A figura 4 representa esse tipo de ML.

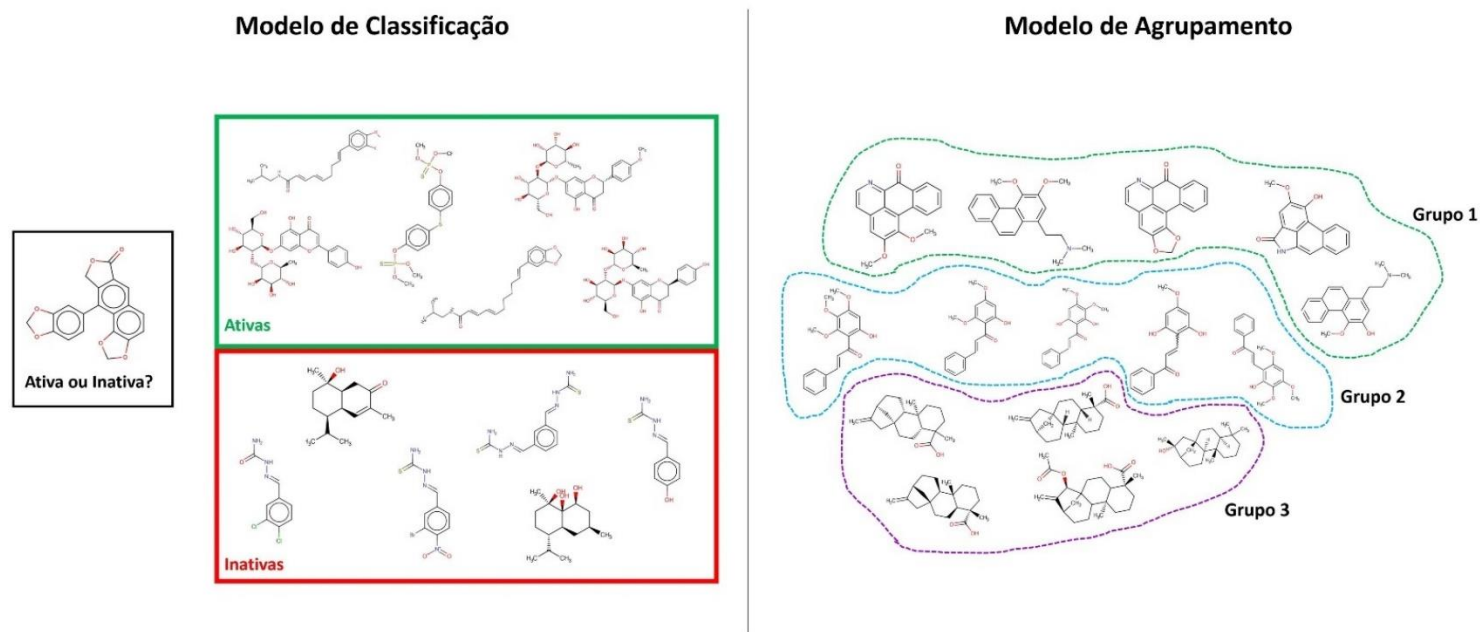


Figura 3: Modelo de classificação e agrupamento

Outro tipo de classificação de ML é com relação a técnica utilizada, podendo ser aprendizado supervisionado, não supervisionado e por reforço. No aprendizado supervisionado, é fornecido ao algoritmo informações de entrada e saída desejada, onde o objetivo será encontrar uma regra geral que mapeia as entradas até as saídas desejadas, ou seja, a classe Y esperada. Um exemplo de aprendizado supervisionado, são os modelos de classificação. No aprendizado não supervisionado, não é fornecido as informações de saída desejada, desta forma, o algoritmo precisa encontrar padrões nos arquivos de entrada. Um exemplo de aprendizado não supervisionado, são os modelos de agrupamento. No aprendizado por reforço, o objetivo é que o algoritmo aprenda com seus erros a tomar a melhor decisão para se chegar ao objetivo. Um exemplo prático para compreender esse tipo de aprendizado, é descobrir o caminho mais eficiente para ir de um ponto A para o ponto B, então o algoritmo irá realizar diversas tentativas a fim de alcançar seu objetivo, onde para cada tentativa será atribuído recompensas ou punição dependendo da decisão que foi tomada, e assim, o algoritmo vai se ajustando até alcançar uma margem satisfatória ou o objetivo proposto. Um exemplo desse aprendizado é um tipo de rede neural artificial.^{30,44,65,68} A figura 4 mostra as classificações do aprendizado de máquina.



Figura 4: Classificação do aprendizado de máquina

Como já foi dito, o ML utiliza algoritmos matemáticos em sua execução. Há uma grande diversidade de algoritmos que são utilizados em ML e alguns foram trazidos para a quimioinformática e consequente para os estudos de QSAR, como árvore de decisão (Decision Tree - DT), florestas aleatórias (Random Forest - RF), máquina de vetor de suporte (Support Vector Machine - SVM), k-ésimo vizinho mais próximo (k-nearest Neighbors - k-NN), naive bayes (NB), redes neurais artificiais (Artificial Neural Network - ANN), que serão brevemente discutidos a seguir.^{7,62,65,69} A figura 5 representa, a estrutura de cada um desses algoritmos.

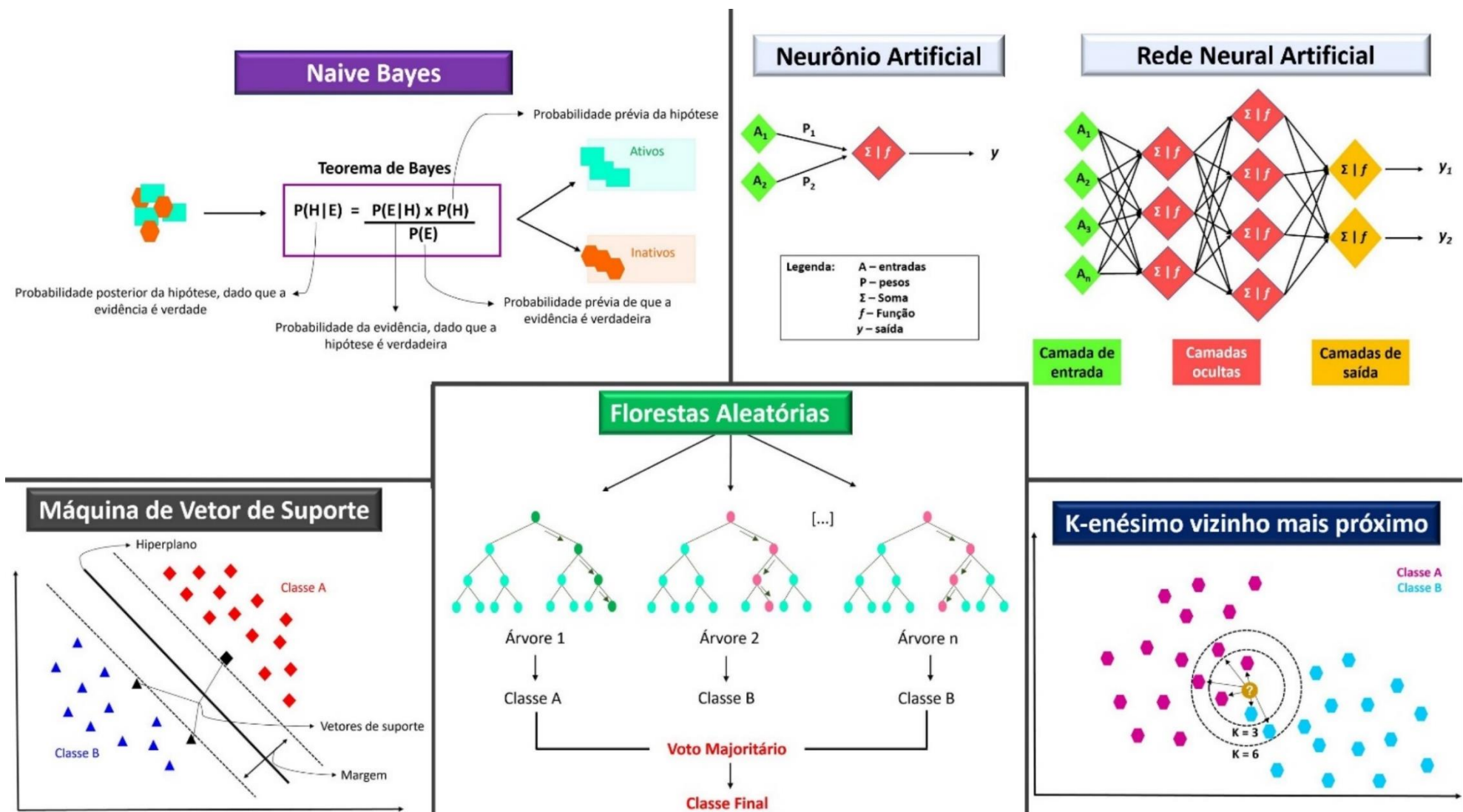


Figura 5: Representação da estrutura dos algoritmos florestas aleatórias, k-ésimo vizinho mais próximo, máquina de vetor de suporte, naive bayes e rede neural artificial.

Árvore de decisão (DT) e florestas aleatórias (RF)

Esses algoritmos se baseiam na estrutura de uma árvore de decisão hierárquica, composta por nós e ramos que se unem para gerar modelos preditivos. São algoritmos de ML supervisionados, mas podem ser utilizados tanto para modelos de classificação como de regressão.^{65,70,71}

A estrutura de uma árvore de decisão é composta por nós, nós raiz, internos e terminais. O nó raiz é normalmente representado no topo da árvore sem galhos chegando até ele, mas com ganhos saindo dele. Os nós internos, possuem galhos que chegam neles e galhos que saem deles em direção ao próximo nível hierárquico. E a árvore, finaliza com os nós terminais que não possuem ramificações que começam a partir deles, uma vez que são o último nível da hierarquia.^{70,72}

A cada novo nó que é iniciado e terminado na árvore, são tomadas de decisões. Na árvore, o resultado de cada curso é ponderado pela probabilidade associada, e o resultado será somado e o valor de cada curso é então determinado. Daí, o curso que fornece o maior valor esperado será o curso preferido. De maneira simplificada a estrutura da DT permite a representação e avaliação de problemas envolvendo decisões sequenciais, destacando os riscos e resultados identificados em cada decisão e rumo tomado.^{65,71,73}

O algoritmo RF, é na verdade um conjunto de uma grande quantidade de DTs que é gerado e, ao final, será realizado uma votação para o caso mais popular, que é considerado o melhor ajuste.^{65,74,75} Uma das vantagens mais significativas do RF é que produz melhores resultados devido à ausência de incremento no viés, pois reduz a variância no modelo.⁷⁰ A figura 5 representa a estrutura do RF.

Máquina de vetor de suporte (SVM)

SVM são algoritmos de ML supervisionado, que pode ser aplicado tanto para classificação quanto para regressão.^{65,70,72} É um algoritmo bastante utilizado devido ao seu desempenho e capacidade de generalização em domínios de alta dimensionalidade.^{72,76,77} É um algoritmo baseado em kernel, logo, se baseia em similaridade entre objetos.^{72,76–78}

Um modelo SVM, representa as amostras do banco de dados como em pontos no espaço, de modo que os pontos de diferentes categorias sejam separados por um hiperplano que seja tão amplo quanto possível.^{72,76–78} As novas amostras serão mapeadas no mesmo espaço e serão previstos para qual lado do hiperplano pertenceram.⁶⁵ As amostras utilizadas

para definir as margens do hiperplano, são aqueles mais semelhantes e são chamados de vetores de suporte.

Uma observação importante é que o SVM pode ainda realizar o ML não supervisionado, ou seja, uma vez que os dados não estejam rotulados (não possam informação da saída desejada), o SVM por meio do algoritmo de vetorização de suporte criado por Hava Siegelman e Vladimir Vapnik, que aplicará estatísticas de vetor suporte, desenvolvidas no SVM, e categorizará os dados não rotulados, criando assim grupos e sendo um dos algoritmos de agrupamento bastante utilizado nas mais diversas aplicações.^{65,71,79} A figura 5 representa o SVM.

K-ésimo vizinho mais próximo (k-NN)

Diferentemente dos outros algoritmos apresentados, o k-NN não gera um modelo. Ele busca em tempo de execução qual nova amostra (instancia) se parece mais com os dados históricos e todo o cálculo é adiado até a classificação. Devido a essa característica, é um algoritmo de alto custo computacional uma vez que cada nova instancia a ser classificada terá que ser comparada com todos os dados do conjunto de treino e avaliar aqueles com menor distância.^{65,80,81}

O k-NN é chamado de algoritmo baseado em instancia, onde sua mecânica é apenas classificar uma nova instancia atribuindo a classe que é mais frequente entre os k vizinhos (amostras) mais próximos do conjunto de treinamento a esse elemento, onde essa distância é determinada através de alguma medida de similaridade (pode ser distancias euclidianas, de manhattan, ponderada etc.). O k é um valor inteiro e será determinado pelo usuário. É um método não paramétrico que pode ser utilizado para classificação, regressão e agrupamentos.^{7,65,81} A figura 5 representa o funcionamento do k-NN.

Naive Bayes (NB)

O algoritmo NB é na verdade uma família de algoritmos classificadores de ML supervisionado. Sua grande vantagem está em sua simplicidade e em sua eficiência, com capacidade de gerar modelos de classificação de boa precisão a partir de um pequeno conjunto de dados, as vezes seu desempenho supera classificadores mais complexos.^{49,65,71}

O NB, se baseia no Teorema de Bayes e supõe que os atributos irão influenciar a classe Y de maneira independente. O teorema de Bayes descreve a probabilidade de um evento, por exemplo a probabilidade de uma molécula ter potencial ativo, com base em um

conhecimento prévio das condições que podem estar relacionadas a esse evento, ou seja, com base naqueles atributos (descritores moleculares) que melhor descrevem as moléculas que tem atividade.^{65,71,72}

Explicando de maneira simples e objetiva esse algoritmo irá construir uma tabela mostrando a importância de cada atributo para a classe Y, assim, ao submeter uma nova instancia (amostra) o NB irá analisar os pesos de cada atributo comparar com os atributos da nova instancia somá-los e ver qual classe teve maior peso, sendo então, classificado com esta classe “vitoriosa”.^{49,71,72} A figura 5 traz a representação do teorema de Bayes e do funcionamento do algoritmo NB.

Rede neural artificial (ANN)

As ANN são algoritmos que geram modelos computacionais inspirados nos neurônios do sistema nervoso central humano. Para compreender melhor uma rede neural, precisamos começar entendendo o que é um neurônio artificial e seu funcionamento. Um neurônio artificial, assim como neurônios do cérebro, pode se comunicar entre si e as conexões dos neurônios artificiais são representadas por pesos que não passam de um valor específico e esse valor tenta expressar a força sináptica dessa conexão entre neurônios.^{17,65,70,72}

Existem diferentes tipos de arquitetura de ANN, elas podem ser supervisionadas, não supervisionadas e por reforço, porém todas possuem em comum alguns elementos em sua estrutura. A estrutura básica de uma ANN possui um conjunto de nós, primeiro são os nós de entrada, são aqueles que obtém informações, esses nós são chamados de camada de entrada. A ANN também precisa de nós de saída que são aqueles que transmitem o resultado da ANN, e por fim, tem os nós da camada oculta, são aqueles que transmitem informações entre os neurônios artificiais.^{17,32,65,72} Vale ressaltar que o importante da estrutura de uma ANN não é apenas a topologia das interconexões entre os neurônios e das funções de ativação, mas uma parte fundamental é a relevância de cada uma das conexões.

O funcionamento básico de uma ANN se dá da seguinte maneira: quando a camada de entrada é determinada, os pesos serão atribuídos. Esses pesos são valores numéricos que auxiliam a determinar a importância de cada variável (em QSAR, de cada descritor molecular) fornecida, onde os maiores pesos serão para aquelas variáveis que contribuem de forma mais significativa para a classe Y, quando comparado as demais variáveis. Assim, todas as entradas serão multiplicadas por seus respectivos pesos, e em seguida, somadas. A próxima etapa é a transmissão da saída através de uma função de ativação, que determina a

saída (classe Y). Se a saída exceder um determinado valor, o nó será ativado, que irá transmitir as informações para a camada seguinte da ANN. Dessa forma, a saída de um nó torna-se a entrada do próximo nó. Caso a saída não exceda o valor determinado, não haverá transmissão de informação. Este processo de transmissão de informação de uma camada para a seguinte define o funcionamento de uma ANN.^{17,32,65,71,72} A figura 5 representa a estrutura básica de uma ANN.

Construindo Modelos E Avaliando Seu Desempenho

A depender do modelo que desejamos construir, seja supervisionado ou não supervisionado, há um caminho básico que devemos seguir. Mas independentemente do tipo de modelo sempre será preciso validá-lo e avaliar o seu desempenho.

Como explicado anteriormente, em um aprendizado de máquina um algoritmo irá aprender a partir de um conjunto de dados. Nesse aprendizado é construído o modelo, que é simplesmente um fórmula criada pelo algoritmo para prever, classificar ou agrupar os novos dados que sejam apresentados para o algoritmo, ou seja, dados que o algoritmo não conheça.⁴⁹

A figura 6 representa o esquema geral da construção de um modelo. Cinco etapas são cruciais para a construção de qualquer modelo utilizando aprendizado de máquina. Inicialmente o banco de dados que será utilizado para construção de modelo, previamente tratado, será particionado de maneira estratificada em dois subconjuntos, 80% do conjunto será treino e 20% será teste. Nesta partição os conjuntos são selecionados de maneira aleatória, mas mantem a mesma proporção de classes entre os conjuntos de treino e teste para um modelo de classificação, por exemplo. Há um consenso em particionar o banco de dados em 80% para treino e 20% para teste, mas há possibilidade de variar um pouco essa proporção para 70% treino e 30% teste.⁴⁹ Na segunda etapa, o conjunto de treino será utilizado para gerar o modelo, ou seja, o conjunto de treino será apresentado ao algoritmo para que ele possa aprender e então gerar o modelo. Na terceira e quarta etapa, o conjunto de teste será utilizado para que possamos compreender o desempenho do modelo, ou seja, esse conjunto terá sua classe ocultada e então os dados descritivos será apresentado ao modelo, em seguida será realizada a predição das amostras do conjunto de teste. Após a predição, será então realizada a análise de performance do modelo no qual iremos avaliar a taxa de acerto do modelo. Uma vez que temos o conhecimento da classe de cada amostra do conjunto de teste, é realizada a comparação com o que foi predito e assim temos conhecimento da taxa de acerto do modelo e sua performance. Um modelo ideal irá acertar

100% em sua predição, caso ele acerte 50% isso nos informa que sua predição foi um mero palpite, o algoritmo não conseguiu aprender com os dados do conjunto de treino. A última etapa do processo da criação de modelos computacionais utilizando aprendizado de máquina será a predição de um novo conjunto de dados, que é o objetivo final da criação do modelo, realizar a predição de um conjunto para o qual não há conhecimento de sua classe.

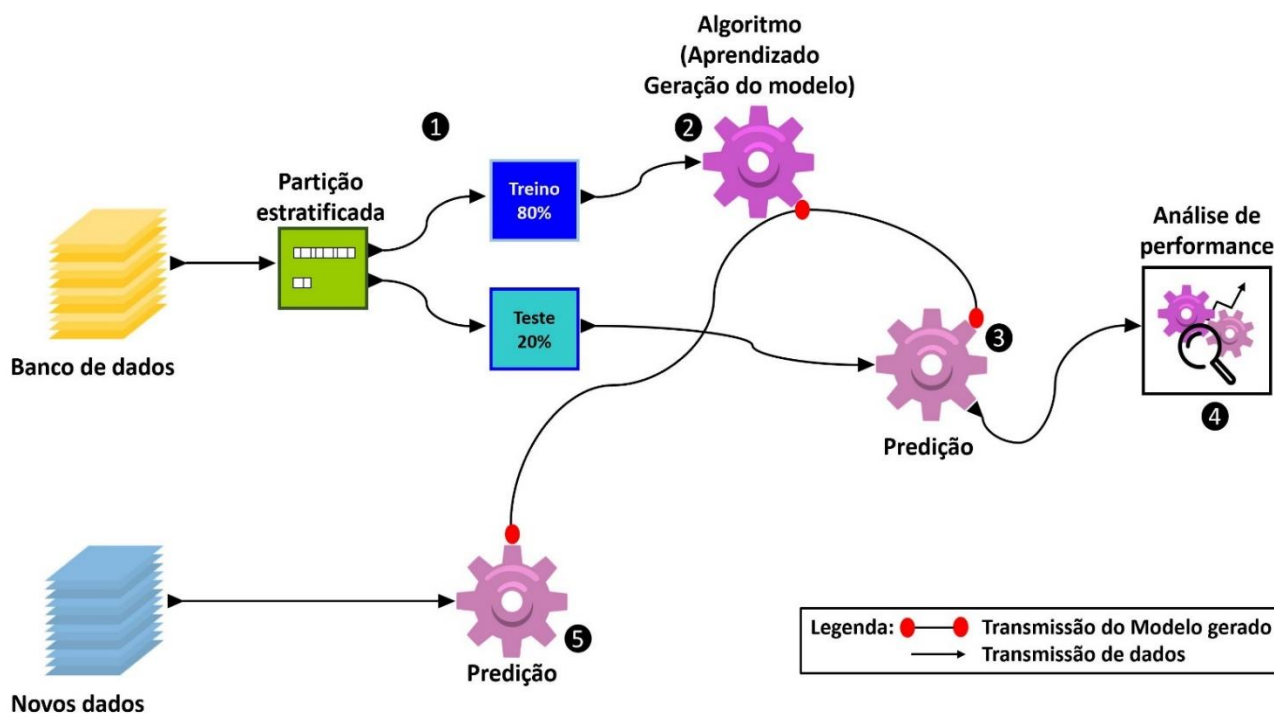


Figura 6: Representação global de um modelo computacional utilizando aprendizado de máquina. 1 – partição estratificada do conjunto de dados em 80% treino e 20% teste. 2 – O conjunto de treino é apresentado ao algoritmo para aprendizado e geração do modelo computacional. 3 – O conjunto de teste é utilizado no modelo para obter sua predição. 4 – A partir da predição do conjunto de teste é realizada a análise de performance do modelo criado. 5 – Predição de novos dados utilizando o modelo criado

Análise de desempenho para modelos de classificação

Em um modelo de classificação existem quatro possibilidades de avaliação de desempenho básicas. Considerando um banco de dados no qual possui duas classes, positivos (moléculas ativos) e negativos (moléculas inativos), vamos ter as seguintes possibilidades: verdadeiros positivos (VP), aqueles que são ativos e foram acertadamente classificados como ativos; falso negativo (FN), aquelas amostras que são verdadeiras positivas, mas foram classificadas como inativas; verdadeiros negativos (VN), amostras inativas sendo classificadas como inativas; e por fim os falsos positivos (FP), as amostras que são inativas

mas que foram classificadas como ativas. Assim, os verdadeiros ativos e verdadeiros inativos são os acertos do modelo, ou seja, a taxa de acerto, enquanto os falsos ativos e falsos inativos são os erros do modelo. Esses valores quando colocados em uma tabela, temos então, a matriz de confusão.^{49,82,83} A figura 7 representa uma matriz de confusão.

		Informação Real	
		Positivo	Negativo
Predição	Positivo	Verdadeiro Positivo (VP)	Falso Positivo (FP)
	Negativo	Falso Negativo (FN)	Verdadeiro Negativo (VN)

Figura 7: Matriz de confusão

A partir da matriz de confusão, várias métricas podem ser calculadas para avaliar o modelo fornecendo informações fundamentais para total compreensão de seu desempenho. Essas métricas são: precisão (acurácia), sensibilidade, especificidade, verdadeiro preditivo positivo, verdadeiro preditivo negativo, coeficiente de correlação de Matthews e a curva ROC (Características Operacionais do Receptor, do inglês “Receiver Operating Characteristics”). Cada uma será explicada detalhadamente a seguir.

Precisão (acurácia - ACC): Se refere a taxa de acerto global do modelo. O quanto que o modelo está classificando corretamente tanto os positivos como os negativos. É calculada da seguinte maneira:

$$ACC = \frac{(VP + VN)}{P + N}$$

Em que P é o total de amostras positivas, e N o total de amostras negativas.

Sensibilidade: Avalia a taxa de acerto do modelo apenas das amostras positivas, ou seja, a capacidade de aprendizado do modelo em classificar uma amostra verdadeiramente positiva como positiva.

$$Sensibilidade = \frac{VP}{(VP + FN)}$$

Especificidade: Temos informação da capacidade de aprendizado do modelo em classificar amostras verdadeiramente negativas como negativas, ou seja, a taxa de acerto das amostras negativas.

$$Especificidade = \frac{VN}{(VN + FP)}$$

Verdadeiro preditivo positivo (VPP): Diz respeito a taxa de acerto de amostras verdadeiros positivos em relação a toda predição de positivos.

$$VPP = \frac{VP}{(VP + FN)}$$

Verdadeiro preditivo negativo (VPN): Informa a taxa de acerto de amostras verdadeiras negativas em relação a toda predição de negativos.

$$VPN = \frac{VN}{(VN + FP)}$$

Coeficiente de correlação de Matthews (MCC): Esse coeficiente nos ajuda a ter informação de performance global do modelo. Seu valor varia entre -1 e 1, onde um coeficiente de valor 1 indica um modelo de predição perfeita, o 0 indica um modelo de predição aleatória, e -1 indica um modelo em total desacordo entre o que previsto e o que é verdadeiro.⁸⁴ Dessa forma, buscamos modelos que possuam um MCC o mais próximo de 1 possível, é calculado da seguinte forma:

$$MCC = \frac{(VP \times VN) - (FP \times FN)}{\sqrt{(VP + FP)(VP + FN)(VN + FP)(VN + FN)}}$$

Curva ROC: A curva ROC nos mostra de maneira bidimensional o desempenho real do modelo com mais clareza que a precisão, conciliando a especificidade e sensibilidade. A curva ROC é expressa através do cálculo da área abaixo da curva (AUC), que é uma porção de um quadrado unitário, assim seu valor pode variar de 0 a 1, mas na prática costuma variar entre 0.5 e 1, onde 1 é um desempenho perfeito e 0,5 um modelo completamente aleatório. Não modelos classificadores piores que os aleatórios por esta razão, não se considera modelos com valores de curva ROC abaixo de 0,5. O gráfico da curva ROC é plotado pelo valor da sensibilidade (eixo Y), o valor dos verdadeiros positivos, em função do valor de falsos positivos, ou seja, 1 menos especificidade.⁸³ A figura 8 representa a plotagem da curva ROC. Quanto menos falsos positivos há no modelo, maior o acerto em verdadeiros negativos, e quanto maior a sensibilidade, menor é o erro em falsos negativos. Desta forma, o valor da AUC será maior quanto mais próximo de 1 no eixo Y e mais próximo de 0 no eixo X.

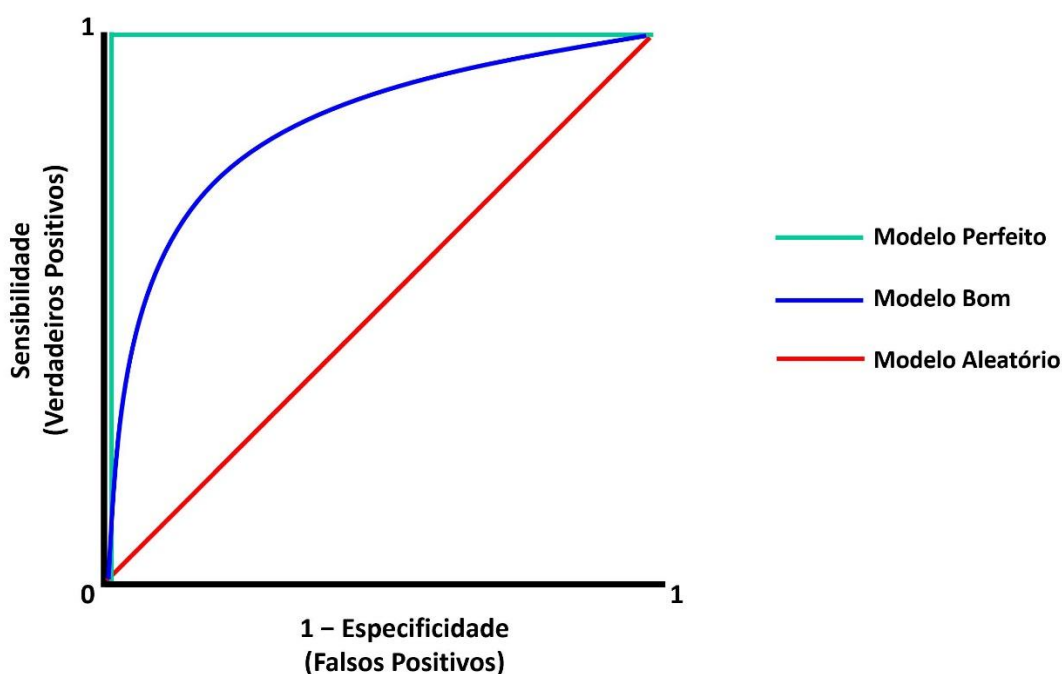


Figura 8. Representação da curva ROC

Há alguns problemas que podem acontecer em modelos de classificação e que afetam diretamente as suas métricas de avaliação de desempenho e que devemos sempre estar atento, são eles: super ajuste e classe rara.

Na construção de modelos buscamos por modelos genéricos, modelos que tenham um bom desempenho e que seja confiável. O contrário desses modelos, são os modelos super ajustado (do inglês: overfitting). Os modelos super ajustado apresentam ótimo desempenho, alta precisão, funcionam muito bem para o conjunto de treino, mas não generaliza bem para novos dados. É como se o modelo decorasse o conjunto de treino, mas não aprendeu de fato a diferenciar as classes e por isso tenho um pobre desempenho para novos dados.

São vários os fatores que podem causar um super ajuste, mas o principal é quando os dados de treino são poucos, aplicando para QSAR, possuem pouca variedade de moléculas e pouca quantidade, o que leva o algoritmo a criar um modelo que não representam eficientemente o conjunto de novas moléculas. Classe rara também pode causar modelos super ajustados.

Classe rara acontece quando a quantidade de uma classe é consideravelmente menor que outra classe. Por exemplo, iremos trabalhar com um banco de dados para construção de um modelo preditivo que possui 1000 moléculas. Dessas 1000 moléculas, 900 são da classe de moléculas ativas e 100 são moléculas inativas, veja que a classe de moléculas inativas representa apenas 10% de todo o conjunto de dados. A consequência disso é que o modelo

aprenderá muito bem a classificar moléculas ativas, mas terá sérios problemas de aprendizado na classificação de moléculas inativas.

A solução desse problema é equilibrar as classes no conjunto de dados, e é de extrema importância que as classes estejam equilibradas no conjunto de treino. Por esse motivo, quando o conjunto de dados é particionado no conjunto de treino e teste, essa partição aconteça de maneira estratificada, ou seja, mantendo a proporção de moléculas ativas e inativas no conjunto de treino e no conjunto de teste. Desta forma, evita-se que na partição aconteça o problema de classe rara devido a uma partição inadequada, sem equilíbrio entre as classes, pois mesmo que o banco de dados tenha equilíbrio entre as classes, o erro na partição pode gerar um problema de classe rara.

Análise de desempenho para modelos de regressão linear

Como já mencionado, a regressão linear é bastante semelhante a classificação, a diferença está na classe, ao invés de um valor nominal ou categórico, é um valor numérico. É preciso entender alguns conceitos para compreender os resultados e a análise de desempenho de um modelo de regressão.

Em um modelo de regressão linear, a variável que desejamos prever não é chamada de classe como nos modelos de classificação, é conhecida por variável dependente e os descritores, as variáveis que utilizaremos para prever, é chamada de variáveis independentes. Dessa forma, na regressão, irá se utilizar de técnicas estatísticas para modelar correlações e prever o valor de uma variável dependente (Y) a partir das variáveis independentes (X).^{49,82,85} Podemos ter dois tipos de regressão linear, a simples onde temos uma variável independente para prever uma variável dependente, e a regressão linear múltipla, onde utiliza-se duas ou mais variáveis independentes para prever uma variável dependente.

Então, o objetivo do modelo de regressão linear é avaliar o grau de associação entre duas variáveis, X e Y, ou seja, mede a “força” de correlação linear entre as variáveis X e Y. Para investigar essa correlação e analisar a qualidade do modelo, pode-se representar os valores das variáveis num gráfico de dispersão. Desta forma, haverá presença de relação linear entre as variáveis dependente e independente se os valores se aproximarem de uma linha reta, e quanto mais os valores estiverem da reta, mais forte será a correlação. Assim, apenas observando o gráfico de dispersão já teremos uma ideia se o modelo está bom ou ruim.^{49,82,85}

A correlação entre as variáveis X e Y pode ser positiva ou negativa, e influencia diretamente na direção da reta. Uma correlação positiva significa que enquanto uma variável

crece, a outra que está correlacionada também cresce. Já na correlação negativa acontece o inverso, enquanto uma variável cresce a outra diminui.^{49,82,85} Influenciando, portanto, no direcionamento da reta, como pode ser observado na figura 9.

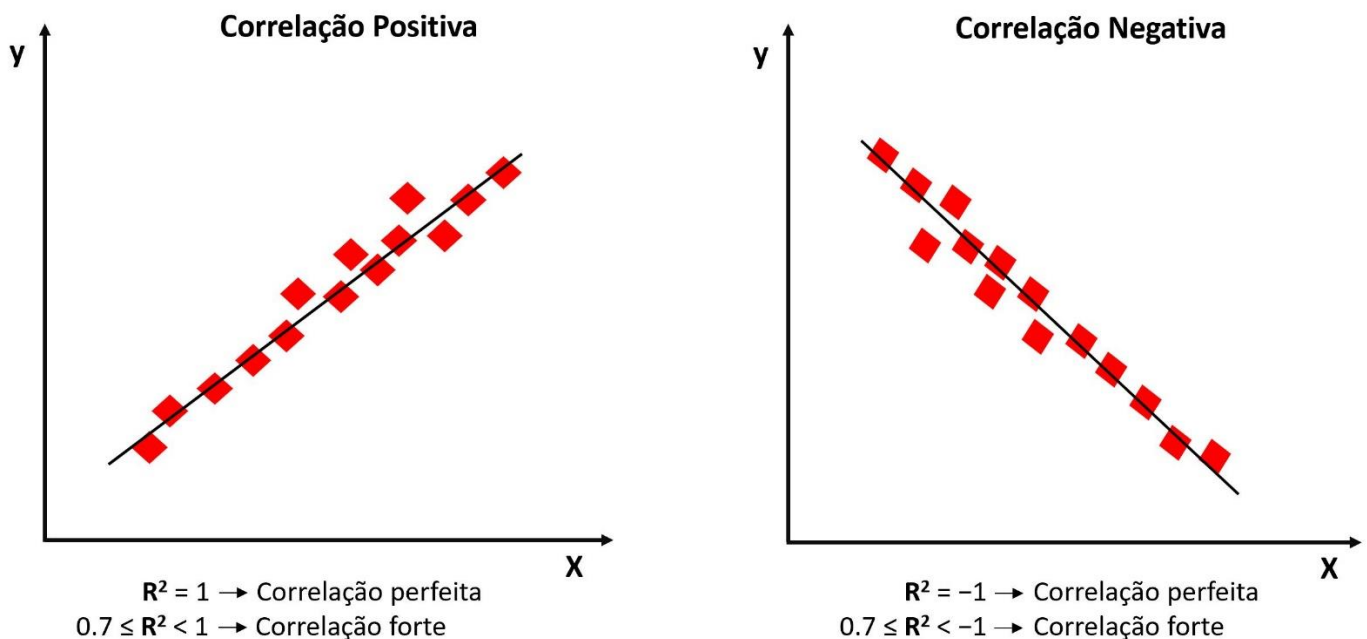


Figura 9. Representação da correlação através do diagrama de dispersão

Para medir e quantificar a explicação da reta no gráfico de dispersão utiliza-se o cálculo do coeficiente de determinação (R^2). O R^2 também pode ser entendido como a porcentagem da variância que pode ser prevista pelo modelo de regressão e consequentemente, informar o quão próximo a predição gerada está dos valores reais. Portanto, em uma regressão linear de correlação positiva o modelo perfeito possui R^2 igual a 1, então neste tipo de correlação quanto mais próximo de 1, melhor é o modelo. Já em uma regressão linear de correlação negativa o modelo perfeito possui R^2 igual a -1, então quanto mais próximo de -1 melhor será o modelo. Um modelo de R^2 igual 0 nos informa não há correlação entre as variáveis, então tanto na correlação positiva quanto na negativa, quanto mais o R^2 se aproximar de 0, mais fraca é a correlação entre as variáveis e pior é o modelo.^{49,82,85,86}

Mas a análise de desempenho de uma regressão linear não se limita apenas ao cálculo de R^2 , pois há limitações que podem acabar gerando mal interpretação do modelo. Por exemplo, caso o modelo esteja super ajustado, o valor de R^2 continuará alto. Por isso, outras métricas precisam ser avaliadas para ter total compreensão e uma boa interpretação da qualidade do modelo.^{49,82,85} Essas métricas são:

Erro quadrático médio (MSE): consiste em calcular a média de erro das previsões ao quadrado, ou seja, calcula-se a diferença entre o valor predito e o valor real e eleva-se o resultado ao quadrado. Isso é feito para todas as amostras, depois é só somar e dividir pelo número total de amostras. Quanto maior for o MSE, pior será o modelo.

Raiz do erro quadrático médio (RMSE): como o próprio nome já diz, essa métrica é a raiz do MSE. Tanto o MSE quanto o RMSE penalizam a presença de outliers nos dados.

Erro absoluto médio (MAE): essa métrica é a média do cálculo das distancias entre os valores preditos de todas as amostras com os valores reais. O MAE diferente do MSE e RMSE é sensível a presença de outliers, ou seja, ele não detecta sua presença. Quanto maior o MAE, pior é o modelo.

Erro percentual absoluto médio (MAPE): essa métrica gera uma porcentagem que é obtida por meio da divisão da diferença entre os valores preditos e real pelo valor real, ou seja, o MAE dividido pela soma de todos os valores reais. Quanto maior essa porcentagem, pior é o modelo.

Análise de desempenho para modelos de agrupamento

A avaliação de desempenho de modelos de aprendizado não supervisionado é muito importante, mas devido a sua natureza, a avaliação ou validação como também é conhecida, não é muito bem desenvolvida e não é tão trivial como avaliar modelos de aprendizado supervisionado.^{87,88} Aqui farei uma breve explicação visto que os modelos de agrupamento não são muito utilizados para QSAR.

Apesar de ser difícil a análise de desempenho de modelos de agrupamento, tem sido proposto na literatura uma vasta quantidade de métricas para quantificar a qualidade dos resultados dos modelos de agrupamento. Essas métricas se dividem em dois grupos: métricas de validação externa, quando há informações externas para avaliar a qualidade dos resultados de agrupamento, semelhante a análise de modelos de classificação, por exemplo; e métricas de validação interna, quando não há informação externa para auxiliar na análise de desempenho.^{82,87,88}

As métricas de validação interna se baseiam na medição da coesão e separação dos clusters (grupos), na análise estatística da matriz de proximidade ou no estudo do dendrograma gerado por algoritmos de agrupamento hierárquico. A depender do tipo de algoritmo utilizado, algoritmo de agrupamento hierárquico e algoritmo particional, há métricas mais específicas para analisar o desempenho. Para algoritmos particionais métricas baseadas em coesão e separação, e na matriz de proximidade, como por exemplo o

coeficiente de silhueta e o índice de Dunn, são os mais utilizados. Já para algoritmos hierárquicos o coeficiente covenético é o mais usual.^{82,87,88} A figura 14 traz informações dos métodos de avaliação dos modelos não supervisionados e são listados alguns dos coeficientes utilizados.

As métricas de validação externa avançam incorporando as informações externas no processo de validação dos grupos, ou seja, rotula as amostras de treino com classes. Há uma grande quantidade de métricas de validação externa na literatura, que podem ser divididas em conjuntos de correspondência, correlação ponto a ponto e índices teóricos de informações (Figura 10).^{82,87,88}

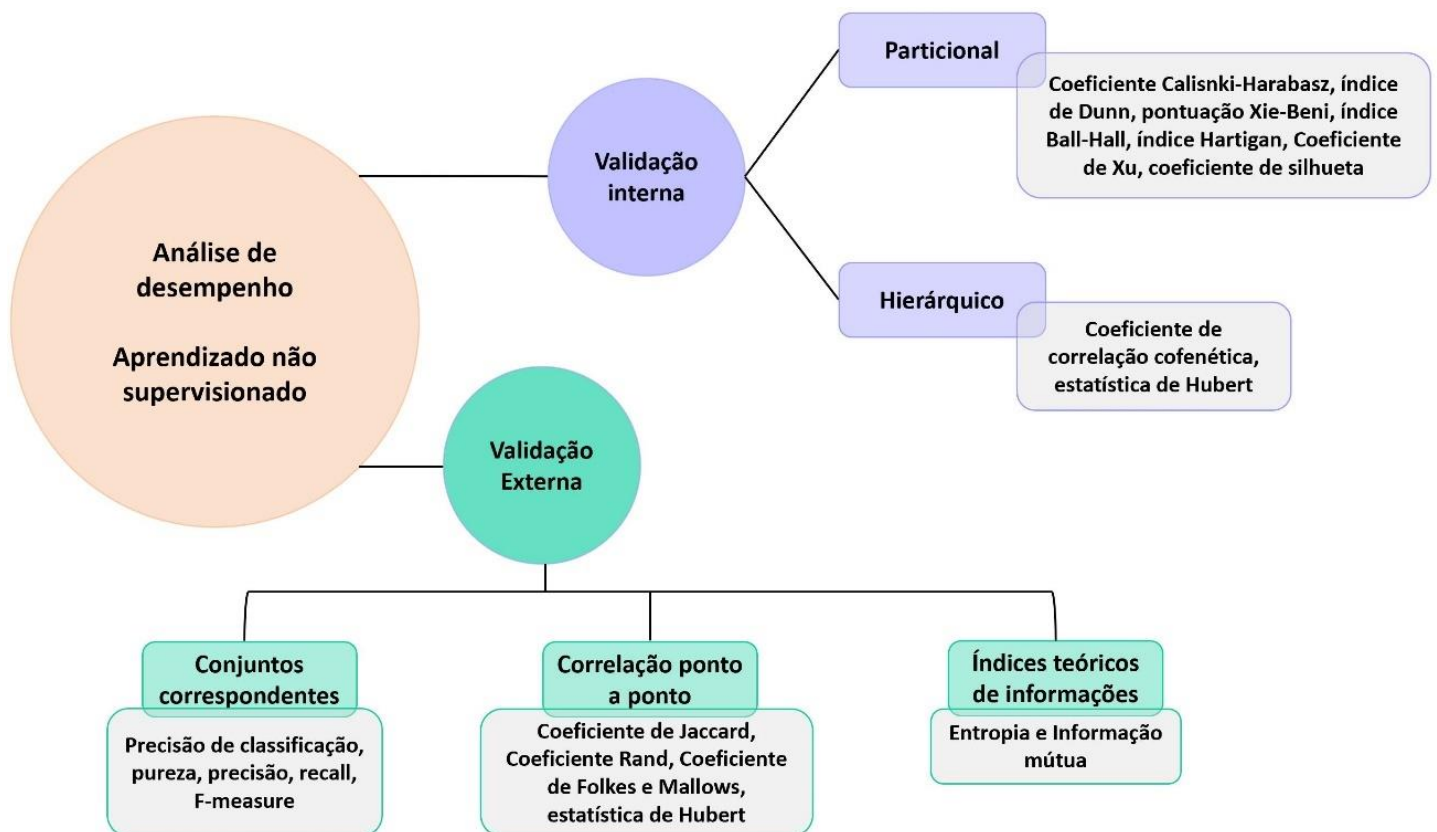


Figura 10: Métodos de análise de desempenho de aprendizado de máquina não supervisionado

Validação De Modelos De Aprendizado De Máquina

Ao particionarmos o banco de dados em treino e teste, o conjunto de teste é utilizado para visualizarmos a qualidade do modelo, sua capacidade preditiva, avaliarmos a sua performance. Esta técnica é conhecida por validação Holdout, mas o problema dessa técnica é que não é suficiente para ser usado como validação do modelo, uma vez que sua

amostragem pode não representar todo o conjunto de treino. Assim, é preciso aplicar outras técnicas de validação para compreendermos a real performance do modelo criado.

A validação de um modelo é de extrema importância para avaliar a capacidade preditiva de modelo, bem como a sua confiabilidade e reprodutibilidade, podendo ser avaliada por um coeficiente de correlação de validação cruzada, o Q^2 .^{17,89} A validação cruzada (CV, do inglês “cross-validation”) utilizando as técnicas de k partes (do inglês “ k -fold”), leave-one-out e estratificada, e validação bootstrap, são os métodos mais utilizados em validação interna.^{17,89} Cada um desses métodos será brevemente explicado a seguir, como também a validação externa.

Primeiro vamos compreender o que é uma validação interna e externa. Na validação interna, o modelo será validado utilizando um conjunto de moléculas que foram utilizadas para criar o modelo, o conjunto de treino. Na validação externa, o modelo é validado com um conjunto de moléculas na qual o modelo não foi apresentado.

A CV é uma técnica fundamental para avaliar a capacidade de generalização de um modelo, bastante importante para modelos de predição. Neste método de validação o modelo é avaliado uma série de vezes e ao final o desempenho é calculado a partir da média aritmética das avaliações.^{49,82,89}

Na validação cruzada interna de k partes o conjunto de treinamento é dividido em k partes semelhantes, de forma aleatória e sem substituição. Então, o processo de validação irá fazer k iterações: a cada iteração $k - 1$ partes será utilizada para treino e gerar um modelo, e uma parte será utilizada para teste. Na próxima iteração uma nova parte será utilizada para teste e as outras $k - 1$ restantes como treino, de forma que ao final, as k partes tenham sido utilizadas como teste e assim avaliar o modelo. Ao final, a média de todas as k iterações será calculada e teremos a performance final do modelo. Na CV interna estratificada de k partes, a única diferença é que a divisão do conjunto de treinamento em k partes ao invés de ser aleatória, é realizada de forma estratificada, ou seja, mantendo o equilíbrio entre as classes, e em modelo de regressão o valor médio da resposta é aproximadamente igual em todos as k partes.

O método de CV estratificada em k partes é a mais utilizada e recomendada para uma validação externa. Nesta técnica o modelo é criado já realizando a validação cruzada, desta forma, o conjunto de treino é particionado em k partes de maneira estratificada e cada modelo será analisado por um conjunto de teste que conterá moléculas que o modelo não conhece, por isso validação externa. De modo que ao final do processo teremos cinco modelos que foram validados separadamente, então realiza-se a média aritmética dos modelos e teremos

a performance do modelo. O conjunto para o qual desejamos prever a atividade será avaliado pelos k modelos criados, e ao final é feita a média aritmética da predição para cada molécula.^{14,66} Normalmente na CV de k partes, $k = 5$ ou 10 são boas opções, uma vez que há um bom equilíbrio entre complexidade computacional e precisão de validação.^{14,66}

A CV leave-one-out é uma variação da CV de k -partes. Neste método, cada amostra do conjunto de dados de treino é separada como um conjunto de teste, assim o número de combinações possíveis é igual ao número de pontos de dados no conjunto de treino. É um método exaustivo, e a depender do tamanho do conjunto de treino pode se tornar bastante custoso computacionalmente.

O último método que será comentado é o método bootstrap. Ao contrário da CV que utiliza amostragem sem repetição, ou seja, mesmas amostras não são incluídas em uma parte, o método bootstrap a amostragem é feita com repetição. Dessa forma, pode haver duplicação de algumas amostras. Embora essa técnica pareça suspeita, uma vez que pode estar gerando uma análise super ajustada, já foi comprovado que para esse caso, nesta técnica de validação, essa repetição pode melhorar a estimativa de performance do modelo.⁸⁸⁻⁹⁰ Este método subdivide conjunto de dados em treino e teste, várias vezes (a ser determinado pelo operador), e a cada nova subdivisão, moléculas estarão repetidas no conjunto de treino.

Domínio De Aplicabilidade

Uma vez que o modelo foi criado e validado, a próxima etapa é a predição. Um conjunto de moléculas que não possui informações de atividades é utilizado para ser avaliado pelo modelo e ter sua predição realizada. Mas para ter confiança no resultado da predição é imprescindível analisar o domínio de aplicabilidade (APD).

O APD corresponde ao espaço químico que envolve os descritores das moléculas utilizadas na construção do modelo. Dessa forma, o APD fornecerá informações sobre a semelhança entre o que está sendo testado e o que foi utilizado para construir o modelo.⁹¹⁻⁹³ A predição de uma molécula só é utilizável/confiável quando estiver dentro do APD do modelo.⁹¹⁻⁹³

O cálculo do APD é feito a partir de uma varredura de similaridade das estruturas presentes no conjunto de teste em relação ao banco de dados do treino, em que o melhor limiar de similaridade é aquele que apresenta o menor erro e o menor número de moléculas abaixo do limiar. As distâncias euclidianas geralmente são as mais utilizadas para calcular a similaridade química.⁹¹⁻⁹³

Perspectivas

A área da descoberta de medicamentos, em especial o QSAR, está constantemente evoluindo e aderindo a novas tecnologias, marcado pelo aumento do uso de mineração de dados, aprendizado de máquina e automação. Neste contexto, é importante que os químicos e farmacêuticos busquem se familiarizar com essas novas abordagens computacionais. Como explicado por Muratov e colaboradores (2020)¹¹ os químicos que utilizam as novas tecnologias e se adaptam a evolução irão substituir aqueles que não utilizam.

Neste artigo de revisão o nosso principal objetivo foi apresentar de forma didática o aprendizado de máquina aplicado ao QSAR, abordando toda a teoria detalhadamente, como construir modelos, os tipos de modelos, algoritmos, abstraindo a complexidade e bem ilustrada.

Agradecimentos

Os autores agradecem ao CNPq e CAPES pelo auxílio financeiro.

Conflito de Interesse

Os autores declaram não haver conflito de interesse.

Apoio financeiro

A pesquisa foi apoiada pelo Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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CAPÍTULO III

Neste capítulo a partir da construção de um banco de dados com os metabolitos secundários já isolados da família Annonaceae entre os anos de 1970 e 2019, foi realizada uma análise quimiotaxonômica utilizando a classe de diterpenos.

A quimiotaxonomia é definida como um método de classificação taxonômica baseado na similaridade química dos compostos identificados nos organismos/plantas sendo classificados. Assim, buscou-se investigar moléculas químicas que sirvam como marcadores taxonômicos da Annonaceae.

Para a realização de uma análise quimiotaxonômica é comum o uso de aprendizado de máquina incluindo algoritmos de redes neurais, máquinas de vetores de suportes e k-ésimo vizinho mais próximo.

Neste estudo foi utilizado mapas auto-organizáveis (SOM), um tipo de rede neural, a fim de encontrar padrão químico entre diterpenos e três tribos de Annonaceae: Annonaceae, Xylopieae e Miliuseae.

Foi observado que através do SOM houve separações claras e indicativas das três tribos de Annonaceae, sendo diterpenos do tipo kauranos mais encontrados na tribo Annonaceae, clerodanos na tribo Miliuseae, e traquilobanos na tribo Xylopieae.

Desta forma, através deste estudo quimiotaxonômico foi possível separar as tribos Annonaceae, Xylopieae e Miliuseae de acordo com a separação morfológica e taxonômica da família. Esse fenômeno permite prever a localização de um determinado diterpeno nas tribos Annonaceae, Xylopieae e Miliuseae das Annonaceae e buscar esses metabólitos secundários e seus potenciais biológicos de forma mais eficaz.

Secondary Metabolites Extracted from Annonaceae and Chemotaxonomy Study of Terpenoids

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Artigo publicado na revista: Journal of the Brazilian Chemical Society

Doi: 10.21577/0103-5053.20210097

Fator de Impacto: 1.838

QUALIS: A2

Abstract: The Annonaceae family of plants is one of the most anatomically and structurally uniform families. Chemotaxonomy is a common practice to determine the chemical patterns within these families at different phylogenetic levels. The aim of this study was to build a dataset of all the secondary metabolites isolated within the Annonaceae family and to perform the respective chemotaxonomic analysis using self-organizing maps (SOMs). This dataset is composed of 5321 botanical occurrences and 1860 unique molecules present in all subfamilies of the Annonaceae. Diterpenes account for 366 unique compounds and 533 botanical occurrences seen in both Annonoideae and Malmeoideae subfamilies. The Annoneae, Xylopieae and Miliuseae tribes had the highest number of botanical occurrences and were therefore selected for the analysis. Molecular descriptors of the diterpenes and their respective botanical occurrences were used to generate the SOMs. These SOMs demonstrated clear and indicative tribe separations, with a match rate higher than 70%. Our results corroborate with the morphological and molecular data. These models can be used to predict the phylogenetic location of certain diterpenes and to accelerate the research of Annonaceae secondary metabolites and their biological potentials.

Keywords: Annonaceae, Secondary Metabolites, Diterpenes, Chemotaxonomy, Self-Organization Maps.

Introduction

The Annonaceae family was first described by Antoine Laurent de Jussieu in 1789 and is known for its striking anatomical and structural uniformity. The family is very consistent morphologically, with a unique primitive group of angiosperms providing easy identification.^{1–4}

Two recent studies relevantly discuss the phylogenetic classification of the Annonaceae family. The first study carried out by Chatrou *et al.*⁵ used eight plastid markers and representatives of 94 genera to formally and scientifically classify the Annonaceae into four subfamilies: Anaxagoreoideae, Ambavioideae, Annonoideae and Malmeoideae. The two largest subfamilies, Annonoideae and Malmeoideae, were divided into 14 tribes. The second study was conducted by Guo *et al.*⁶ and considered the phylogenetics of the Annonaceae based on a super matrix of eight chloroplast loci and 749 accessions representing 705 species (29% of ca. 2,400 species of 105 genres; 98% of 107 genres currently accepted). This matrix included almost four times more species as well as representatives of 15 additional genera compared to the first large study of phylogenetic importance by Chatrou *et al.*⁵

In addition to rebuilding the most comprehensive Annonaceae evolutionary tree, Guo *et al.*⁶ also determined the phylogenetic position of five genera, Bocageae, Boutiquea, Cardiopetalum, Duckeanthus and Phoenicanthus, that were not included in any previous phylogenetic reconstruction. Their work assessed the monophyletic status and phylogenetic relationships within each major clade highlighting possible non-monophylides of genera and evaluating alternative resolutions for nomenclatural problems. Additionally, they identified and discussed unresolved problems such as the phylogenetic location and taxonomy of two genera, Froesiodendron and Melodorum, which have not yet been sampled. Finally, they provided an updated view of the genera currently recognized in the family using their wealth of species.

Overall, Guo *et al.*⁶ reorganized the phylogenetics and taxonomy of Annonaceae and concluded their study stating that the family contains four subfamilies, 15 tribes, 107 genera and 2400 species.

Annonaceae are very important economically given the multitude of ways the derivatives are used; the fruits are used in cooking and the production of ropes, the great diversity of chemical compounds shown to have pharmacological activities inspire new medicines, and the wood that is both light and durable.^{7–9} These chemical compounds, also

known as secondary metabolites, have great structural diversity in this family and represent many chemical classes including but not limited to alkaloids, terpenes, acetogenins, and steroids.^{10–12}

One of the most common classes of Annonaceae is the terpenes. Terpenes are a very diverse class of substances and in addition to their important natural defense mechanisms in plants, terpenes display several therapeutic uses for humans.^{9,13}

In the natural biosynthetic route, terpenes are formed from isoprene units, which are considered the basic units for the formation of both terpenes and steroids. Subclasses of terpenes include monoterpenes (two isoprene units - 10 carbons in their structure), sesquiterpenes (15 carbons), diterpenes (20 carbons) and triterpenes (30 carbons).^{9,14}

The information gathered from chemical structures of both different species and genera has been and continues to be used in chemotaxonomy, that is, to determine the chemical phylogenetic patterns of a given family.^{15–17} For chemotaxonomy studies, it is common practice to use machine learning with either supervised or unsupervised algorithms. A few examples of these machine learning techniques include Neural Networks (NN), Support Vector Machine (SVM) and k-Nearest Neighbors (k-NN).^{15–17}

Self-organizing maps (SOMs), which were developed by Kohonen,¹⁸ are the main algorithm used in this study. A SOM is an unsupervised neural network that recognizes patterns and performs groupings based on exploratory analysis of the input data to generate non-linear relationships.^{18–20} The SOM learning phase is competitive as there is no convergence or minimization criteria, and it works with a defined number of iterations and weight adjustments. In addition, each variable is mapped in a finite space of neurons organized in a typically two-dimensional arrangement (Kohonen Map).^{19–21}

In order to generate the SOM model, the model must first be trained on a portion of the established data previously separated for training. Then, the second set called the test set evaluates the training of the model. Using the results from the test set evaluation, we then isolate models capable of correctly mapping the test set, since the test data instances are not present in the training data,^{20–24}

Vesanto *et al.*²⁵ created a unified distance matrix (U-Matrix) that uses Euclidean distances to further analyze the SOM. In this matrix, it is possible to better visualize the possible groupings of the analyzed data.^{25–27}

The goal of this study is to compile and integrate secondary metabolites isolated from Annonaceae into one curated dataset and to perform a chemotaxonomic analysis of diterpenes.

Results and Discussion

We collected and processed all Web of Science-indexed research papers published between 1970 and 2019 to create a database of secondary metabolites isolated from Annonaceae, except for the acetogenin class that is exclusive to this family. As seen in figure 1, the interest in studying the Annonaceae plants has grown over time. One explanation for this growth is the abundant and diverse biological activity of the Annonaceae that comes from the structural diversity of the secondary metabolites. Alkaloids, for example, exhibited a wide variety of pharmacological activities and have been clinically studied for the treatment of cancer, Parkinson's disease, cardiovascular diseases, and various viral infections.^{1-4,8,28,29}

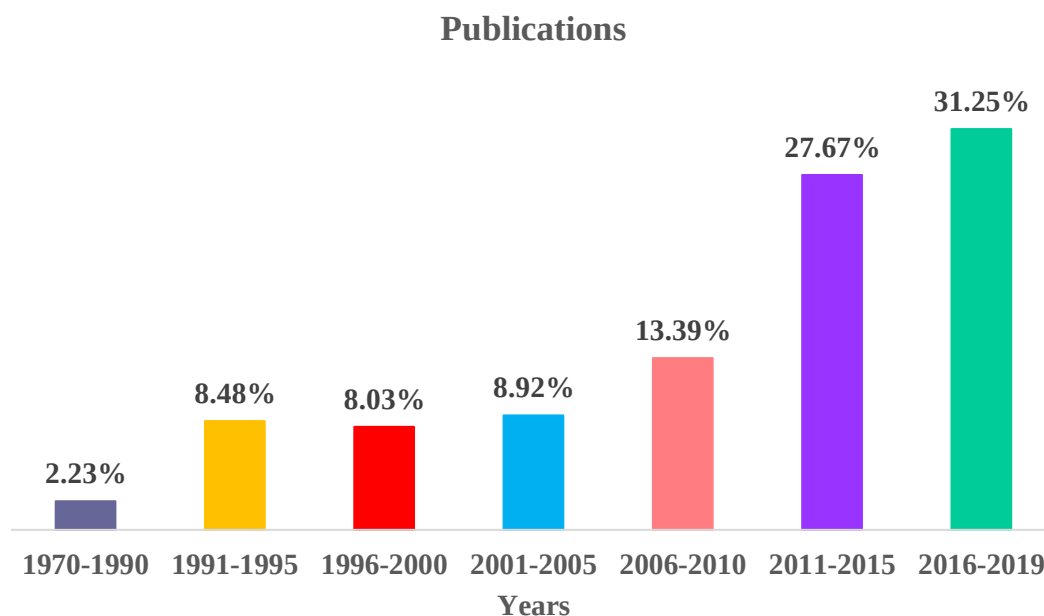


Figure 1: Distribution of published phytochemical studies of the Annonaceae plant family over time.

Our database consisted of 5321 botanical occurrences and 1860 unique molecules present in all subfamilies, 12 tribes, 64 genera and 380 species of the Annonaceae. Terpenes and alkaloids are the largest classes present in these plants (figure 2).

It is important to note that although Annonaceae has 107 genera and 2400 species, only a small percentage of them have been studied chemically and therefore our database was considered comprehensive.

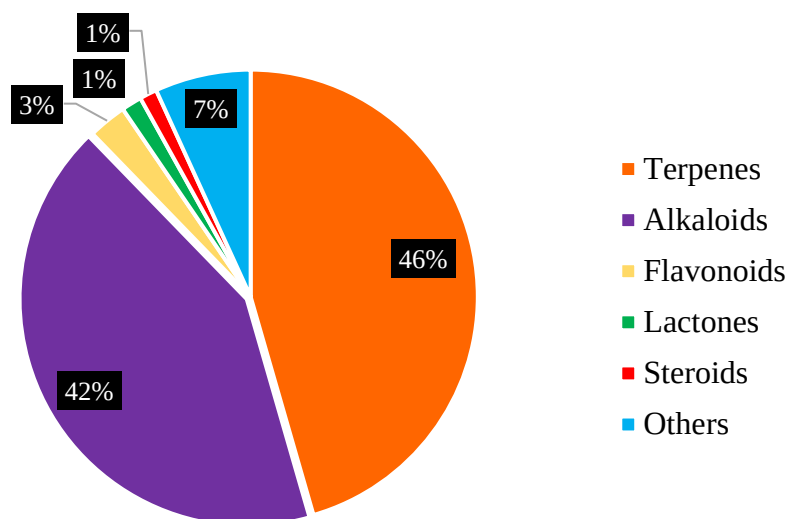


Figure 2: Secondary metabolite classes isolated from the Annonaceae family.

The alkaloids present in the Annonaceae are isoquinolines but the biosynthetic origins of the main nuclei occurring in the Annonaceae are the simple isoquinoline, proaporphine, aporphine, benzyloisoquinoline, protoberberine, and phenanthrene.^{30,31}

Terpenes, the second most common class in Annonaceae, occur in all subclasses (mono-, di-, sesqui-, and triterpenes), with the diterpenes being the most abundant. The most frequent diterpenes are kaurene, trachylobane, labdane, and atisane, wherein kaurane is the most common. Figure 3 shows the skeletons of some of the most present alkaloids and diterpenes in this family.

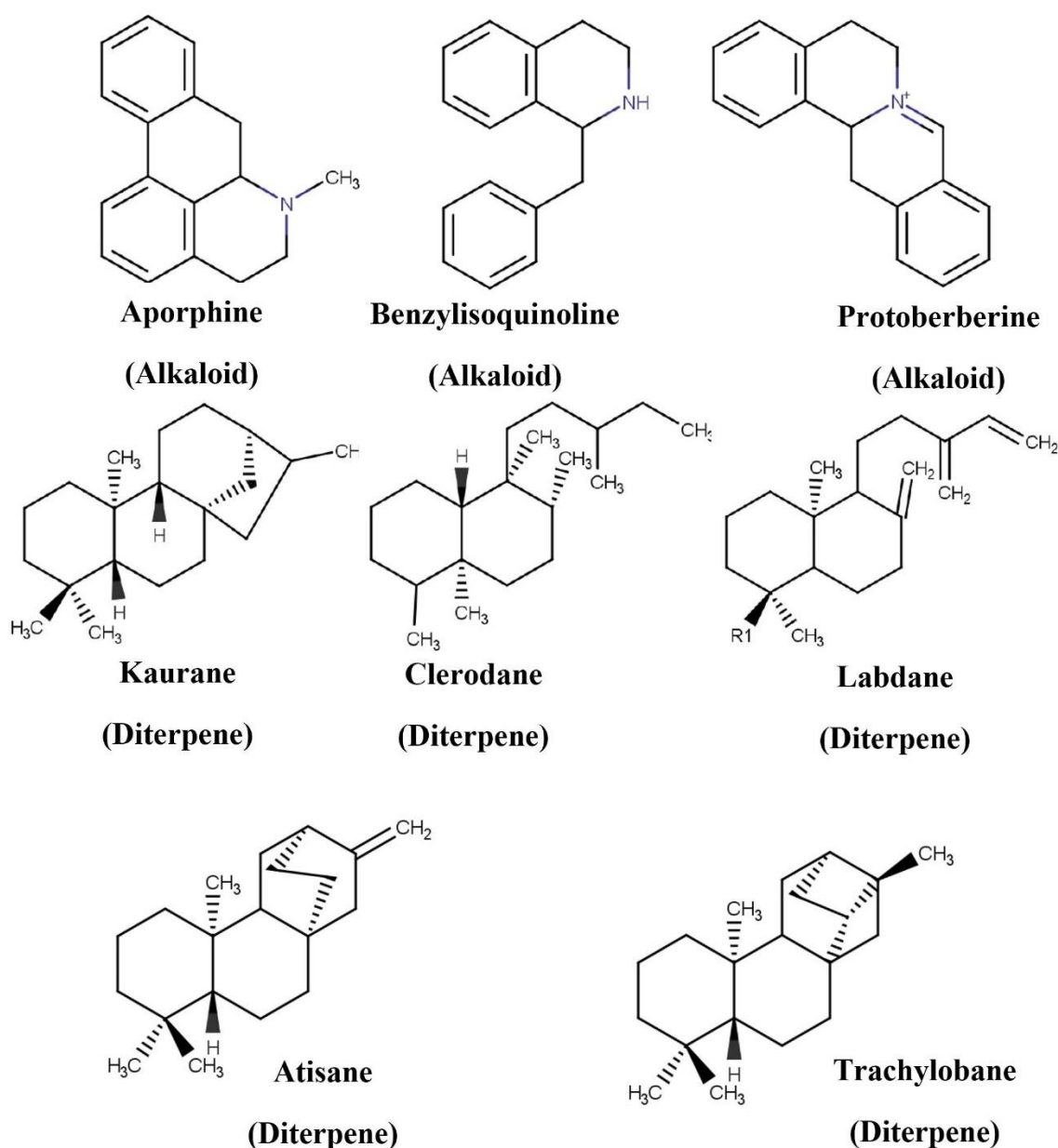


Figure 3: Skeletons of the most abundant alkaloids and diterpenes in the Annonaceae family.

Once the database was compiled and the classes and skeletons of the secondary metabolites most present in Annonaceae were identified, the chemotaxonomic analysis was performed.

Chemotaxonomy is defined as a taxonomic classification method based on the chemical similarity of compounds identified in the organisms/plants being classified.³² Thus, we sought to investigate chemical molecules that serve as taxonomic markers of the Annonaceae.

Given the assortment of the secondary metabolites collected, the terpenes were

selected for the chemotaxonomic studies because they were the predominant class (46% of metabolites). As mentioned earlier, terpenes can be classified into mono-, di-, sesqui-, and triterpenes.

Among these four subclasses, about 50% of the terpenes were diterpenes . Annonaceae diterpenes have promising anti-inflammatory activity, making compounds of this class excellent candidates for clinical trials in anti-inflammatory therapy.³³

Diterpenes represented a total of 366 unique chemical structures and 533 botanical occurrences; a botanical occurrence indicates that the compounds are present in several species.

These 533 botanical occurrences are distributed in two subfamilies, Annonoideae and Malmeoideae, which are the largest subfamilies of the Annonaceae and are distributed in 8 tribes, 13 genera and 50 species. The phylogenetic classification of the Annonaceae family proposed by Guo *et al.*⁶ was utilized.

The three tribes with the highest number of botanical occurrences and molecules were then selected for the self-organizing neural maps, as the high number of diterpenes allows for the recognition of chemical pattern among the tribes. These tribes were Annoneae, Xylopieae and Miliuseae, and table 1 contains the botanical characteristics and quantities of the selected molecules.

The genera represented in each selected tribe are: *Annona* (Annoneae), *Xylopia* (Xylopieae), *Polyalthia*, *Pseudouvaria*, *Piptostigma* and *Greenwayodendron* (Malmeoideae). Malmeoideae is the most studied genera of these tribes.

Table 1: Botanical characteristics and occurrences of the diterpenes of the tribes Annoneae, Xylopieae and Miliuseae.

Tribes	Subfamily	Genus	Species	Diterpenes	Occurrences
Annoneae	Annonoideae	1	11	150	179
Xylopieae	Annonoideae	1	14	179	241
Miliuseae	Malmeoideae	4	13	95	101
					TOTAL: 521

For the 521 molecules of the three selected tribes, molecular descriptors were calculated using the DRAGON 7.0 software,³⁴ which has 5270 descriptors organized in 30 logic blocks. From these three blocks of descriptors, 60 molecular descriptors were selected

to consider ring descriptors, functional groups, and fragments of central atoms.

The botanical occurrences were classified in the three selected tribes and the values of the 60 molecular descriptors were used as input data in the SOM Toolbox software.²⁵ The self-organized matrix of diterpenes was then generated, classified into the three aforementioned tribes according to the chemical similarity between them. Then, the classification generated was compared with the phylogenetic classification proposed by Guo *et al.*⁶ The phylogenetic classification of Guo *et al.*⁶ can be seen in figure 4.

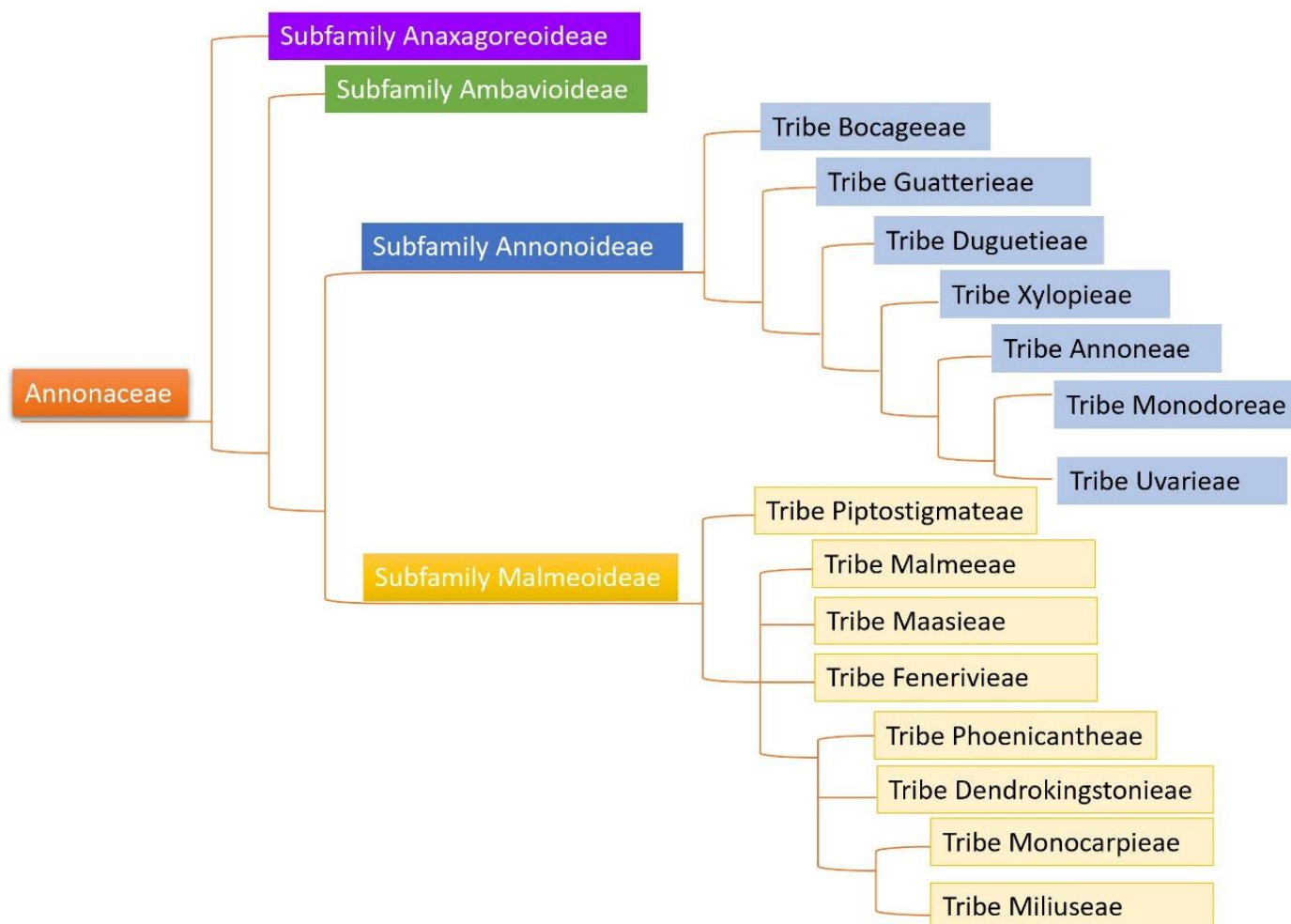


Figure 4: Phylogenetic diagram of the Annonaceae family (Adapted from Guo *et al.*⁶).

In the generated maps, the hit rate using the two types of DRAGON 7.0 descriptors was > 77%. Thus, the 5-fold validation was performed for the generated SOM model, in which the diterpenes were divided into five training groups and five test groups, always maintaining the proportion of molecules from the three tribes (Annoneae, Xylopieae and Miliuseae). The results of the validation are described in table 2.

Table 2, like table 3, also describes the accuracy values for each training and test.

Accuracy provides us with information about the overall performance of the model, indicating the overall hit rate. The values of this metric vary between 0 and 1, and the closer to 1 it indicates that the model is getting more correct in its classification of molecules in terms of their tribes, that is, correctly classifying a molecule of the Annoneae tribe in the Annoneae tribe. Models with an accuracy greater than 0.70 are already considered models of excellent performance.²⁴

Table 2: Accuracy statistics of the training and tests groups the 5-fold cross-validation of the Self-Organizing Map from the Annoneae, Xylopieae and Miliuseae tribes.

Tribes	Training 1	Training 2	Training 3	Training 4	Training 5	Average
Annoneae	0.89	0.90	0.80	0.80	0.78	0.83
Miliuseae	0.90	0.86	0.86	0.90	0.90	0.88
Xylopieae	0.77	0.76	0.85	0.86	0.85	0.82
Accuracy	0.83	0.83	0.83	0.84	0.84	0.83
Tribes	Test 1	Test 2	Test 3	Test 4	Test 5	Average
Annoneae	0.83	0.70	0.77	0.86	0.70	0.77
Miliuseae	0.90	0.94	0.85	0.95	0.80	0.88
Xylopieae	0.71	0.89	0.83	0.67	0.85	0.79
Accuracy	0.79	0.83	0.81	0.79	0.78	0.80

After analyzing table 2, it is observed that the hit rate was overall > 70%, with the best hit rate of 95% for the Miliuseae tribe. The average hit rate of the test sets was 80% and is very close to the average hit rate for the training, which was 83%, revealing not only the good predictive power of the model, but that the model is robust. The applicability domain was also analyzed and was > 99% of the predictions of the test sets.

To verify the tribes dependence on chemical similarity and the ability to separate them accordingly, chemotaxonomy analysis was performed using other machine learning algorithms such as the Support Vector Machine (SVM) and the k-nearest neighbors'

algorithm k-NN, in addition to neural maps generated using the fingerprint descriptors calculated by the DRAGON 7.0 software. The results are shown in Table 3 for this SOM analysis of the Annoneae, Xylopieae and Miliuseae tribes and like those in Table 2, the hit rates are excellent.

Table 3: Summary of test averages corresponding to 5-fold cross-validation using the different machine learning algorithms and Self-Organizing Map (SOM) with the fingerprint descriptors for the Annoneae, Xylopieae and Miliuseae tribes.

Tribes	SOM Molecular descriptors	SOM fingerprint descriptors	SVM ^a	k-NN ^b
	Average	Average	Average	Average
Annoneae	0.766	0.77	0.70	0.70
Miliuseae	0.88	0.92	0.81	0.85
Xylopieae	0.79	0.89	0.87	0.81
Accuracy	0.80	0.85	0.80	0.78

^aSupport Vector Machine; ^b k-nearest neighbors' algorithm.

To visualize the generated SOM, we utilize a U-matrix and display it alongside a principal component analysis (PCA) which was developed from the correlation matrix of the database used in the generation of SOM. PCA is measured using eigenvectors with higher eigenvalues. In the projection of the PCA, the neighboring map units are connected by lines to make the visualization of the data on the map more clear and defined. The PCA performed has an explained variance of 37.04%, that is, using only two variables it is possible to visualize one third of the entire variance.

Figure 5 shows the U-matrix of the generated SOM where we can see a chemical pattern separating the three tribes Annoneae (blue), Xylopieae (red) and Miliuseae (green), which are best observed in the principal component analysis chart (PCA).

PCA

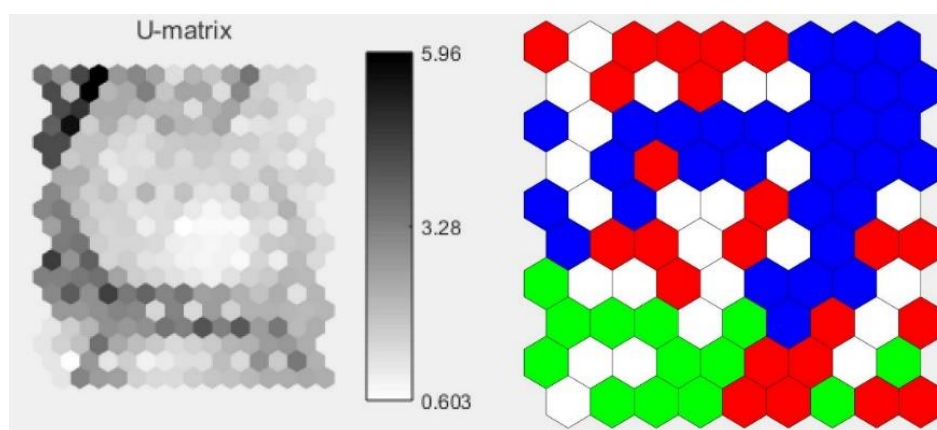


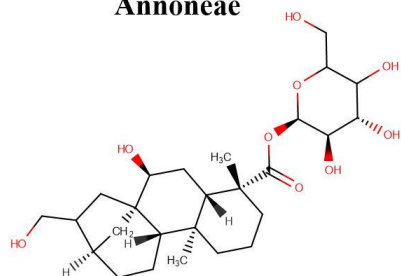
Figure 5: Visualization of the SOM of Annonaceae diterpenes data. In the upper corner we have the U-matrix. The left U-matrix does not identify the tribes while the right U-matrix identifies the tribes by color; Annoneae is blue, Xylopieae is red, and Miliuseae is green. The values shown on the scale between the two U-matrix represent the values of the molecular descriptors of the diterpenes, varying between 0.603 and 5.96. These values were used to group the diterpenes by tribes. At the bottom, we have the PCA projection of the SOM measured by its two eigenvectors with higher eigenvalues. The tribes were plotted using the same identification colors as the U-matrix.

We can see that the Miliuseae tribe, despite having the fewest number of diterpenes and, consequently, the fewest botanical occurrences, was the tribe with the best hit rates (greater than 85% in all algorithms and different descriptors in SOM) and is more structurally distant from the Annoneae and Xylopieae tribes, corroborating Guo's⁶ phylogenetic classification, seen in figure 4.

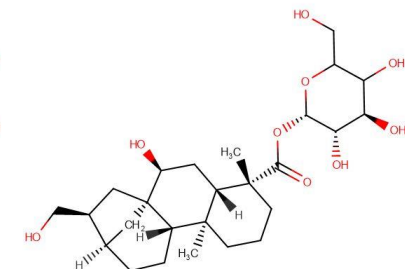
Annoneae and Xylopieae are part of the same subfamily, Annonoideae, explaining the proximity of the two tribes in the SOM, while Miliuseae is part of the Malmeoideae subfamily, and is therefore further away. When observing the diterpenes present in the tribes present in the SOM (figure 6), we can see that each tribe has a higher frequency of a certain subtype of diterpene. The subtypes present in the Annoneae and Xylopieae tribes, although different, maintain a certain chemical similarity in their skeletons, explaining once again the approximation of these two tribes in the SOM.

Figure 6 shows some of the isolated diterpenes in each of the analyzed tribes, focusing on the most frequent skeletons identified from each tribe. The Miliuseae tribe has a clerodane subclass of diterpenes. The clerodane diterpene is able to undergo structural changes and generate some subtypes,³⁵ and the kolava subtype is present in the Miliuseae tribe. The Annoneae and Xylopieae tribes have kaurane and trachylobane diterpenes, respectively. Although different, these subclasses similarities in their chemical skeletons, even further supporting the closeness of the two tribes in the SOM.

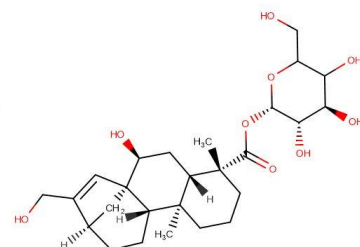
Annoneae



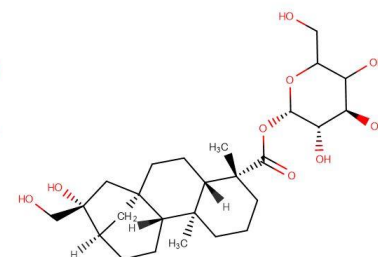
7β,17-dihydroxy-ent-kaur-15-en-19-oic acid 19-O-β-D-glucopyranoside ester



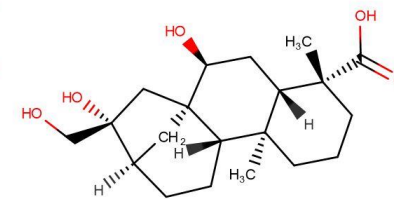
7β,17-dihydroxy-16α-ent-kauran-19-oic acid 19-O-β-D-glucopyranoside ester



7β,17-dihydroxy-ent-kaur-15-en-19-oic acid 19-O-β-D-glucopyranoside ester

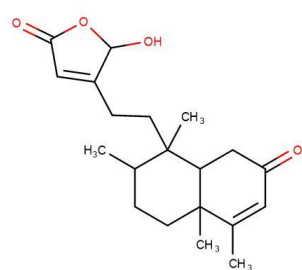


Paniculose IV

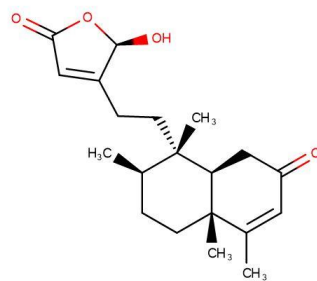


7β,16α,17-trihydroxy-ent-kauran-19-oic acid

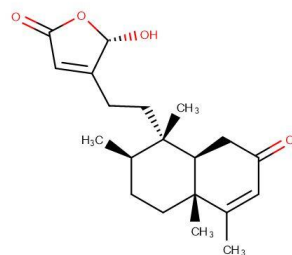
Miliuseae



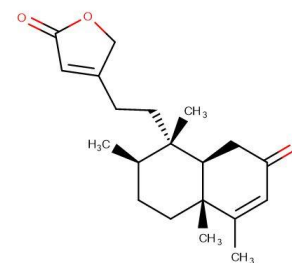
Cleroda-3-13-dien-15-16-olide-16-e-hydroxy-2-oxo



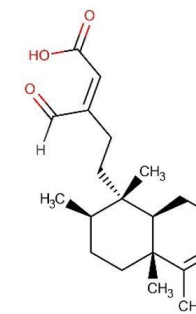
(16R)-hydroxy-Kolava-3-13-cis-dien-16-15-olide-2-one



(16S)-hydroxy-Kolava-3-13-cis-dien-16-15-olide-2-one

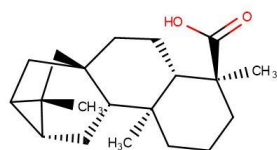


Kolava-3-13-cis-dien-16-15-olide-2-one

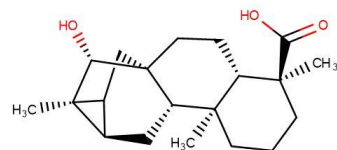


Kolava-3-13-cis-dien-15-al-16-oic acid

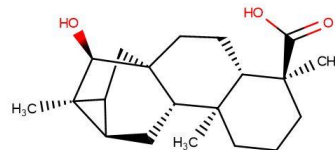
Xilopieae



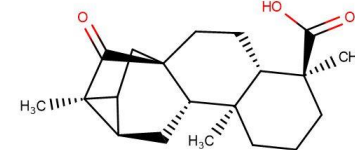
(-)-Trachyloban-19-oic acid



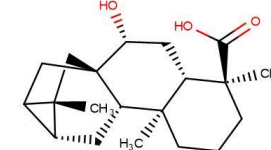
15-α-hydroxy-Trachyloban-19-oic acid



15-β-hydroxy-(-)-Trachyloban-19-oic acid



15-oxo-(-)-Trachyloban-19-oic acid



7-α-hydroxy-(-)-Trachyloban-19-oic acid

Figure 6: Diterpenes of the most present subtypes in the Annoneae, Xylopieae, and Miliuseae tribes.

The most significant descriptors in the separation of each cluster (each tribe in SOM) are represented in figure 7. For the Annoneae tribe, the descriptors that presented a high value were 1) NROH, which describes hydroxyl groups (OH) linked to aliphatic groups, 2) nOHp descriptor that points to primary alcohols, 3) C-006, which indicates CH₂ carbons attached to a radical and that radical attached to an OH, and 4) the descriptor O-056 that describes the alcohol function. Thus, these descriptors report that the diterpenes of this tribe are distinguished by the large number of hydroxyls in their chemical structure (figure 6).

For the Xylopieae tribe, the most representative descriptors were nCIR, which indicates the number of circuits (rings / cycles connected to each other) present in the molecule, the RFD descriptor of ring melting density, and the RCI descriptor that provides information about the ring complexity of the molecule. These descriptors point to the presence of molecules with a large number of interconnected rings/cycles; as seen in figure 6, the diterpenes of this tribe have many interconnected rings/cycles and a certain degree of complexity.

For the Miliuseae tribe, the descriptors with the highest values were nConj, a descriptor that expresses the presence of non-aromatic C conjugates (sp²), NNRS, the normalized number of ring system, which accounts for both the ratio between the number of ring systems (NRS) and the cyclomatic number (nCIC - discriminates cyclic compounds from acyclics) to provide information related to the presence of aromatic rings in the chemical structure, and lastly the ARR descriptor. The ARR, aromatic ratio, is the ratio of the number of aromatic bonds to the total number of bonds in the molecule. These descriptors reveal that the diterpenes of this tribe have an aromatic ring and conjugated non-aromatic bonds, which can also be seen in figure 6.

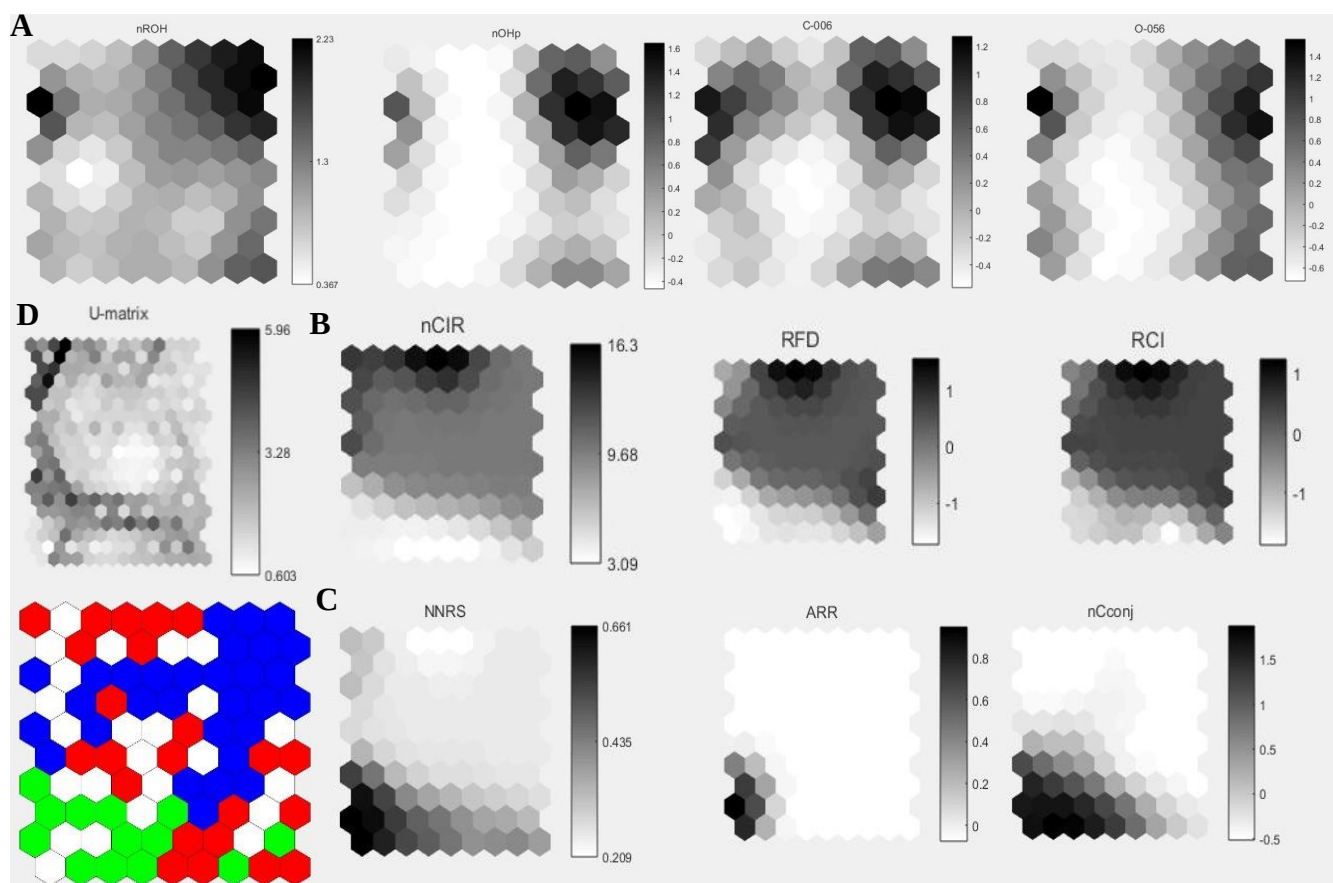


Figure 7: The most significant descriptors for the Annoneae, Xylopieae and Miliuseae tribes. In A we have the U-matrix for the four most significant descriptors in the grouping of the diterpenes of the Annoneae tribe. In B, the U-matrix is shown for the three most significant descriptors of the Xylopieae tribe. C shows the U-matrix for the three most significant descriptors of the Miliuseae tribe. Finally, in D we have the U-matrix of the self-organizing map generated in the study, with the upper U-matrix not identifying the tribes and the lower U-matrix identifying the tribes by color; Annoneae is blue, Xylopieae is red, and Miliuseae is green.

An article by Scotti *et al.*¹⁵ constructed a SOM with nuclear magnetic resonance (NMR) data of 118 diterpenes from three genera of the Annonaceae, the genera *Xylopia*, *Polyalthia* and *Annona*. The SOM was able to separate the diterpenes of the three genera with the NMR data and specific chemical displacement values of ^{13}C were observed for the skeletal carbons of each type of diterpenes of each genus. Kauranes skeletons were found for *Annona*, while trachylobans were found for *Xylopia* and clerodanes were found for *Polyalthia*.

Review papers concerning the *Annona* genus and some of its species have suggested that ent-kauranes are the most abundant diterpenes.^{36–38} A review by Barbosa and Vega,⁹ highlights that diterpenes are the second most common class of secondary metabolites in species of the *Xylopia* genus, with kaurane, labdane, atisane and trachylobane diterpenes

being the most frequent. Of these, trachylobanes are considered as chemotaxonomic markers of *Xylopia* as they are the most abundant in *Xylopia* and are difficult to find elsewhere in Annonaceae.^{9,39}

The four genera selected from the Miliuseae tribe are those with the most phytochemical studies, with the *Polyalthia* and *Pseuduvaria* genera being the most chemically and biologically studied of the tribe. As in the other genera, there are studies in the literature that show that the most isolated diterpenes of *Polyalthia* and *Pseuduvaria* species are clerodanes.^{40–43}

Conclusions

This literature corroborates the information obtained in this study. In this way, this study of Annonaceae diterpenes establishes a way to separate the Annoneae, Xylopieae and Miliuseae tribes in accordance with the family's morphological and taxonomic separation. This phenomenon makes it possible to predict the location of a certain diterpene in the Annoneae, Xylopieae and Miliuseae tribes of the Annonaceae and to search for these secondary metabolites and their biological potentials more effectively.

Methodology

Construction of the Annonaceae database

The articles used for the construction of the database were selected by means of an electronic search in the Web of Science research base, and using this the database was composed of studies and literature reviews on secondary metabolites isolated in plants of the Annonaceae. The following terms were used in the search for scientific articles: Annonaceae, secondary metabolites, terpenes, alkaloids, flavonoids. All secondary metabolites, the species from which they were isolated, and the geographic locations will be registered on the SISTEMATX⁴⁴ web tool located at: <http://sistemtx.ufpb.br> and developed by the Chemistry Laboratory of the Postgraduate Course on Natural and Bioactive Synthetic Products.

Obtaining structures in three dimensions of compounds

For all structures, SMILES codes were used as input data for Marvin v. 19.27.0.⁴⁵ It also used the Standardizer software⁴⁶ which made it possible to convert the various chemical structures into personalized canonical representations. This standardization is extremely important to create libraries of consistent compounds, in addition to canonizing the structures, adding hydrogens, aromatizing molecules, generating the 3D structures, and saving the compounds in SDF format.

Obtaining the molecular descriptors

Molecular descriptors are used to calculate the physicochemical properties of the molecules of each set of molecules. To obtain the molecular descriptors, the DRAGON 7.0 program³⁴ was used.

The DRAGON 7.0 software³⁴ can calculate 5270 molecular descriptors, covering several approaches. These molecular descriptors are arranged in 30 logic blocks.³⁴ Of the 30 blocks of molecular descriptors available in the Dragon 7.0 software,³⁴ only the ring descriptors, functional groups, and fragments of central atoms blocks were selected.

Pre-processing of data

In this step, the variables / descriptors were selected. This selection tactic is used to identify those descriptors that are most important for the grouping of the diterpenes and in this case were mostly related to the tribes. The selection of descriptors is an important step that must be carried out before the generation of the model, since it is useful for reducing the dimensionality of the data, helping to obtain a generic and not over-adjusted model, reducing computational cost, simplifying extraction processes and transformation of data, and further simplifying the presentation and demonstration of data.⁴⁷ In short, this step helps to reduce overfitting, increases the accuracy of the model, and reduces training time.

The pre-treatment criteria removed descriptors that had equal values in the series, ones that only a different value, and ones that had a correlation greater than 0.99. The majority of descriptors end up being removed, as many were inter-correlated, such that the independent variable remained the most correlated with the dependent variable.

Self-organizing maps (SOMs)

For the realization of the neural maps, the selection of molecular descriptors was performed for the bank of isolated molecules of the Annonaceae. The functional group, central atom, and ring descriptors were selected. Then, the constant variables for each block of descriptors and those with a different value in the series were excluded.

The molecular descriptors selected were analyzed with SOMs in Matlab 6.5 and SOM Toolbox 2.0.^{25,26,48} The SOM Toolbox tool is a set of Matlab functions that can be used for the elaboration and implementation of neural networks, since it contains functions for the creation, visualization, and analysis of self-organizing maps. The data set was presented to the network before any adjustments were made. Subsequently, the data group was partitioned according to the regions of the weight vectors of the map, in each training stage. Then the correct prediction of these sets and the total correct predictions of the compounds were evaluated. In the most relevant models, the set was divided into training and test sets to assess the forecasting capacity. Training and test performance were assessed by calculating the proportion of the number of samples correctly classified by SOM. For each map, 5 cross-validations were performed, being partitioned into 80% training and 20% testing. In the SOM, sites containing molecules for each descriptor were identified to highlight existing chemical patterns.

SVM and k-NN models

Knime 3.6.2 software⁴⁹ was used to perform all the following analyzes. The class descriptors and variables were imported from the Dragon 7.0 software³⁴ and, for each, the data was divided into the “partitioning” node with the “stratified sample” option to create a training set and a set of tests, covering 80% and 20% of the compounds, respectively. Although the compounds were selected at random, the same proportion of active and inactive samples was maintained in both sets. Two models were generated using the Support Vector Machine (SVM) algorithm⁵⁰ and the K-nearest neighbors’ algorithm (k-NN).⁵¹ An external cross-validation was modeled 5 times.

SVM is a supervised machine learning algorithm that analyzes data and recognizes patterns.^{50,52} The parameters selected for the SMV for all the models generated were polynomials, with power 1.0, bias 1.0, and range 1.0.

K-NN consists of instance-based machine learning as the function and is approximated only locally (neighbors) so the entire calculation is postponed until

classification.^{52,53} It is a technique that gives weight to the contributions of neighbors, so that the closest neighbors contribute more to the average than the more distant ones.^{51–53} The parameters selected for the SVM for all the generated models were $k = 3$.

Acknowledgments

We thank the CNPq for financial Support, grant numbers 309648/2019-0 and 431254/2018-4.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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The background of the page features a decorative border at the bottom. This border consists of a network of interconnected nodes and lines, resembling a molecular structure or a complex network diagram. The nodes are colored in shades of blue, purple, and green. Interspersed among these network elements are several faint, light-colored icons, including what appears to be a camera or a similar device. The overall aesthetic is scientific and technological.

CAPÍTULO IV

A doença de Chagas é uma das principais doenças tropicais negligenciadas, endêmica em 21 países latino-americanos. Particularmente prevalente no Brasil, a doença de Chagas é responsável por cerca de 10 mil mortes por ano na América do Sul. É um problema de saúde pública e tem se tornado ainda maior por se expandir para áreas não endêmicas, como América do Norte e Europa, devido ao movimento migratório de pessoas infectadas.

A infecção é causada pelo *Trypanossoma cruzi*, um protozoário flagelado, que é transmitido para os seres humanos a partir de insetos vetores, principalmente pelo inseto *Triatoma infestans*, conhecido como “barbeiros”.

Não há vacina contra a doença de Chagas, e os medicamentos utilizados no tratamento da doença, Benznidazol e Nifurtimox, apesar de serem pouco eficazes na fase crônica da doença, eles exibem sérios efeitos colaterais. Além de já haver estudos comprovando cepas resistentes a esses medicamentos. Desta forma, a busca por novos drogas para o tratamento da doença de Chagas é extremamente relevante. Neste contexto os produtos naturais se destacam como fonte promissora de novos fármacos, com destaque para a família Annonaceae.

Diante do exposto, o objetivo desse estudo foi utilizar de técnicas computacionais, a triagem virtual baseada no ligante (LBVS), para obter as estruturas de potenciais moléculas antichagásicas de um banco de dados de metabólitos secundários de Annonaceae e testar as moléculas mais promissoras *in vitro*.

Por meio da triagem virtual baseada em ligantes, foram identificadas 57 moléculas com potencial atividade contra a forma epimastigota do *T. cruzi*. Em seguida, 16 moléculas foram analisadas no estudo *in vitro*, das quais, seis moléculas apresentaram atividade antiepigastigota até então desconhecida. Também foi avaliado se essas seis moléculas possuem atividade anti-amastigota e anti-tripomastigota. Foi observado que todas as seis moléculas têm atividade potencial contra a forma amastigota, mas nenhuma molécula foi ativa contra a forma tripomastigota. O ácido 13-epicupressico parece ser o mais promissor, pois foi previsto como composto ativo no estudo *in silico* contra a forma amastigota do *T. cruzi*, além de possuir atividade *in vitro* contra a forma epimastigota.

Natural Products from Annonaceae as Potential Antichagasic Agents

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Artigo publicado na revista: ChemMedChem

Doi: doi.org/10.1002/cmdc.202200196

Fator de Impacto: 3.540

QUALIS: A2

Abstract: Chagas disease, a neglected tropical disease, is endemic in 21 Latin American countries and particularly prevalent in Brazil. Chagas disease has drawn more attention in recent years due to its expansion into non-endemic areas. The aim of this work was to computationally identify and experimentally validate the natural products from an Annonaceae family as antichagasic agents. Through the ligand-based virtual screening, we identified 57 molecules with potential activity against the epimastigote form of *T. cruzi*. Then, 16 molecules were analyzed in the *in vitro* study, of which, six molecules displayed previously unknown antiepipimastigote activity. We also evaluated these six molecules for trypanocidal activity. We observed that all six molecules have potential activity against the amastigote form, but no molecules were active against the trypomastigote form. 13-Epicupressic acid seems to be the most promising, as it was predicted as an active compound in the *in silico* study against the amastigote form of *T. cruzi*, in addition to having *in vitro* activity against the epimastigote form.

Keywords: Natural Products, Annonaceae, Ligand-Based Virtual Screening, Machine Learning, Antichagasic activity

Introduction

Trypanosoma cruzi is a single-celled protozoan that causes Chagas disease.^{1–3} Chagas disease, a neglected tropical disease, is endemic in 21 Latin American countries. Particularly prevalent in Brazil, Chagas disease is responsible for the death of around 10,000 people every year in South America.^{3–7} According to the World Health Organization (WHO), up to seven million people worldwide are infected by *T. cruzi*, characterizing it as an important health and socioeconomic problem.^{4,6,7} And this problem has been getting bigger and more attention in recent years due to its expansion to non-endemic areas, such as North America and Europe, due to the increase in the migratory flow of infected people.^{4,6,7}

The *T. cruzi* parasite is transmitted to humans by domiciled hematophagous triatomine vector insects, mainly *Triatoma infestans* and *Rhodnius prolixus*.^{8,9} Although recent vector control programs such as the National Program for the Control of Chagas Disease have helped to reduce infection rates in endemic areas, non-vector transmission routes such as contaminated food, blood transfusions and congenital transmission are becoming most common.^{3,4,7,9,10}

Vector transmission occurs when the insect feeds on the blood of an infected mammal and ingests the circulating trypomastigote form of *T. cruzi*. Once inside the midgut of the insect, *T. cruzi* differentiates into its replicative epimastigote (epim.) form.^{6,9,11–14} After replication, epim. differentiate into infectious trypomastigotes, this parasitic form will be eliminated by the insect vector through its feces and urine during the bite.^{15,16} Then, when inoculated into human or other mammalian hosts, trypomastigotes enter the bloodstream and infect different types of cells.^{9,11} The trypomastigote form differentiates into the amastigote form once inside host cells and replicates in the cytoplasm for 3–5 days before differentiating again into highly motile trypomastigotes still within the cells.^{9,11} Trypomastigotes cause the lysis of infected cells and therefore spread the infection by entering new cells or being ingested by the vector.^{6,9,11–13}

There is no vaccine against Chagas disease and the available treatment, benznidazole and nifurtimox, have limited efficacy in the chronic phase of the disease and exhibit serious side effects.^{8,9} For this reason, the search for new drugs for the treatment of Chagas disease is extremely relevant. And natural products stand out as a promising source of new drugs, since so many therapeutic drugs are natural products or inspired by their chemical structures.¹⁷

The Annonaceae family stands out for being one of the richest families in the Magnoliales order, with roughly 107 genera and 2400 species currently recognized.¹⁸ Annonaceae has a great structural diversity of secondary metabolites present in its species, a fact that results from the biogenetic diversity of the family.¹⁹ Alkaloids and acetogenins, for example, have exhibited a wide variety of pharmacological activities and have been clinically studied for the treatment of cancer, Parkinson's, cardiovascular disease, and various viral infections.^{20–26} Standing out then as a promising family for the search for natural products as antichagasic agents.

The use of computational techniques for the discovery and development of new drugs has become increasingly common, since through *in silico* techniques there is a reduction in costs, time, and waste of material.^{27–29} One of the most prominent techniques in drug discovery is virtual screening, which seeks to select potentially active compounds based on computational predictions and is divided into two techniques: ligand-based virtual screening (LBVS) and structure-based virtual screening (SBVS). In LBVS, the technique used in this work, there is information on chemical structures that have scientifically proven biological activity. From this, molecular descriptors can be calculated and, with the use of computational algorithms, the explanation that correlates the relationship between chemical structure and biological activity will be sought. This information will be extremely important so that predictions can be made and thus find molecules with active potential against the target disease.^{29–32}

The aim of this work was to use LBVS to obtain the structures of potential antichagasic molecules from an Annonaceae secondary metabolite database and to test the most promising molecules *in vitro*.

Results and Discussion

For the construction of predictive models, chemical structures with known activity for *T. cruzi* were selected from the ChEMBL database.[33] The dataset was subdivided into each form of *T. cruzi* protozoan: amastigote, trypomastigote and epimastigote. Details of the dataset can be found in Table 1. Compounds from each dataset were classified based on pIC₅₀ (-log IC₅₀ (mol/l)), where IC₅₀ represents the concentration required for 50% inhibition of the protozoan.

Table 1: Molecular dataset from ChEMBL of each form of *T. cruzi*.

Protozoa	Forms	Total molecules	Active	Inactive	ChEMBL ID
<i>T. cruzi</i>	Tryp. ^[a]	1694	pIC ₅₀ ≥ 5	pIC ₅₀ < 5	ChEMBL368
	Ama. ^[b]	1157	pIC ₅₀ ≥ 4.7	pIC ₅₀ < 4.7	
	Epim. ^[c]	1884	pIC ₅₀ ≥ 4.7	pIC ₅₀ < 4.7	

[a] Trypomastigote. [b] Amastigote. [c] Epimastigote

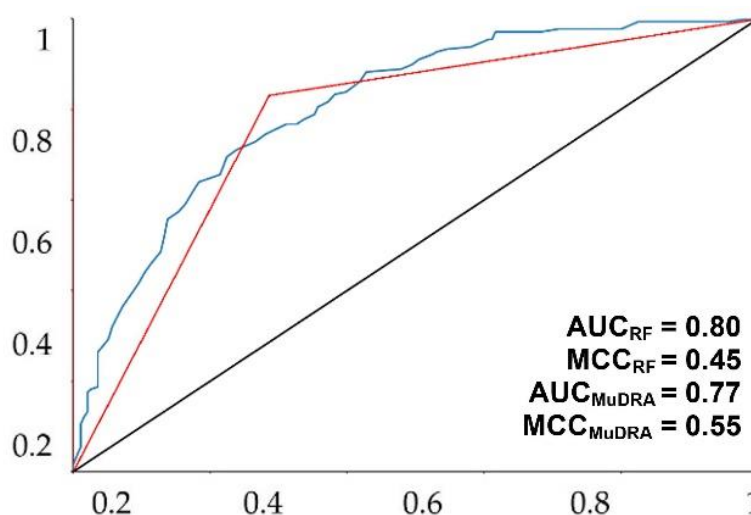
The random forest (RF) predictive model was constructed using 5200 DRAGON 7.0 descriptors. Likewise, Morgan, Avalon, MACCS and RdKit descriptors were used in the MuDRA models.

The performance characteristics of the predictive models are described in Table 2, revealing the predictive power and reliability of the models. Figure 1 shows the receiver operating characteristic (ROC) curve and Mathew's coefficient, providing information on the performance and robustness of the models.

Table 2: Summary of the parameters of the results obtained in the RF and MuDRA models for the epimastigote forms of *T. cruzi*.

Models		Specificity	Sensitivity	Accuracy	PPV ^[a]	NPV ^[b]
RF	Epimastigote	0.70	0.78	0.72	0.70	0.76
MuDRA		0.72	0.84	0.77	0.73	0.83

[a] Positive Predictive Value. [b] Negative Predictive Value

**Figure 1:** ROC curve, true positive versus false positive, generated for RF and MuDRA model. AUC – area under the curve, MCC – Mathew's coefficient, red line – MuDRA, blue line – RF.

Through these results, we observed that for the epimastigote form of *T. cruzi*, the MuDRA and RF models performed similarly, with an accuracy of 0.72 for RF and 0.77 for MuDRA. This result is also reflected in the similarity of both models' ROC and MCC curves.

After verifying the models, the virtual screening of the Annonaceae secondary metabolite bank was run to select molecules with potential activity against the epim. *T. cruzi*.

The RF model selected 673 molecules as active, with probability ranging between 51–87%. The MuDRA model selected only 51 molecules, with probabilities between 51–99%.

To select the secondary metabolites that are most likely to be active against epim. *T. cruzi*, a ligand-based virtual screening consensus analysis was performed using the following formula:

$$P_{cm} = \frac{(p_{RF} * ESP_{RF}) + (p_{MuDRA} * ESP_{MuDRA})}{ESP_{RF} + ESP_{MuDRA}}$$

where P_{cm} is the combined probability between the models, P_{RF} is the probability that a molecule is active in the “Random Forest” model, ESP_{RF} is the RF model specificity, P_{MuDRA} the MuDRA model probability, and finally ESP_{MuDRA} is the MuDRA model specificity. Note that in this equation, the activity probability score of each model is conditioned by a decrease in the false positive rate with increasing specificity. Thus, the probability of selecting inactive molecules as active molecules is minimized.^{34,35}

From this consensual analysis, 57 molecules (probability between 51–83%) were selected to test against epim. *T. cruzi*. By performing this consensus analysis, we increased the probability of selecting molecules with active potential, since these molecules were screened as active in both models (RF and MuDRA).

Through partnerships with internal and external professor of the Post-Graduate Program of Bioactive Natural and Synthetic Products, who work with isolation and identification of secondary metabolites, 16 substances were given to us. Most of these substances have already been isolated from the Annonaceae species in the literature.

Some of these substances were not reported in the Annonaceae species; lithocholic acid, morolic acid, tirucallol, lanosterol, hecogenin acetate and diosgenin. Thus, these molecules were not present in the bank of molecules that was screened. Molecular descriptors were obtained from their structures and were then run through virtual screening.

Figure 2 shows the chemical structure of the molecules selected for the *in vitro* test of antiepipimastigote activity of *T. cruzi*. Information on IC₅₀ values from the *in vitro* test is

available in the Table 3 and the prediction information, applicability domain, and pIC₅₀ are available in Table 4.

Table 3: Antiepipimastigote activity.

ID	Molecule name	IC ₅₀ (μM)
1	Lithocholic acid	9.99
2	Morolic acid	11.83
3	Lupeol	12.6
4	Xylodiol	13.19
5	13-Epicupressic acid	18.9
6	Trachyloban-18-oic acid	26.9
7	<i>Ent</i> -kauran-16-β-ol	>100
8	Labda-8-17-12-13-trien-18-oic acid	>100
9	Kaurenoic acid	>100
10	β-Amyrin	>100
11	Lanosterol	>100
12	Pregnandiol	>100
13	Tirucallol	>100
14	Diosgenin	>100
15	Stigmasterol	>100
16	Hecogenin acetate	>100

Table 4: Antiepipimastigote activity and prediction information (*T. cruzi*).

Molecule	Class	APD ^[a]	P _{RF} ^[b]	P _{MuDRA} ^[c]	P _{cm} ^[d]	Pred. ^[e]	pIC ₅₀
1	Biliar acid	Re ^[f]	0.57	0.65	0.61	A ^[h]	5.00
2	Triterpene	Re	0.58	0.59	0.59	A	4.92
3	Triterpene	Re	0.60	0.58	0.59	A	4.89
4	Diterpene	Re	0.60	0.54	0.57	A	4.87
5	Diterpene	Re	0.58	0.57	0.56	A	4.72
6	Diterpene	Re	0.57	0.55	0.55	A	4.57
7	Diterpene	Re	0.48	0	0.24	I ^[i]	<4
8	Diterpene	Re	0.25	0	0.12	I	<4
9	Diterpene	Re	0.41	0.35	0.38	I	<4
10	Diterpene	Un ^[g]	0.62	0	0.31	I	<4
11	Triterpene	Re	0.56	0.40	0.48	I	<4
12	Steroid	Re	0.44	0.36	0.40	I	<4
13	Triterpene	Un	0.61	0.23	0.42	I	<4
14	Steroid	Un	0.61	0.16	0.38	I	<4
15	Steroid	Re	0.49	0.36	0.42	I	<4
16	Steroid	Un	0.58	0.99	0.78	I	<4

[a] Applicability domain. [b] Random Forest model probability. [c] P_{MuDRA} the MuDRA model probability [d] combined probability between the models. [e] Prediction activity. [f] Reliable. [g] Unreliable. [h] Active. [i] Inactive.

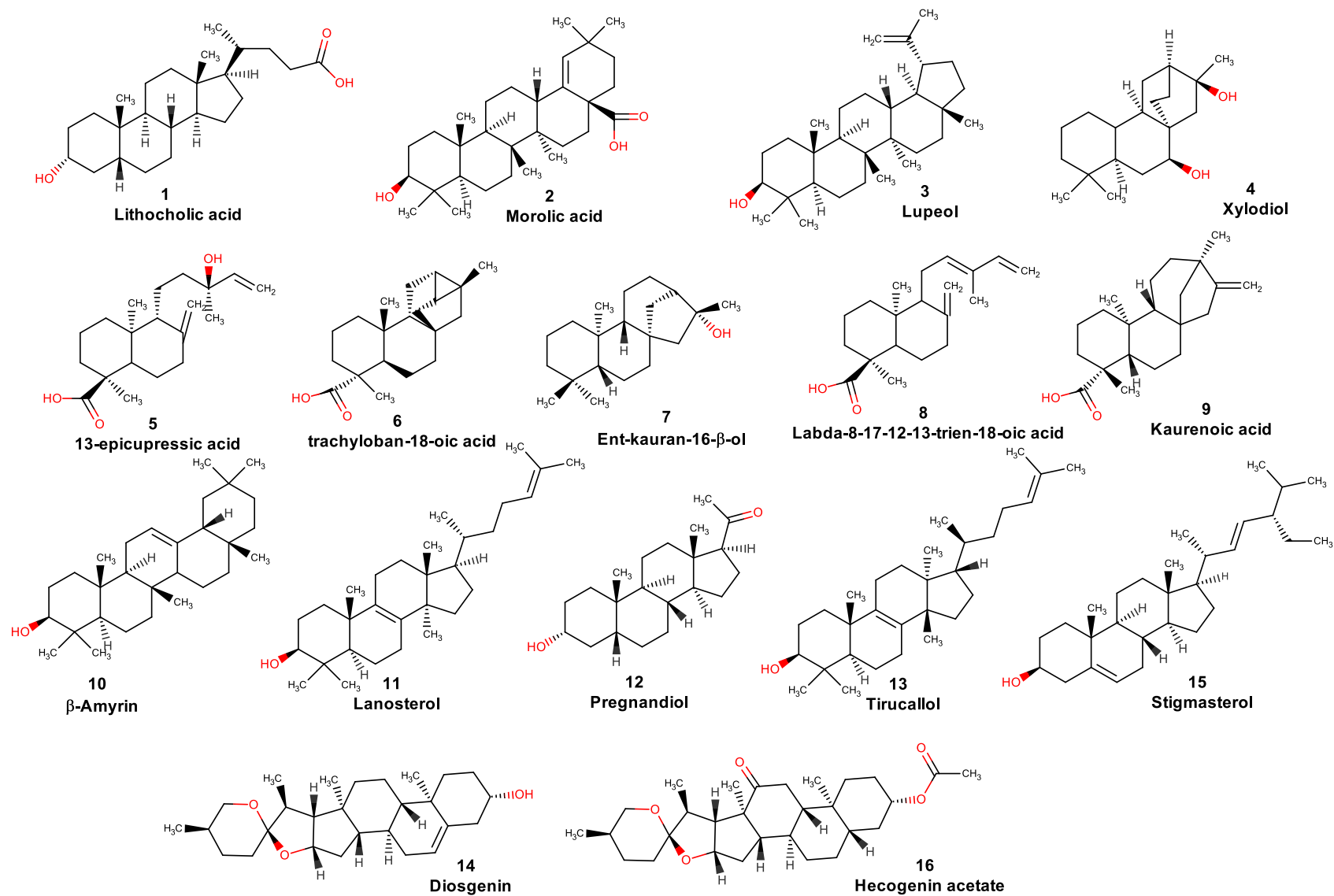


Figure 2: Chemical structures of the molecules selected for *in vitro* testing.

Among the 16 molecules tested *in vitro*, six showed activities against the epimastigote form of *T. cruzi*. The six molecules that showed activity *in vitro* are the same molecules that were predicted as active in the combined analysis between the models. That means that the models were 100% correct in their predictions. Translationally, virtual screening, when combined with machine learning, has great predictive power in the identification of molecules with a desired biological activity.

Among the six molecules that showed activity, four have already been isolated from the Annonaceae species: lupeol (3), xylodiol (4), 13-epicupressic acid (5) and trachyloban-18-oic acid (6). The other two (1 and 2), have not been reported in the literature under the Annonaceae family.

Through our virtual screening study, we were able to select six compounds predicted to have activity against the epimastigote form of *T. cruzi* and applying this result in an *in vitro* study, the six selected compounds also showed activity.

Therefore, we decided to evaluate the activity profile of these six molecules against the amastigote and trypomastigote forms of *T. cruzi*, which are the forms that cause Chagas disease in humans, to find a multitarget molecule, that is, one that is active against all parasitic forms of *T. cruzi*.

Then, from the selected ChEMBL database with molecules of known activity also for the amastigote and trypomastigote forms (details in Table 1) the predictive models were created, using Dragon descriptors of RF model and Morgan, Avalon, MACCS and RdKit descriptors for MuDRA models. Table 5 summarizes the statistical performances of the models.

Table 5: Summary of the parameters of the results obtained in the RF and MuDRA models for the amastigote and trypomastigote forms of *T. cruzi*.

Models	form	Spec. ^[c]	Sens. ^[d]	ACC ^[e]	PPV ^[f]	NPV ^[g]	MCC ^[h]	ROC ^[i]
RF	Ama. ^[a]	0.75	0.81	0.77	0.77	0.79	0.55	0.86
MuDRA		0.80	0.84	0.81	0.70	0.82	0.63	0.81
RF	Tryp. ^[b]	0.83	0.80	0.82	0.83	0.81	0.64	0.89
MuDRA		0.80	0.83	0.82	0.81	0.82	0.63	0.82

[a] Amastigote. [b] Trypomastigote. [c] Specificity. [d] Sensitivity. [e] Accuracy. [f] Positive Predictive Value. [g] Negative Predictive Value. [h] Mathew's coefficient. [i] ROC curve (Area under the curve).

Through these results, we observed that the models for these two forms of the parasite had excellent reliability and predictive power. Both the RF and MuDRA models proved to

be remarkably similar, with an average accuracy of 80%. This result is also reflected in the ROC and MCC curves of the models, where the areas under the curve were similar, but with a small increase for the trypomastigote model. Thus, the same behavior can also be observed for the MCC, whose trypomastigote RF model was the one with the best result. Overall, the models for both amastigote and trypomastigote activity performed well.

With the models built and validated, we then performed the prediction of the six selected compounds and analyzed whether they were within the applicability domain of each model. The six compounds are reliable (within the applicability domain) in the amastigote and trypomastigote models. The results show that the six compounds also have potential activity against the amastigote form, which is the parasitic form of the chronic phase of Chagas disease in humans, 13-epicupressic acid (5) being the most promising among them (70% probability of active potential). None of the six compounds was likely to have activity against the trypomastigote form. Table 6 represents the probabilities of activity for the amastigote form that was calculated through consensus analysis between the RF and MuDRA models.

Table 6: Antiamastigote activity prediction.

Molecule	P_{RF}^[a]	P_{MuDRA}^[b]	P_{cm}^[c]	Prediction activity
1	0.67	0.7	0.67	Active
2	0.63	0.6	0.63	Active
3	0.58	0.99	0.58	Active
4	0.55	0.52	0.55	Active
5	0.7	0.55	0.70	Active
6	0.53	0.56	0.53	Active

[a] Random Forest model probability. [b] P_{MuDRA} the MuDRA model probability [c] combined probability between the models.

A study by Newman and Cragg¹⁷, revealed that more than 68.8% of new drugs approved for commercial use between the years 1981 and 2019 are of direct or indirect origin from natural products. This reveals the importance of natural products in the identification of new substances with therapeutic potential and low toxicity.^{17,36,37} And in this context, the antichagasic activity has been studied for more than 20 years in the Annonaceae family, from 2000 to 2021, about 21 articles were found in the literature reporting the antichagasic activity of 21 species of Annonaceae.

Most studies evaluate extract activity, fractions, and essential oils.^{38–48} Among the articles that report the activity of isolated substances, some analyzed class are terpenes, which shows us that Annonaceae terpenes are potential antiepipimastigote and

antitrypomastigote agents.^{49–51} The study carried out by Armenio et al.⁵⁰ brings us the activity of six terpenes with activity ranging between 16.3 and 47.5 μM (IC_{50}), very promising results.

As already mentioned, one of the great advantages of finding secondary metabolites as antichagasic agents is the greatly reduced toxicity of natural products when compared to the drugs available today for the treatment of Chagas disease.^{17,36,37} Thus, we evaluated the druglikeness profile of the six substances selected in this study, as well as their respective toxicity profiles.

We started the analysis by calculating and analyzing the parameters of the Lipinski alert index, using the Dragon 7.0 software.⁶⁰ This alert from Lipinski is the first filter that predicts the oral bioavailability of compounds based on molecules that have reached phase II clinical trials, and also predicts that malabsorption or permeation is more likely when more than one rule is violated.^{53–57} In this analysis, it was observed that none of the six compounds selected in our study violated any of the rules. We then proceeded with the analysis of the drug similarity consensus score. This score is an average of seven similarity indices of drugs already available in the therapeutic market, that is, these seven indices are parameters that have model variables used as filters to select candidates for new drugs.^{55–60} As a result, it was observed that 13-epicupressic acid reached the maximum score, standing out as a great drug candidate, followed by lithocholic acid, xylodiol, lupeol, Morolic acid and trachyloban-18-oic acid.

Finally, we analyze the toxicity profile of these compounds through the DataWarrior program, a program that has the ability to perform chemical analyzes in silico through a database, being possible, among other analyses, to generate predictions of toxicity of chemical compounds that is calculated using algorithms and a bank of fragments with known toxicity.⁶¹ By comparing the molecules that we want to predict toxicity with the bank of fragments with known toxicity, DataWarrior will predict their respective toxicity profiles. We thus evaluated the toxicity profile for mutagenicity, tumorigenicity, reproductive toxicity and skin irritation. The predictions are classified into no risk, low risk, medium risk and high risk. The results of this analysis showed that 13-apicupressic acid and lithocholic acid have a low probability of tumorigenic risk and lithoclic acid has a high probability of mutagenic risk, the other molecules did not present any probability of toxic risk.

No reports of antichagasic activity were found in the literature for these six selected compounds, even if isolated from other botanical families. Therefore, this is the first time that the antiepipimastigote activity of these compounds has been validated. And the toxicity

and druglikeness studies only reinforce the importance of these results, as they have shown to be quite promising.

Conclusions

In summary, the virtual screening performed in this study was able to identify six compounds (lithocholic acid, morolic acid, lupeol, xylodiol, 13-epicupressic acid and trachylobane-18-oic acid) with potential activity against the epimastigote and amastigote forms of *T. cruzi*. The selected molecules are active when evaluated in vitro against *T. cruzi* epimastigote. Once again, the use of ligand-based virtual screening (LBVS) proved to be quite effective and saved time, effort, and money in the selection of potential antichagasic agents. 13-Epicupressic acid showed to be the most promising from the druglikeness point of view and has low toxicity, therefore being a potential antichagasic agent. Our results demonstrate that natural products from the Annonaceae family, especially terpenes, can serve as a promising starting point for the development of new antichagasic agents.

Experimental Section

Annonaceae database

The Annonaceae database was built¹⁹ and uploaded to the SISTEMATX^{62,63} web tool developed by the Chemistry Laboratory of the Postgraduate Course on Natural and Bioactive Synthetic Products. The Annonaceae dataset contains 1885 molecules from the most varied classes of natural products.

Data curation

For all structures of the ChEMBL database and the Annonaceae dataset, SMILES codes were generated and used as the input data for Marvin 19.27.0, 2019, ChemAxon (<http://www.chemaxon.com>)⁶⁴ and Standardizer software [JChem 19.27.0, 2019; ChemAxon (<http://www.chemaxon.com>)]⁶⁵ to convert the chemical structures into curated and canonical representations. This standardization is of paramount importance to create consistent compound libraries and is done through the following steps: addition of hydrogens, aromatization, generation of 3D structure and exporting the compounds in SDF format. For a more detailed description on how the dataset was curated, please refer to the workflows described by Fourches et al.^{66–68}

Dragon 7 descriptors

Molecular descriptors are used to calculate the physicochemical properties of the molecules of each set of molecules, ChEMBL database and Annonaceae database. To obtain the molecular descriptors, the DRAGON 7.0 program⁵² was used. 5270 molecular descriptors, covering several approaches, are arranged in 30 logic blocks.⁵²

Random forest model

Knime 4.4.0 software (Knime 4.4.0 the Konstanz Information Miner Copyright, 2003–2021, www.knime.org)⁶⁹ was used to perform the analyses and to generate the *in silico* model. Datasets of molecules, along with their calculated descriptors and class variables, were imported from the DRAGON 7.0 software. Each dataset was divided using the “Partitioning” tool, with the “stratified sample” option, to create a training set and an external test set, which represented 80% and 20% of the compounds, respectively. Although the compounds were selected randomly, the same proportion of active and inactive samples was maintained in both sets.

For external validation, we employed 5-fold cross-validation^{70,71} using randomly selected, stratified groups. The distributions according to activity class variables were found to be maintained in all validation groups and in the training set. Descriptors were selected and modeled following a 5-fold external cross-validation procedure using the Random Forest algorithm (RF). The entire data set is partitioned five times into a modeling set (training set) including 80% of the compounds the set, and the external cross-validation data set, comprising the remaining 20% of the compounds the data set. After this, only the modeling set is used to build the models and then the models are validated with the external cross-validation technique. There were 100 parameters selected for RF for all generated models, which is the total number of trees constructed and the random (1550953075932) seed was used in the generation of the models.

Using Knime nodes, the most important descriptors in the generation of each prediction model were evaluated. The external performances of the selected models were analyzed for sensitivity (true positive rate, i. e., active rate), specificity (true negative rate, i. e., inactive rate) and accuracy (overall predictability). The positive (PPV) and negative (NPV) predictive values inform us about the probability of predicted positives (PPV) and negatives (NPV) being the true positives and negatives, respectively. In addition, the sensitivity and specificity of the Receiver Operating Characteristic (ROC) curves were found to describe true performance with more clarity than accuracy.

The models were also analyzed by the Matthews coefficient, a way to evaluate the model globally from the results obtained from the confusion matrix. The MCC is a correlation coefficient between observed and predictive binary classifications. It results in a value between -1 and $+1$, where a coefficient of $+1$ represents a perfect forecast, 0 is nothing more than a random forecast, and -1 indicates total disagreement between forecast and observation.⁷²

The Matthews correlation coefficient can be calculated from the following formula:

$$MCC = \frac{VP \times VN - FP \times FN}{\sqrt{(VP + FP)(VP + FN)(VN + FP)(VN + FN)}}$$

where VP is the value of true positive, VN is the value of true negative, FP is the value of false positives and FN of false negatives.

The applicability domain (APD) was used to analyze the compounds of the test sets to evaluate whether their predictions were reliable. The APD is based on Euclidean distances, and similarity measures between the descriptors of the training set are used to define the applicability domain. Meaning, if a test set compound has distances and similarity beyond this limit, its prediction is not reliable. The APD calculation is performed behind the formula:

$$APD = d + Z\sigma$$

where d and σ are the Euclidean distances and the standard mean deviation, respectively, of the compounds in the training set. Z is an empirical cut-off value, and in this work the Z value was used as 0.5 .^{73,74}

MuDRA model

A second prediction model was constructed from the bank of molecules with known antichagasic activity, to predict the activity of the Annonaceae bank. The model was constructed according to the methodology of Alves et al.⁷⁵ The model is called MuDRA and uses instance-based machine learning, meaning, it compares new instances with seen instances in the training set, rather than performing explicit generalizations. This method provides an alternative to the set-modeling approach, facilitating the implementation and lower computational cost.⁷⁵⁻⁷⁷

We used the Knime software to generate the MuDRA model. We followed a 5-fold external cross-validation procedure^{70,71} using the complete datasets in the original publications, and randomly selected 20% of the dataset as an external set using stratified sampling to standardize the analysis within this study. For MuDRA modeling, four different types of molecular descriptors are used: Morgan, Avalon, MACCS and RDkit. The MuDRA plating depends on the chemical similarity and comparisons of biological responses, where

structural similarity is identified within a chemical space defined by the different types of molecular descriptors. In each chemical space, several nearest neighbors are selected based on their similarity to the compounds that have known activity, and the predicted activity is calculated based on the Tanimoto coefficient as a function of the Jaccard distance.^{75–77}

Virtual screening

Virtual screening was performed using the two predictive models created, Random Forest and MuDRA. The Annonaceae dataset was screened in the two models and the results were analyzed through a consensual analysis between them.^{34,35,78}

Toxicity and druglikeness assessment

Molecular descriptors from Dragon 7.0 software⁶⁰ were used to evaluate Drug-Likeness properties of the five alkaloids selected in the virtual screening. To analyze the *in silico* toxicity of these alkaloids, the DataWarrior v4.7.2 software,⁶¹ OpenMolecules, was used, evaluating the mutagenic, tumorigenic, reproductive, and irritant effects.

Maintenance of parasites

Parasites of the species *Trypanosoma cruzi*, strain Y, were maintained in *liver infusion tryptose* (LIT) medium supplemented (10% FBS and 100 U/mL of penicillin and 100 µg/mL of streptomycin) pH 7, at 26 ± 1°C in BOD.

Drug preparation

For the *in vitro* tests, stock solutions of the substances were prepared at concentrations of 40 mg/mL in dimethyl sulfoxide – (DMSO) (Mallinckrodt Chemicals, St. Louis, USA). For each test, the stock solution was diluted in the appropriate culture media until reaching the desired final concentrations, not exceeding the concentration of 0.2% DMSO.

Activity assay against epimastigotes of *T. cruzi*

Growth inhibition of epimastigote forms of *T. cruzi* was evaluated based on Santoro et al., 2007.⁷⁹ Epimastigote forms in logarithmic phase of growth were cultured in 96-well plates (1×10⁶ parasites/ well) containing 100 µL of supplemented LIT medium, in triplicate, containing serial concentrations of test substances (50 to 1.56 µM) and control positive (benznidazole 200 to 1.56 µM). The plates were incubated in BOD at 26°C for 72 h. After the treatment period, 10 µL of MTT (5 mg/mL) was applied to each well to establish the colorimetric assessment of growth inhibition. The plate was then incubated again for another 4 h, followed by the addition of 50 µL of 10% sodium dodecyl sulfate (DSS) solution (w/v in distilled water) to solubilize the formazan crystals. The reading was performed in a microplate spectrophotometer at 540 nm (model Biosystems ELx800, Curitiba, PR, Brazil).

The negative control was performed with Schneider medium supplemented and containing 0.2% DMSO, considered as 100% viability of epimastigotes.

Statistical analysis

The different mean concentrations on promastigote forms (IC₅₀) were calculated by non-linear regression. Variance was calculated using ANOVA, followed by Tukey's test through a statistical program, taking the value of $p < 0.05$ as the maximum level of statistical significance.

Acknowledgements

We would also like to thank the CNPq (Brazilian National Council for Scientific and Technological Development – Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq), grant numbers 309648/2019-0 and 431254/2018-4, and Capes for financial Support.

Conflict of Interest

The authors declare no conflict of interest.

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The background of the page features a decorative border at the bottom. This border consists of a network of interconnected nodes and lines, resembling a molecular structure or a complex network diagram. The nodes are colored in shades of blue, purple, and green. Interspersed within this network are faint, light-colored logos that appear to be stylized representations of a camera or a similar device. The overall aesthetic is technical and scientific.

CAPÍTULO V

A leishmaniose é uma doença tropical negligenciada causada por protozoários do gênero *Leishmania*. É uma doença crônica e transmitida pela picada de mais de 30 espécies de flebotômíneos infectados com espécies de *Leishmania*. A doença tem sido reportada em cerca de 98 países e afeta mais de um milhão de pessoas anualmente.

Há duas formas clínicas da doença: a leishmaniose visceral, que é a mais grave, causada principalmente pela espécie *L. donovani*, e a leishmaniose tegumentar, que é causada pelas espécies *L. amazonensis* e *L. major*, podendo ser subdividida em leishmaniose cutânea e leishmaniose mucocutânea.

Os tratamentos medicamentosos incluem antimoniais pentavalentes, como estibogluconato de sódio e antimoniato de meglumina, bem como anfotericina B e pentamidina, mas esses medicamentos estão desatualizados e questionáveis devido à variabilidade em sua eficácia entre as diferentes espécies de *Leishmania*.

Assim, a busca por novos medicamentos é imprescindível e a utilização de produtos naturais como fonte se destaca. Em um estudo, Newman e Cragg (2020), constataram que cerca de 68.8% dos novos medicamentos aprovados para o uso terapêutico estão direta ou indiretamente associados a produtos naturais.

Nesta perspectiva, o objetivo deste trabalho foi utilizar técnicas de aprendizado de máquina e triagem virtual baseada em ligantes (VS) para selecionar moléculas potencialmente ativas contra *Leishmania amazonensis* a partir de um banco de dados interno de metabólitos especializados da família da pinha ou graviola (Annonaceae).

Os modelos obtiveram uma precisão de 63% para amastigota e 81% para a forma promastigota, permitindo a triagem virtual baseada em ligantes do banco de dados de Annonaceae. Foi realizado uma análise de consenso entre os dois modelos para selecionar 16 substâncias a serem submetidas a testes *in vitro* contra a forma promastigota de *L. amazonensis*. Quatro substâncias, a saber, lupeol, ácido morólico, ácido litocólico e xilodiol, mostraram atividade leishmanicida. O triterpeno lupeol, apresentou a melhor atividade em ensaios biológicos *in silico* e *in vitro*. A atividade prevista contra a forma amastigota de *Leishmania amazonensis* excede 77% para lupeol, ácido morólico e ácido litocólico. Os resultados encontrados e propostos podem servir como um promissor ponto de partida para o desenvolvimento de novos produtos naturais a base de agentes terapêuticos leishmanicidas.

Annonaceae Terpenoids as Potential Leishmanicidal Agents

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Artigo publicado na revista: Brazilian Journal of Pharmacognosy

Fator de Impacto: 2.464

QUALIS: A4

Abstract: Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*. Current drug treatments for leishmaniasis are outdated and questionable due to variability in their effectiveness between different *Leishmania* species. In this perspective, we combined two predictive ligand-based virtual screening models to select potentially active molecules against *Leishmania amazonensis* from specialized metabolites of Annonaceae. To construct the predictive models, compounds with known activity for *L. amazonensis* were selected from the ChEMBL database, and subdivided into *L. amazonensis* protozoal, amastigote, and promastigote datasets. Our models obtained an accuracy of 63% for amastigote and 81% for the promastigote form, enabling ligand-based virtual screening of the Annonaceae databank. We performed a consensus analysis between the two models to select 16 substances to undergo *in vitro* testing against the promastigote form of *L. amazonensis*. Four substances, namely lupeol, morolic acid, lithocholic acid, and xylodiol, showed leishmanicidal activity. The triterpene lupeol, showed the best activity *in silico* and *in vitro* biological assays. The predicted activity against the amastigote form of *Leishmania amazonensis* exceeds 77% for lupeol, morolic acid, and lithocholic acid. The hits proposed here could serve as a promising starting point for the development of new natural products based leishmanicidal therapeutical agents.

Keywords: Custard apple family, Diterpenes, Secondary Metabolites, Soursop family, Triterpenes, Virtual Screening

Introduction

Leishmaniasis is a neglected tropical disease (NTD) caused by protozoa of the genus *Leishmania*. Among the NTDs, leishmaniasis has the highest incidence in tropical and subtropical Latin American countries ¹⁻⁴. Leishmaniasis has been reported in ~98 countries worldwide and affects more than one million people annually. Leishmaniasis is a chronic disease that is transmitted through the bite of a sandfly (Phlebotominae) infected with *Leishmania* sp. There are two clinical forms of the disease: visceral leishmaniasis, which is the most serious, primarily caused by the specie *Leishmania donovani*, and tegumentary leishmaniasis, which are caused by the species *Leishmania amazonensis* and *Leishmania major*, and can be subdivided into cutaneous leishmaniasis and mucocutaneous leishmaniasis ^{1,3-7}.

Current treatments for leishmaniasis include pentavalent antimonials, such as sodium stibogluconate and meglumine antimoniate, as well as amphotericin B and pentamidine ^{1,5-10}. However, these drugs are outdated, expensive, and can caused severe side effects. They exhibit high variability in effectiveness between different *Leishmania* species, the need for parenteral administration and the high toxicity makes these drugs questionable in the treatment of leishmaniasis. Thus, there is a strong need for new leishmaniasis treatments.

Medicinal plants are a rich source of bioactive molecules. In a recent review, Newman and Crag found that about 68.8% of new drugs approved for therapeutic use are directly or indirectly associated with natural products ¹¹. Annonaceae is a family rich in natural products and there are several studies in the literature proving the biological activity of several of its metabolites, mainly alkaloids ^{8,12-16}. Consequently, the aim of this work is to use machine learning techniques and ligand-based virtual screening (VS) to select potentially active molecules against *Leishmania amazonensis* from an in-house database of specialized metabolites from the custard apple or soursop family (Annonaceae).

Materials and Methods

Database construction

Our database is composed of specialized metabolites isolated from members of the Annonaceae family. These metabolites were extracted from the phytochemical literature within the Web of Science research base using the following search terms: “Annonaceae”, “secondary metabolites”, “terpenes”, “alkaloids”, “flavonoids”. All secondary metabolites, their plant species, and the geographic locations were registered on the SISTEMATX^{17,18}

web tool and developed by the Chemistry Laboratory of the Postgraduate Course on Natural and Bioactive Synthetic Products.

Predictive Model

A set of chemical structures with known activity for *L. amazonensis* were selected from the ChEMBL database to construct the predictive model. The dataset was subdivided into the *L. amazonensis* protozoal, amastigote, and promastigote forms (Table 1). The compounds of each dataset were classified based on the pIC_{50} ($-\log IC_{50}$ mol/l). For all structures, SMILES codes were used as input data for Marvin 19.27.0, 2019, ChemAxon (<http://www.chemaxon.com>)¹⁹ and Standardizer software [JChem 19.27.0, 2019; ChemAxon (<http://www.chemaxon.com>)]²⁰ to convert the chemical structures into curated and canonical representations. Standardization is of paramount importance to create consistent compound libraries and is done through the following steps: addition of hydrogens, aromatization, generation of 3D structure, and exporting the compounds in SDF format. For a more detailed description on how the dataset was curated, please refer to the workflows described by Fourches et al.^{21–23}

Table 1: Molecules dataset from ChEMBL Databases to each form of *L. amazonensis*.

Forms	Total molecules	Active molecules	Inactive Molecules	ChEMBL ID
Amastigote	226	103 ($pIC_{50} \geq 5.00$)	123 ($pIC_{50} < 5.00$)	ChEMBL612877
Promastigote	576	242 ($pIC_{50} \geq 4.80$)	334 ($pIC_{50} < 4.79$)	

Dragon 7 Descriptors

Molecular descriptors are used to calculate the physicochemical properties of each molecule. To obtain the molecular descriptors, the DRAGON 7.0 program was used (Kode Chemoinformatic, Italy). The DRAGON 7.0 software calculates 5270 molecular descriptors, arranged in 30 logic blocks²⁴.

Random Forest Model

The Knime 4.4.0 software (Knime 4.4.0 the Konstanz Information Miner Copyright, 2003–2020, www.knime.org)²⁵ was used to perform the analyses and to generate the *in silico* model. Datasets of molecules, along with their calculated descriptors and class variables were imported from the DRAGON 7.0 software. Each dataset was divided using the “Partitioning” tool, with the “stratified sample” option, to create a training set and an external test set, which represented 80% and 20% of the compounds, respectively. Although the compounds were selected randomly, the same proportion of active and inactive samples were maintained in both sets.

For external validation, we employed 5-fold cross-validation^{26,27} using randomly selected, stratified groups using the Random Forest algorithm (RF). The entire data set was partitioned five times into a modeling set (training set) including 80% of the compounds the set, and the external cross-validation data set with the remaining 20% of the compounds. Here, the modeling set is used to build the models and the models are validated with the external cross-validation technique.

We selected 100 parameters for each RF model (the total number of trees constructed) and used seed 1550953075932 in the generation of random numbers for the model. The most important descriptors in the generation of each prediction model were evaluated using KNIME. The external performances of the selected models were analyzed for sensitivity (true positive rate, i.e., active rate), specificity (true negative rate, i.e., inactive rate), and accuracy (overall predictability). The positive (PPV) and negative (NPV) predictive values inform us about the probability of predicted positives (PPV) and negatives (NPV) being the true positives and negatives, respectively. In addition, the sensitivity and specificity of the Receiver Operating Characteristic (ROC) curve were found to describe true performance with more clarity than accuracy.

The model was also analyzed by the Matthews correlation coefficient (MCC), MCC is a correlation coefficient between observed and predictive binary classifications. This is used to evaluate the model globally from the results obtained from the confusion matrix. It results in a value between -1 and +1, where a coefficient of +1 represents a perfect forecast, 0 is a random forecast, and -1 indicates total disagreement between forecast and observation²⁸.

The Matthews correlation coefficient can be calculated from the following formula:

$$MCC = \frac{VP \times VN - FP \times FN}{\sqrt{(VP + FP)(VP + FN)(VN + FP)(VN + FN)}}$$

where VP is the value of true positive, VN is the value of true negative, FP is the value of false positives and FN of false negatives.

The applicability domain (APD) was used to analyze the compounds of the test sets to evaluate whether their predictions were reliable. The APD is based on Euclidean distances. Similarity measures between the descriptors of the training set are used to define the applicability domain. Meaning, if a test set compound has distances and similarity beyond this limit, its prediction is not reliable. The APD calculation is calculated using:

$$APD = d + Z\sigma$$

where d and σ are the Euclidean distances and the standard mean deviation, respectively, of the compounds in the training set. Z is an empirical cut-off value, and in this work the Z value was used as 0.5.^{29,30}

MuDRA Model

A second predictive model (MuDRA) was constructed from the bank of molecules with known leishmanicidal activity, to predict the activity of the Annonaceae dataset, according to the methodology previously published by Alves et al.³¹ MuDRA uses instance-based machine learning, meaning, it compares new instances with known instances in the training set, rather than performing explicit generalizations. For MuDRA modeling, four different types of molecular descriptors are used: Morgan, Avalon, MACCS and RDkit. The MuDRA plating depends on the chemical similarity and comparisons of biological responses, where structural similarity is identified within a chemical space defined by the different types of molecular descriptors. In each chemical space several nearest neighbors are selected based on their similarity to the compounds that have the known activity and the predicted activity is calculated based on the Tanimoto coefficient as a function of the Jaccard distance.^{32–34} This method provides an alternative to the set-modeling approach, facilitating the implementation and lower computational cost.

We used the KNIME software to generate the MuDRA model. We followed a 5-fold external cross-validation procedure^{26,27} using the complete datasets in the original publications, and randomly selected 20% of the dataset as an external set using stratified sampling for standardize the analysis within this study.

Virtual Screening

Virtual screening was performed using the two predictive models created, Random Forest and MuDRA. The Annonaceae dataset was screened in the two models and the results were analyzed through a consensual analysis between them.^{35–37}

Parasites Cultures

Leishmania amazonensis (IFLA/BR/67/PH8) were maintained *in vitro* in promastigote forms in supplemented Schneider's 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/l streptomycin) (Sigma-Aldrich, St. Louis, EUA), pH 7, with a temperature of 26 ± 1 °C in a Biochemical Oxygen Demand (BOD) incubator (Eletrolab EL202, São Paulo, Brazil) and peaked weekly.

Drug Preparation

For the *in vitro* tests, stock solutions of the substances were prepared at concentrations of 40 mg/ml in dimethylsulfoxide (DMSO) (Mallinckrodt Chemicals, St. Louis, USA). For each test, the stock solution was diluted in the appropriate culture media until reaching the desired final concentrations, not exceeding the concentration of 0.2% DMSO.

Through partnerships with internal and external professors of the Post-Graduate Program of Natural and Synthetic Bioactive Products, sixteen substances were obtained for the *in vitro* tests.

Leishmanicidal Activity

Growth inhibition of promastigote forms of *L. amazonensis* was evaluated based on Rodrigues et al.³⁸ Promastigote forms in logarithmic growth phase were cultivated in 96-well plates (1×10^6 parasites/well) containing 100 μ L of supplemented Schneider medium, in triplicate, containing serial concentrations of test substances (50 to 1.56 μ M) and control positive (miltefosine 200 to 1.56 μ M). Plates were incubated in BOD at 26 °C for 72 h. After the treatment period, 10 μ L of MTT (5 mg/mL) was applied to each well to establish the colorimetric assessment of growth inhibition. Then, the plate was again incubated for another 4 h, then receiving the addition of 50 μ L of 10% sodium dodecyl sulfate (DSS) solution (w/v in distilled water) for the solubilization of the formazan crystals. The reading was performed in a microplate spectrophotometer at 540 nm (model Biosystems ELx800, Curitiba, PR, Brazil). The negative control was carried out with Schneider medium supplemented and containing 0.2% of DMSO, considered as 100% of *Leishmania* viability.

Statistical Analysis

The different mean concentrations on promastigote forms (IC_{50}) were calculated by non-linear regression. For comparisons between groups, ANOVA analyzes of variance were performed, followed by Tukey's test through a statistical program, taking the value of $p < 0.05$ as the maximum level of statistical significance.

Results and Discussions

Despite having a significant impact on human health, no sufficient treatment options for leishmaniasis exist. To identify novel natural products-based compounds with the potential to treat leishmaniasis, in this study, predictive models were built for the amastigote and promastigote forms of *L. amazonensis*. We constructed random forest (RF) models using

5200 DRAGON 7.0 descriptors and MuDRA models, using four different molecular descriptors (Morgan, Avalon, MACCS and RdKit).

The performance characteristics of the four models created are described in Table 2. The results showed that the performance for promastigote models were better than those for the amastigote form. For the amastigote form, the MuDRA model showed better performance and robustness than the RF model, with an accuracy of 0.72 and 0.63, respectively. Whereas all models for the promastigote form were similar, with an accuracy of 0.81 for the RF and 0.84. for MuDRA models. Figure 1 shows the ROC curve and Mathew's coefficient which provides information on performance and robustness. The ROC and MCC curves of the models show that better results were obtained for the promastigote form over the amastigote form. Thus, we decided to focus on the promastigote form for further analysis.

Table 2: Summary of the parameters of the results obtained in the RF and MuDRA models for the amastigote and promastigote forms of *L. amazonensis*.

Models		Specificity	Sensitivity	Accuracy	PPV	NPV
RF	Amastigote	0.65	0.60	0.63	0.60	0.65
MuDRA		0.68	0.76	0.72	0.66	0.77
RF	Promastigote	0.88	0.79	0.81	0.85	0.80
MuDRA		0.83	0.85	0.84	0.78	0.88

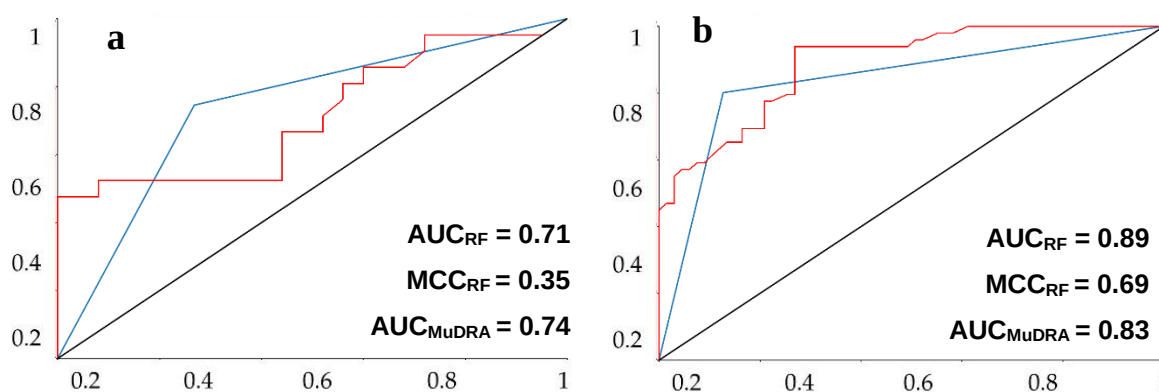


Figure 1. ROC curve, true positive versus false positive, generated for RF and MuDRA model. AUC, area under the curve; MCC, Mathew's coefficient (blue line); MuDRA, (red line) RF; a) amastigote models, b) promastigote models

Using the models, we performed a virtual screening of the bank of secondary metabolites of Annonaceae, to select molecules with potential activity against the promastigote form of *L. amazonensis*. If possible, we intended to select multitarget molecules that may have active potential against the two infective forms of the parasite. The RF model selected 1029 molecules potentially active against the promastigote form, with a probability varying between 50-91% respectively. The MuDRA model selected 864 chemical structures against the promastigote with an active potential probability varying between 50-99%.

To select metabolites with higher probability to be active against the promastigote form of *L. amazonensis*, a consensual ligand-based virtual screening analysis was performed to obtain active molecules in the two models (RF and MuDRA) using the following formula:

$$P_{cm} = \frac{(p_{RF} * ESP_{RF}) + (p_{MuDRA} * ESP_{MuDRA})}{ESP_{RF} + ESP_{MuDRA}}$$

where P_{cm} is the combined probability between the models, P_{RF} is the probability that a molecule is active in the “Random Forest” model, ESP_{RF} is the specificity of the RF model, P_{MuDRA} the probability of the MuDRA model and finally ESP_{MuDRA} the specificity of the MuDRA model. Note that in this equation, each model's activity probabilities score is constrained by a decrease in the false positive rate with increasing specificity. Thus, the probability of selecting inactive molecules as active molecules is minimized.^{35,36} From this consensual analysis it was possible to select 803 molecules with potential activity between 50% and 95% against the promastigote form. Accordingly, we increased the probability of selecting effective molecules, since they were screened as active agents in both models (RF and MuDRA).

Sixteen substances were obtained to the *in vitro* analysis, which are: lupeol (1), lithocholic acid (2), morolic acid (3), xylodiol (4), tirucallol (5), pregnandiol (6), β -amyrin (7), *ent*-kauran-16- β -ol (8), kaurenoic acid (9), trachyloban-18-oic acid (10), lanosterol (11), labda-8(17)-12*E*-14-trien-18-oic acid (12), 13-epicupressic acid (13), stigmasterol (14), hecogenin acetate (15) and diosgenin (16). These substances have mostly been reported in the literature isolated from species of Annonaceae. Substances which have not been reported in Annonaceae species include: lithocholic acid (1), morolic acid (3), tirucallol (5), lanosterol (11), hecogenin acetate (15), and diosgenin (16). These molecules were not present in the bank of molecules that were screened. Thus, these molecules were designed, their 3D structures and molecular descriptors obtained, and submitted to virtual screening. The

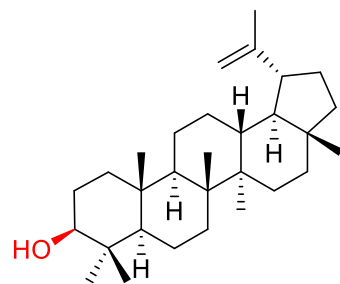
substances obtained were then subjected to biological tests for anti-leishmanial activity of the promastigote form. Prediction information, applicability domain and pIC₅₀ are available in Table 3.

Table 3. Prediction information and anti-promastigote activity against *Leishmania amazonensis*

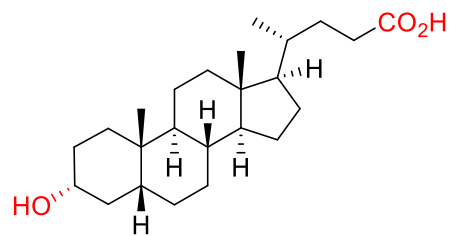
Molecule	Class	Applicability Domain	P _{RF}	P _{MuDRA}	P _{cm}	pIC ₅₀
Lupeol	Triterpene	Reliable	0.9	0.99	0.95	5.07
Lithocholic acid	-	Reliable	0.87	0.78	0.82	4.93
Morolic acid	Triterpene	Reliable	0.87	0.70	0.78	4.80
Xylodiol	Diterpene	Reliable	0.77	0.66	0.71	4.26
Tirucallol	Triterpene	Reliable	0.88	0.51	0.69	<4
Pregnandiol	Steroid	Reliable	0.78	0.6	0.69	<4
β-Amyrin	Diterpene	Reliable	0.85	0.54	0.69	<4
Ent-Kauran-16-β-ol	Diterpene	Reliable	0.8	0.57	0.68	<4
Kaurenoic acid	Diterpene	Reliable	0.77	0.57	0.67	<4
Trachyloban-18-oic acid	Diterpene	Reliable	0.76	0.56	0.66	<4
Lanosterol	Triterpene	Reliable	0.82	0.51	0.66	<4
Labda-8(17)-12E-14-trien-18-oic acid	Diterpene	Reliable	0.57	0.56	0.56	<4
13-epicupressic acid	Diterpene	Reliable	0.6	0.51	0.55	<4
Stigmasterol	Steroid	Reliable	0.83	0.03	0.42	<4
Hecogenin acetate	Steroid	Unreliable	0.91	0.82	0.86	<4
Diosgenin	Steroid	Unreliable	0.93	0.4	0.66	<4

Abbreviations: P_{RF} Probability to be active in the random forest model, P_{MuDRA} Probability to be active in the MuDRA model, P_{cm} Combined probability between the models, pIC₅₀ Calculated from the IC₅₀ in mol/l.

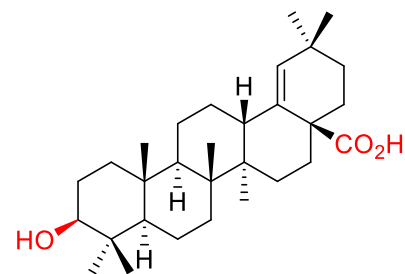
Among the sixteen molecules tested *in vitro*, only four showed activities against the promastigote form of *L. amazonensis*. Of the four molecules that showed anti-leishmanial activity, two were already isolated from species of Annonaceae, lupeol (**1**), xylodiol (**4**), and the lithocholic acid (**2**) and Morolic acid (**3**) have no reports in the literature of their presence in Annonaceae. Molecules which obtained combined probability (P_{cm}) between the two models, RF and MuDRA, greater than 0.7 showed *in vitro* activity (Table 3). This means that the combined analysis between the models hit 100% of the molecules with a prediction greater than 0.7 and had an excellent classification and prediction of molecules to be active.



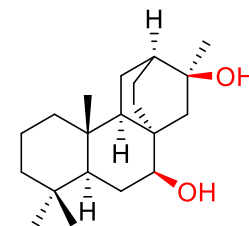
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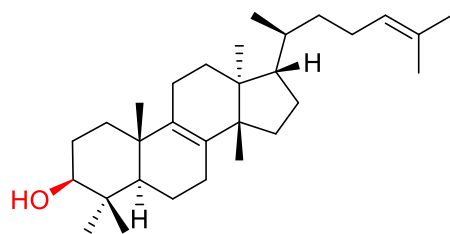
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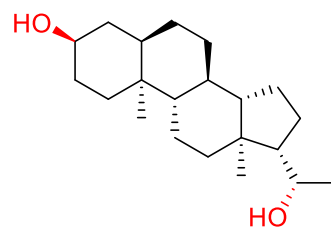
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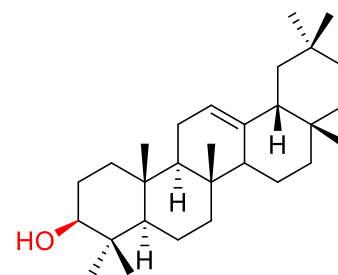
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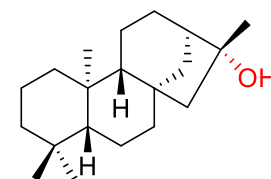
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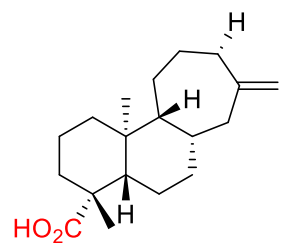
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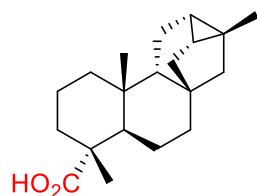
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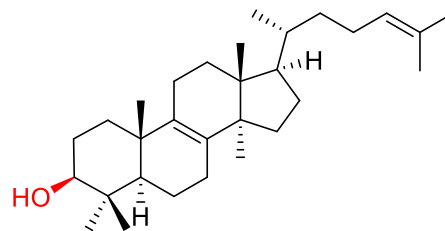
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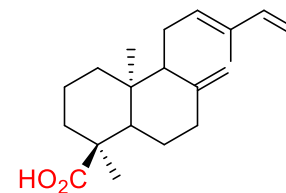
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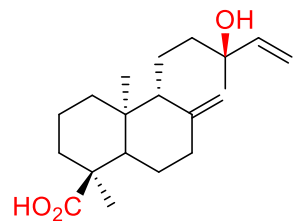
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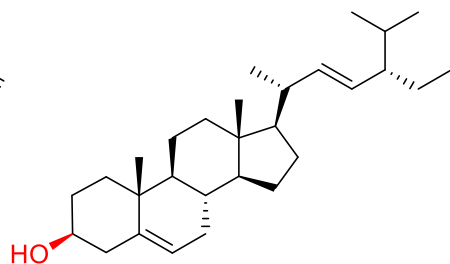
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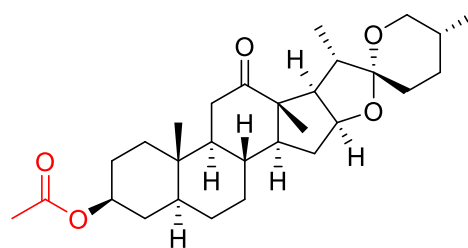
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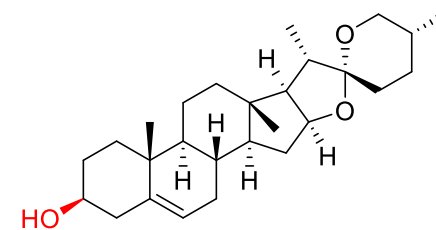
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In addition, the value of the *in vitro* activity (the potency), was directly proportional to the value of the combined prediction of the molecules. This means that the molecule with the highest active potential, lupeol (**1**; $P_{cm} = 0.95$) was also the molecule with the highest leishmanicidal activity, followed by lithocholic acid (**2**), morolic acid (**3**), and xylodiol (**4**). Molecules with predictions less than 0.7 were inactive in the *in vitro* biological activity analysis. The combined analysis of the models was able to identify the inactive molecules well. Hecogenin acetate (**6**), despite having a combined prediction higher than 0.7, did not have anti-leishmanial activity. Probably, because the molecule was not classified within the applicability domain, and therefore its prediction was not reliable.

By comparing the structures of the molecules that have *in vitro* activity with molecules that were not active, we can divide them into three groups: triterpenes, diterpenes and steroids. Two of the molecules form of *L. amazonensis* are triterpenes, lupeol (**1**) and morolic acid (**3**), a diterpene, xylodiol (**4**), and a bile acid, lithocholic acid (**2**). Any of the analyzed steroids showed *in vitro* activity.

The ten most important descriptors in the generation of the predictive model are shown in figure 2. In the figure it is also possible to observe the values of the Cohen's kappa coefficient, accuracy, and the standard error. The higher the Cohen's kappa coefficient and accuracy values, the greater the importance of the descriptor in creating the predictive model.

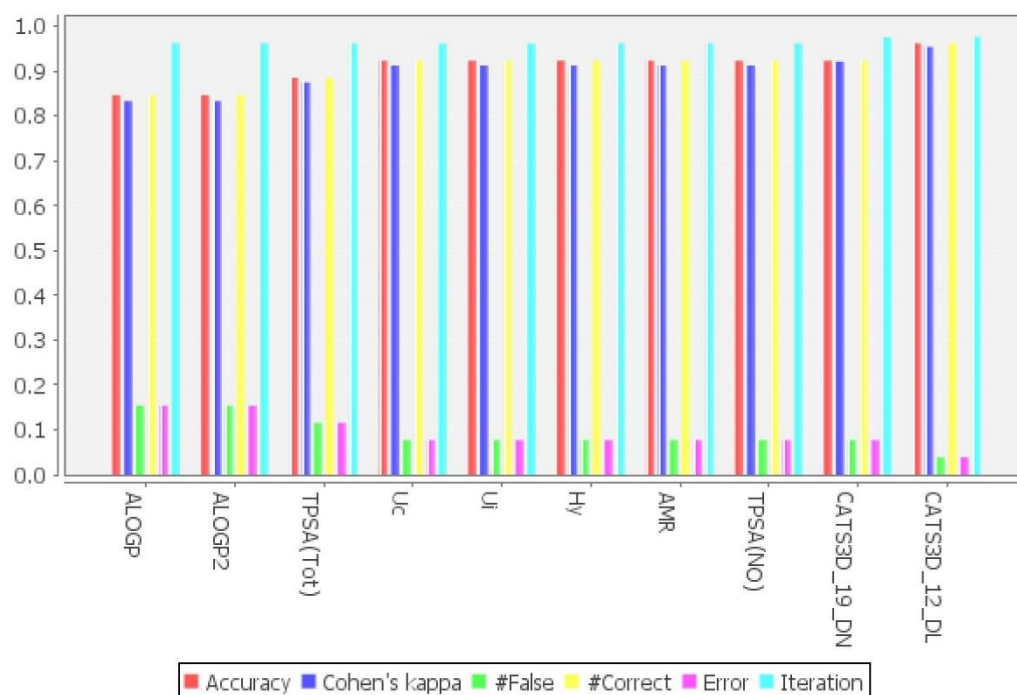


Figure 2: Most important descriptors in the generation of the RF model against *L. amazonensis* promastigotes.

The ten most important descriptors are: Ghose-Crippen molar refractivity (AMR), topological polar surface area using N,O polar contributions (TPSA) and using N, O, S, P (TPSA_{tot}), Ghose-Crippen octanol-water partition coeff. (logP)(ALOGP), squared Ghose-Crippen octanol-water partition coeff. (logP²)(ALOGP²), hydrophilic factor (Hy), unsaturation index (Ui), unsaturation count (Uc), and two CATS3D descriptors that are descriptors based on geometrical distances between the potential pharmacophore point pairs, the CATS3D_12_DL (donor-lipophilic) and CATS3D_19_DN (donor-negative). All these descriptors indicate that the molecules that have activity against *L. amazonensis* promastigotes have in common an equilibrium lipophilic/hydrophilic relationship, and are heterogeneous (they have N, O, S and P atoms) and are unsaturated., they are heterogeneous molecules (they have N, O, S and P atoms) and are unsaturated.

By analyzing the chemical structure of the most active compounds with those that were inactive, selected by the RF model, it is possible to see that polarity is important for activity, as the lipophilicity/hydrophilicity balance proved to be a critical consideration for activity against *L. amazonensis* promastigotes, as demonstrated by the ten most important descriptions in the predictive model.

Finally, the multitarget potential of the four molecules active against the amastigote form of *L. amazonensis* was evaluated. It was performed a virtual screening of these four molecules in the amastigote form's RF and MuDRA models. We analyzed the consensus of both models to obtain the final prediction of the activity of these molecules for the amastigote form. The results indicated that active compounds against the promastigote form also have active potential against the amastigote form of the parasite. Thus, lupeol (**1**), morolic acid (**3**), and lithocholic acid (**2**) have predicted active probability greater than 77% (Table 4).

Table 4. Prediction information and anti-amastigote activity against *Leishmania amazonensis*.

Molecule	Applicability Domain	P _{RF}	P _{MuDRA}	P _{cm}	Prediction Activity
Lupeol	Reliable	0.94	0.60	0.77	Active
Lithocholic acid	Reliable	0.74	0.81	0.78	Active
Morolic acid	Reliable	0.94	0.60	0.77	Active
Xylodiol	Reliable	0.45	0.2	0.32	Inactive

Abbreviations: P_{RF} Probability to be active in the random forest model, P_{MuDRA} Probability to be active in the MuDRA model, P_{cm} Combined probability between the models.

Conversely, the triterpene lupeol has leishmanicidal activity reported in literature. A review carried out by Raimundo et al.³⁹ summarizes the terpene classes of natural products

efficient in the treatment of leishmaniasis, especially the triterpene subclass. The authors highlight those terpenes exhibit leishmanicidal activity acting directly on the parasite and indirectly modulating the immune response and promoting the pro-inflammatory response through the release of cytokines. Specifically, the authors highlight the effective *in vivo* anti-leishmaniasis activity of lupeol, through the production of NO in macrophages.^{39–41}

Overall, triterpenes proved to be excellent leishmanicidal agents. Terpenes were not toxic in treatments against *Leishmania* species and showed equivalent or superior activity compared to current drugs. While literature reported anti-HIV-1,⁴² antibacterial and antifungal activity,⁴³ and anti-inflammatory activity⁴⁴ for the triterpene morolic acid (**3**) there were no reports of leishmanicidal activity. Thus, morolic acid (**3**) could be a promising drug in the treatment of leishmaniasis. Future studies should be carried out to verify its potential in the treatment of this disease.

Conclusion

Using an integrated computational approach for virtual screening of a Annonaceae dataset, we have identified 803 molecules with potential leishmanicidal activity against promastigote form of *L. amazonensis*. Out of 16 molecules, two triterpenes (lupeol and morolic acid), one diterpene (xylodiol) and one bile acid (lithocholic acid) showed leishmanicidal activity *in vitro*. The triterpene, lupeol, shows the best activity in both the *in silico* and biological analyses. We also evaluated the potential activity of these four molecules for leishmanicidal activity against the amastigote form. We observed that three of them, lupeol, morolic acid and lithocholic acid, are highly active against the amastigote form of *Leishmania amazonensis*. The hits proposed from this study could serve as a promising starting point for the development of new leishmanicidal compounds based on natural products.

Author Contributions

Conceptualization, formal analysis, and data curation: RPBM, MTS and LS; methodology, software application and writing: RPBM, MTS, LS, and EM; validation: RPBM, MTS, LS, EM, and HJM; *in vitro* analysis, KAFR, ALSS, and FARC; experimentation, Menezes RPCM, MTS, LS, JF, MJK. All authors have read the final manuscript and approved the submission.

Funding

This research was funded by National Council for Scientific and Technological Development (CNPq: 309648/2019-0) and Coordination of Superior Level Sta Improvement (CAPES).

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The background of the page features a decorative border at the bottom. This border consists of a network of interconnected nodes and lines, resembling a molecular structure or a complex network diagram. The nodes are colored in shades of blue, purple, and green. Interspersed within this network are several faint, stylized logos that appear to be the letters 'Q123RF' and a hexagonal shape with a central circle.

Capítulo VI

Neste capítulo também é abordado a leishmaniose como atividade biológica. O objetivo foi investigar o potencial leishmanicida de extratos de quatro espécies de *Annona*, *Annona glabra*, *Annona mucosa*, *Annona sylvatica* e *Annona dolabripetala*, por meio de uma abordagem metabolômica utilizando análise estatística multivariada (PCA e PLS) e dados de LC-MS, para correlacionar dados espectroscópicos com atividade leishmanicida, buscando sugerir compostos ou grupos de compostos responsáveis pela atividade biológica.

Para cada espécie foi produzido um total de 7 amostras: 1 extrato etanólico bruto, 4 extratos resultantes de uma partição líquido-líquido (hexano, diclorometano, acetato de etil e butanol), 1 resíduo aquoso, e 1 precipitado originado na fase hexânica. Resultando num total de 28 amostras.

Todas as amostras foram testadas *in vitro* contra a forma amastigota de *L. donovani*. Três amostras de *A. sylvatica* apresentaram atividade, sendo o extrato hexano o mais ativo entre eles, com $IC_{50} = 7,9 \mu\text{g/mL}$. *A. mucosa* apresentou atividade relevante para 4 de seus extratos, sendo o extrato de diclorometano o mais ativo desta espécie ($IC_{50} = 17 \mu\text{g/mL}$). Quatro amostras de *A. glabra* apresentaram atividade leishmanicida, mas o extrato mais ativo foi o etanólico $IC_{50} = 11 \mu\text{g/mL}$, o que nos informa que existe um sinergismo de compostos químicos influenciando a atividade desse extrato. *A. dolabripetala* não apresentou atividade.

As 28 amostras foram submetidas a análise de LC-MS no modo negativo com o objetivo de, juntamente com as análises quimiométricas, PCA e PLS, prever compostos potencialmente ativos contra a forma amastigota de *L. donovani*.

A análise de PCA permitiu separar as amostras quanto a sua atividade, nos informando que as espécies de *Annona* analisadas possuem perfil químico semelhante quando a atividade leishmanicida. Em uma segunda análise de PCA foi possível separar as amostras quanto ao solvente, nos informando mais uma vez a semelhança química entre as espécies.

O modelo PLS apresentou uma variância explicada de 67,724%, ou seja, utilizando 27,5% das variáveis X, foi possível explicar quase 70% de toda a variância. O modelo apresentou um R^2 de 0,90 e um Q^2 de 0,72, o que indica um bom modelo com capacidade preditiva confiável. O modelo também apresentou um valor baixo de Root Mean Square Error (RMSE) de 0,3. Nesta análise foi possível identificar 12 íons que estão contribuindo para a atividade dos extratos ativos das quatro espécies de *Annona*. O trabalho será continuado a fim de identificar os íons selecionados no modelo PLS e publicar os resultados obtidos em uma revista científica com Qualis no mínimo A4.

LC-MS Screening of Four Species of *Annona* for Secondary Metabolites with Anti-leishmanial Activity

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Artigo em fase de finalização.

Abstract: Leishmaniasis, caused by protozoa of the genus *Leishmania*, being considered by the World Health Organization the most important protozoan public disease. The available treatment for leishmaniasis have high toxicity and serious side effects. In this perspective, the aim of this study was to investigate the leishmanicidal potential of extracts from four species of *Annona* (*A. glabra*, *A. mucosa*, *A. sylvatica* and *A. dolabripetala*) through a metabolomic approach using multivariate statistical analysis and data from LC-MS, to correlate spectroscopic data with leishmanicidal activity, seeking to suggest compounds or groups of compounds responsible for the biological activity. For each species, a total of 7 samples were produced, totaling 28 samples. All samples were tested in vitro against the amastigote form of *L. donovani*. Except for the species *A. dolabripetala*, the other species present leishmanicidal activity in some of their samples. The 28 samples were submitted to LC-MS analysis in negative mode. The PCA analysis allowed to separate the samples according to their activity and regarding the solvent, informing us that the *Annona* species analyzed have a similar chemical profile in terms of leishmanicidal activity. The PLS model presented an explained variance of 67.724%, that is, using 27.5% of the X variables, it was possible to explain almost 70% of all the variance. The PLS model presented an R² of 0.90 and a Q² of 0.72, which indicates a good model with reliable predictive capacity. In this analysis it was possible to identify 12 ions that are contributing to the activity of the active extracts of the four species of *Annona*.

Keywords: Annonaceae, Chemometrics, Natural Products, Anti-leishmanial, Partial Least Squares.

1. Introduction

Neglected tropical diseases are infectious diseases, mostly caused by microorganisms, and affect mainly the poorest population with limited access to health services, being responsible for high rates of morbidity and mortality worldwide.¹⁻⁴ One of these diseases is Leishmaniasis, caused by protozoa of the genus *Leishmania*, being considered by the World Health Organization (WHO) the most important protozoan public disease.^{1-3,5} Leishmaniasis manifests itself clinically in three ways: visceral, cutaneous and monocutaneous, with visceral leishmaniasis being the most lethal.^{2,6,7} The genus *Leishmania* has about 20 species, and any one of these species can cause leishmaniasis.^{1,2,5,6}

Treatment for leishmaniasis is divided into two categories. First-line drugs are pentavalent antimony salts, sodium stibogluconate and meglumine antimoniate. The second-line drugs are amphotericin B and pentamidine, these drugs have high toxicity and serious side effects.^{1,5,6,8,9} Despite these treatment options, there is a growing number of reports and clinical manifestations of strains resistant to these drugs.^{5,9}

Due to the precarious scenario in the treatment of leishmaniasis, there is a great search for new drugs for the treatment of leishmaniasis, involving secondary metabolites from plants. In this context, the Annonaceae family, composed of 107 genera and about 2400 species, stands out for being one of the most uniform families from an anatomical and structural point of view.¹⁰⁻¹⁴ Annonaceae gains prominence among botanical families due to its richness of fruit species, which makes it the target of many studies.^{11,15,16}

A genus of great prominence is the *Annona*. *Annona* species are widely used in traditional medicine and have aroused the interest of several researchers who have been developing several scientific studies reporting the different biological activities of these species.¹⁷⁻¹⁹

Metabolomics analysis is one of the most successful tools used to find bioactive molecules of plant origin, using quantitative and qualitative datasets of all metabolites in each matrix of biological origin. Such data can be obtained, for example, by analytical methods such as mass spectrometry combined with liquid chromatography (LC-MS) and direct injection mass spectrometry (DI-MS). Based on this information, metabolomic analyzes can determine which metabolites are responsible for a given biological activity. but not necessarily a separate preliminary.²⁰⁻²²

This type of analysis combines multivariate statistical analysis with spectroscopic analysis techniques such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression, which can use data sets to assess important activities. In this way,

chemometrics has become a useful tool in metabolomics. There are many successful examples of the use of these techniques in studies involving biological activity, especially in the search for herbal agents present in complex mixtures of natural origin.^{20,22,23}

The aim of this work was to investigate the leishmanicidal potential of extracts from four *Annona* species (*Annona glabra*, *Annona mucosa*, *Annona sylvatica* and *Annona dolabripetala*) through a metabolomic approach using multivariate statistical analysis (PCA and PLS) and LC-MS data, to correlate spectroscopic data with leishmanicidal activity, seeking to suggest compounds or groups of compounds responsible for the biological activity.

2. Materials and Methods

a. Plant material

The aerials parts of four *Annona* species were collected in March 2021 from Jardim Botânico do Rio de Janeiro arboretum, Rio de Janeiro, Brazil. *Annona glabra* L. (plat 20J, arboretum registered 4398), *Annona mucosa* Jacq. (plat 9E, arboretum registered 2343), *Annona sylvatica* A.St.-Hil (plat 9E, arboretum registered 2369) and *Annona dolabripetala* Raddi (plat 37A, arboretum registered 7347). The material collected has study authorization registered in the Sistema Nacional de Gestão do Patrimônio Genético e do conhecimento tradicional Associado under number A5A49CA.

b. Processing of material – extraction and partition

The material was dried under the sun in the laboratory and at controlled temperature (22°-24°C) with the aid of air conditioners for about 15 days. After drying, the material was subjected to grinding in an Arno 650W processor. Total mass dry and ground material: *Annona glabra* (AG) – 310.68g, *Annona mucosa* (AM) – 311.03g, *Annona sylvatica* (AS) – 39.03g, *Annona dolabripetala* (AD) – 78.1g. Plants were extracted exhaustively by static maceration at room temperature using 96°GL hydrated alcohol as extracting solvent in an approximate ratio of 5:1 (solvent: vegetable drug; volume/mass). Part of the ethanol extracts were separately suspended with water and then submitted to liquid-liquid partition with organic solvents to afford hexane, dichloromethane, ethyl acetate, and n-butanol extracts. All samples showed a precipitate still in the hexane phase. This was separated and the residual solvent present was evaporated. For each plant species there is: 1 crude ethanolic extract; 4 extracts (hexane, dichloromethane, ethyl acetate and butanol); 1 aqueous residue; 1 precipitate. A total of 28 samples.

c. Antileishmanial activity assay

i. Sample preparation

Extracts were diluted in DMSO to a 50 mg/ml stock solution. For each assay, a primary solution was made by diluting the stock solution in GRACE culture medium, pH 5.3, 10% FBS. The final concentration of DMSO in each well during the assay was 0.8%. The antibiotic amphotericin B was used as a control.

ii. *Leishmania infantum* axenic anti-amastigote activity

Late log phase (96 h) promastigote forms of *L. infantum* (strain 579) were centrifuged and resuspended in GRACE culture medium, pH 5.3, with 10% FBS and incubated at 35°C for 96 h for differentiation into axenic amastigote form.

After the differentiation period, the extracts were previously diluted in 96-well microplates [400 to 6.25 µg/mL]. Then, amastigote forms were added at a final concentration of 2×10^7 /mL and the microplates were incubated at 35°C for 48 h.

After the treatment period, the viability indicator, resazurin, was added to all wells at a final concentration of 100 µM and the microplates were incubated at 35°C for 5 h. Resazurin fluorescence was read with excitation/emission at 555 and 585 nm. The percentage of viability was calculated considering the untreated wells with 100% viability and the inhibitory concentration of 50% (IC₅₀) was calculated based on the dose-response curve. Assays were performed in independent triplicates.

iii. Cytotoxicity Assays

Cytotoxicity was evaluated using cells commonly used as parasite hosts. For this, RAW 264.7 (macrophages lineage) obtained from the Cell Bank of Rio de Janeiro (BCRJ/INMETRO), were cultivated in DMEM medium supplemented with FBS (10%), Penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C with a 5% CO₂ atmosphere.

The treatments were carried out according to *Leishmania infantum* axenic anti-amastigote activity assays. After the incubation time, the macrophages were submitted to treatment with different concentrations of plant extracts (1000 – 7.8 µg/ml) and incubated for another 48h. The DMSO concentration was equalized in all treatment wells and in the positive control to 2%.

After the treatment period, macrophage viability was determined by colorimetric assay using MTT, absorbance was read at 570 nm. The percentage of viability was performed considering the positive control as 100% of viability and the cytotoxic concentration of 50% (CC₅₀) was calculated through the regression analysis of the dose-response curve.

Selectivity index (IS) of each extract was calculated using the CC₅₀/CI₅₀ ratio. Assays were performed in independent triplicates.

d. LC-MS profiling

Samples were solubilized in acetonitrile until a final concentration of 2 mg/mL. Then, 2 μ L of each sample was analyzed in an UHPLC Proeminence - Shimadzu Corp. using Acquity CSH C18 (100mm x 2.1mm, 1.7 μ m, 130Å) column, coupled with Q-TOF Impact HD - Bruker Daltonics mass spectrometer with an electrospray ionization source (ESI), operated in positive and negative modes. Samples were analyzed in 0.45 mL/min flow with following gradient: (0 min, 5%B), (20 min, 95%B), (25 min, 95%B), (31 min, 100%B), (37 min, 95%B) and (5 min, 0%B). Other parameters used in the MS analysis were a high purity nitrogen (N₂) as sheath gas (35 arbitrary units) and auxiliary gas (10 arbitrary units). High purity helium (He) was also used as collision gas, where the source voltage was 5 kV, source current was 100 μ A, source temperature was 450 °C, capillary voltage was 7 V, tube lens voltage was 65 V, and capillary temperature was 400 °C. MS spectra were acquired with a range of m/z 100-1000.

e. Multivariate statistical analysis

The LC-MS spectra of each fraction of *Annona*'s species were converted in Proteowizard toolkit - MSConvert version 3.02 and processed in MZmine 2.53 software. The following parameters were used in data processing: mass was detected as 1.0 E4 for MS1 and 1.0 E2 for MS2, and ADAP Chromatogram builder algorithm was used to chromatogram construction and to perform wavelets (ADAP) deconvolution. Isotopes were removed using Isotopic Peaks Grouper, using the most intense isotope as representative, and aligned with join aligner. Identification proceeded using a custom database search comparing precursor exact masses and comparing online with KEGG database. The data was saved to an ASCII text file. The classified data was then exported to "The Unscrambler" software version 9.7 where Principal Component Analysis and Partial Least Squares, PCA and PLS respectively, were performed. For PLS the tested IC₅₀ values were used as the dependent variable.

3. Results and Discussion

The samples of the four *Annona* species were submitted to *in vitro* tests to evaluate their leishmanicidal profiles. A total of 28 samples were evaluated *in vitro*, with seven samples from each species. Table 1 summarizes the results of the *in vitro* test.

Table 1: Anti-amastigote activity of *L. donovani* and cytotoxic of *Annona* sp. samples.

Species	Samples / Extracts	ID	IC ₅₀ (µg/mL) ± SD	pIC ₅₀	Activity classification	CC ₅₀ (µg/mL) ± SD	Selectivity Index
<i>Annona glabra</i>	Ethanolic	AGEF	11 ± 2.5	1.95	Active	250 ± 20.8	22.7
	Hexane	AGHF	29.4 ± 10.8	1.53	Active	21.8 ± 0.5	<1
	Dichloromethane	AGDF	46.1 ± 8.2	1.33	Active	<7.8	<1
	Ethyl acetate	AGAF	80.15 ± 6.5	1.09	Inactive	267.1 ± 13.2	3.3
	Butanol	AGBF	199.8 ± 4.9	0.69	Inactive	145.15 ± 11.8	<1
	Aqueous residue	AGRF	316.6 ± 9.4	0.49	Inactive	114.16 ± 58.7	<1
	Precipitate	AGPF	39.81 ± 17.5	1.4	Active	<7.8	<1
<i>Annona mucosa</i>	Ethanolic	AMEF	25.85 ± 4.4	1.58	Active	41.45 ± 0.9	1.6
	Hexane	AMHF	33.8 ± 5.01	1.47	Active	198.9 ± 41.3	5.8
	Dichloromethane	AMDF	17 ± 0.24	1.76	Active	50.3 ± 4.6	2.9
	Ethyl acetate	AMAF	126.2 ± 17.3	0.89	Inactive	135 ± 8.7	1.07
	Butanol	AMBF	174.9 ± 25.8	0.75	Inactive	277.5 ± 21	1.6
	Aqueous residue	AMRF	>400	>0.39	Inactive	<7.8	<1
	Precipitate	AMPF	20.4 ± 1.2	1.69	Active	<7.8	<1
<i>Annona Sylvatica</i>	Ethanolic	ASEF	16.9 ± 3.7	1.77	Active	81.54 ± 5.4	4.8
	Hexane	ASHF	7.9 ± 0.004	2.10	Active	51.87 ± 2.17	6.6
	Dichloromethane	ASDF	35.54 ± 8.7	1.44	Active	91.4 ± 9	2.6
	Ethyl acetate	ASAF	>400	>0.39	Inactive	265.4 ± 3.2	<1
	Butanol	ASBF	>400	>0.39	Inactive	789 ± 61.2	n.d.
	Aqueous residue	ASRF	>400	>0.39	Inactive	>1000	n.d.
	Precipitate	ASPF	180.9 ± 35	0.74	Inactive	82 ± 1.7	<1
<i>Annona dolabripetala</i>	Ethanolic	ADEF	308.5 ± 31.4	0.51	Inactive	166.1 ± 17.5	<1
	Hexane	ADHF	246.25 ± 21.4	0.60	Inactive	63.95 ± 0.4	<1
	Dichloromethane	ADDF	95.2 ± 18.8	1.02	Inactive	62 ± 4.3	<1
	Ethyl acetate	ADAF	>400	>0.39	Inactive	437.6 ± 2.7	<1
	Butanol	ADBF	>400	>0.39	Inactive	>1000	n.d.
	Aqueous residue	ADRF	>400	>0.39	Inactive	>1000	n.d.
	Precipitate	ADPF	>400	>0.39	Inactive	158.9 ± 71.2	<1
Positive Control							
Amphotericin B			4.37 ± 0.07			58.625 ± 2.5	13.4

Analyzing the data in table 1, no samples of *A. dolabripetala* showed significant activity. Three samples of *A. sylvatica* showed activity, ASEF, ASHF and ASDF, the ASHF extract (hexane) being the most active among them, with IC₅₀ = 7.9 µg/mL. *A. mucosa* showed relevant activity for 4 of its samples (AMPF, AMHF, AMDF and AMEF), with AMDF (dichloromethane) being the most active extract of this species with IC₅₀ = 17 µg/mL. Four samples of *A. glabra* showed leishmanicidal activity, but the most active extract was

the ethanolic one (AGEF) $IC_{50} = 11 \mu\text{g/mL}$, which informs us that there is a synergism of chemical compounds influencing the activity of this extract. Since the other extracts, resulting from the partition of the ethanolic extract, which were active, have IC_{50} activity higher than $11 \mu\text{g/mL}$.

The *Annona* genus is widely used in the popular treatment of leishmaniasis. Brígido et al., 2020⁶ carried out a literature review in which biological studies of the *Annona* genus with leishmanicidal activity are highlighted. Through this review, the authors concluded that alkaloids and acetogenins are the most isolated secondary metabolites of *Annona* species with leishmanicidal activity.

The 28 samples of the four *Annona* species were also subjected to LC-MS analysis in negative mode with the aim of, together with chemometric analyses, PCA and PLS, to predict potentially active compounds against the amastigote form of *L. donovani*.

LC-MS data were processed in MZmine v. 2.53 and generated a table with information ions (m/z) and retention time of all samples. The activity values (pIC_{50}) of each samples were added to this table, so that they could then be submitted to The Unscrambler X v. 10.4 to perform PCA and PLS analyses.

The first analysis performed by chemometrics was the PCA, to find patterns among the samples. In this analysis, it was possible to find patterns in the samples, separating them into two clusters, active samples (those with $pIC_{50} \geq 1.33$, total of 11 extracts) and inactive samples ($pIC_{50} < 1.33$, total of 17 extracts). Figure 1 represents the main component score chart where PC-1 and PC-2 clearly separate the active and inactive samples. The explained variance of the PCA plot is 76%.

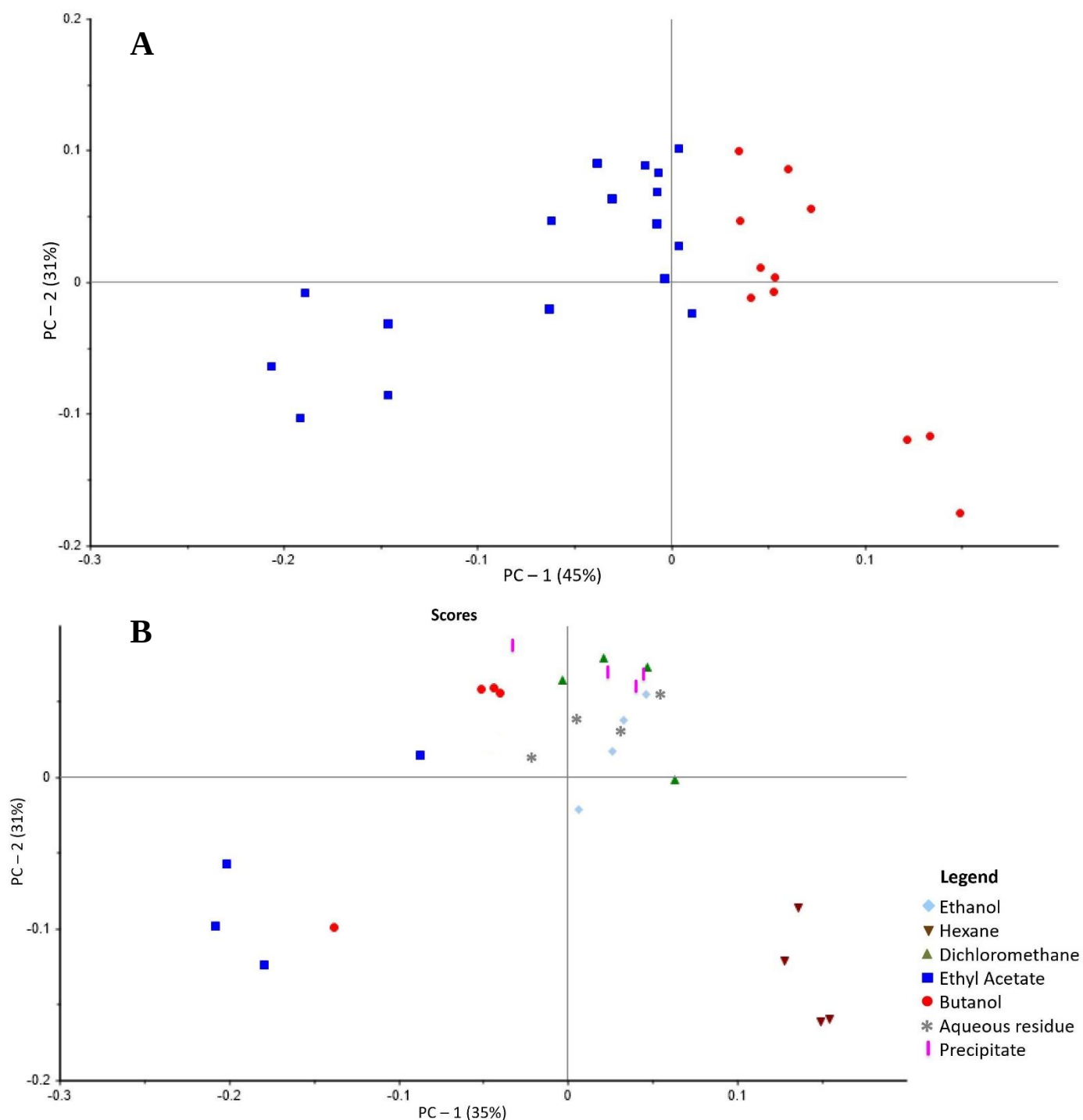


Figure 1: Score plots for PCA model based on LC-MS negative mode of the 28 samples of four *Annona* species. A – Leishmanicidal activity clusters, blue – inactive samples; red – active samples. B – Solvents clusters.

Through the PCA analysis it was possible to have a separation of the samples by solvents, that is, the extracts of the same solvent present similarities of ions. The explained variance for this separation was 66%. The extracts with solvent ethyl acetate and hexane were in well separated groups. Butanol extracts were also well grouped. The ethanol extracts,

aqueous residue, precipitate, and dichloromethane were grouped very closely, but still distinct groups.

To correlate the LC-MS data and the leishmanicidal activity, a partial least squares (PLS) analysis model was built. PLS is an algorithm that addresses both PCA and linear regression, looking for latent variables that describe the maximum correlation between predictors, in this case ions (m/z), with biological activity.^{24,25}

The PLS model created used as independent variables (X) the LC-MS dataset of the 28 samples, with a total of 80 parameters (m/z ions), and the dependent variable (Y) was the pIC_{50} value of the activity *L. donovani* anti-amastigote. The PLS model presented an explained variance of 67.724%, that is, using 27.5% of the X variables, it was possible to explain almost 70% of all the variance.

The PLS model was validated using the cross-validation technique, analyzing the values of R^2 and Q^2 . The model presented an R^2 of 0.90 and a Q^2 of 0.72, which indicates a good model with reliable predictive capacity. The model also presented a low Root Mean Square Error (RMSE) value of 0.3.

In the Score chart of the PLS model, it was possible to observe a good separation of active and inactive samples (figure 2A). In the Loadings chart (figure 2B) it is possible to observe the ions that are correlated with the active samples, being, therefore, molecules with leishmanicidal potential.

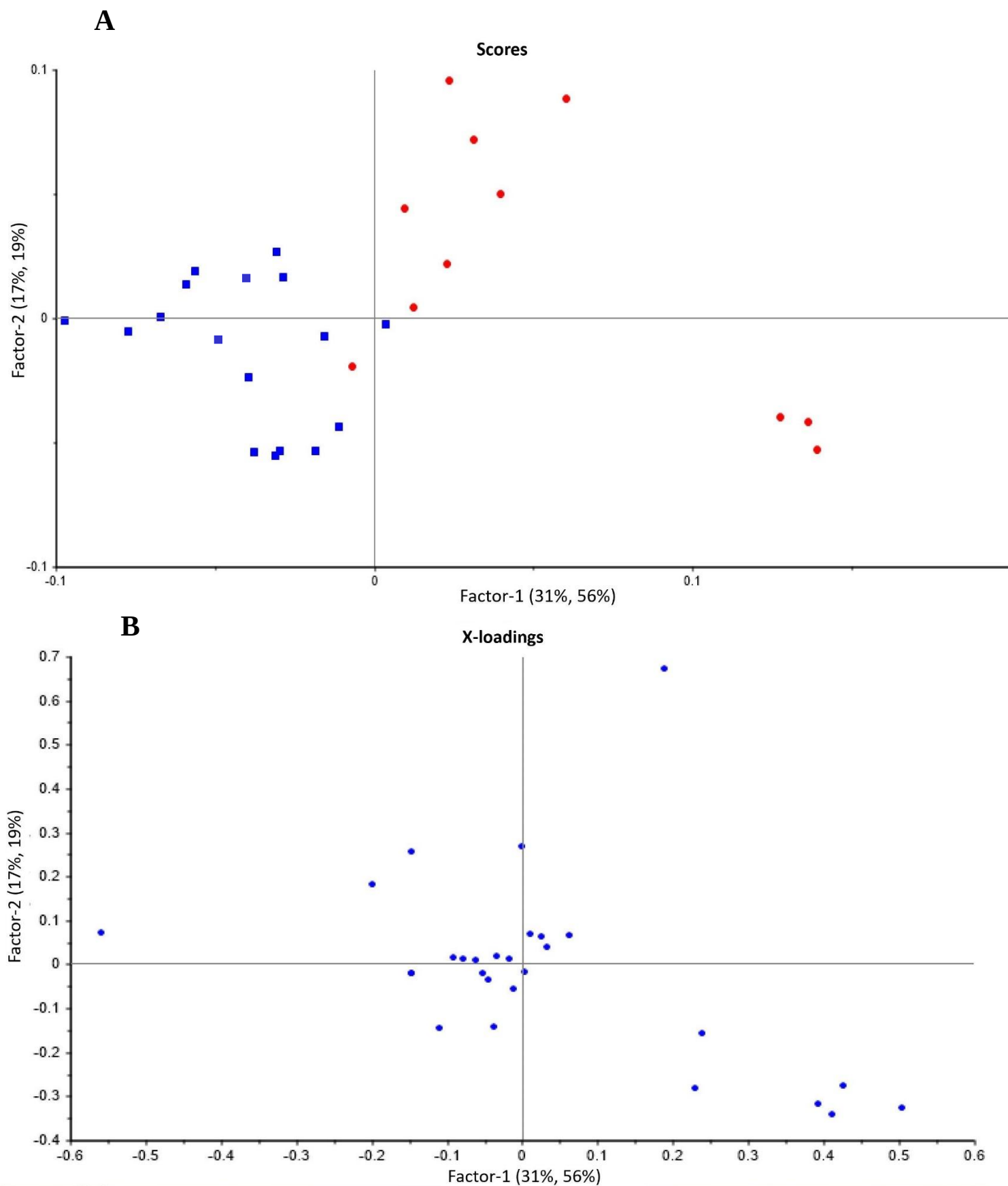


Figure 2: Score (A) and X-loadings (B) plots for PLS model based on the LC-MS negative mode of the 28 samples of four *Annona* species and the leishmanicidal activity.

As can be seen in Figure 2B, the ions that are in the right positive and negative quadrants correlate with the active samples, which are also in the positive and negative quadrants (Figure 2A). In this way, 12 ions were identified that may be contributing to the activity of the samples. The 12 ions are described in Table 2, along with their respective retention times and the sample in which they are present.

Table 2: Selected ions in the PLS model.

Ions (m/z)	Average retention time (Min.)	Samples / Extract
239.0608	24.35	AMHF, AGEF, ASEF and AMDF
255.2339	17.19	AMEF, AGEF, AGHF and AMHF
277.2183	15.44	ASHF, AGHF and AMHF
279.2339	16.47	AGHF and AMHF
281.2497	17.60	ASHF, AGHF and AMHF
297.1871	14.63	AGPF, AGEF and AMHF
312.1254	6.16	ASDF and AMDF
313.0794	21.69	AMHF
367.3598	22.12	ASHF, AGHF and AMHF
395.3909	23.22	AGHF and AMHF
658.4649	16.78	AMEF
684.4711	16.79	AMPF and AMHF

4. Perspectivas

Continue the work, identifying the ions selected in the PLS model to publish the work in a scientific journal with Qualis at least A4.

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Conclusão

Conclusão

A revisão de literatura das atividades biológicas da família Annonaceae evidenciou quão versátil e promissora é essa família na busca por novas drogas. o conteúdo dessa revisão oferece uma grande diversidade de dados aptos para serem explorados em estudos de descoberta de novas drogas. Abrindo um grande leque de exploração dessa família a fim de identificar, isolar e testar substâncias *in vitro* e *in vivo* visando a identificação de novas drogas.

A revisão de literatura sobre aprendizado de máquina aplicado a QSAR irá suprir a necessidade de artigos científicos com esse tema em língua portuguesa. A revisão aborda o assunto de forma simples e didática para estudantes e pesquisadores que estão iniciando nesta área tão promissora e importante. Descrevendo a teoria detalhadamente explicada de aprendizado de máquina aplicado a QSAR, abstraindo a complexidade, e bem ilustrada.

O levantamento bibliográfico dos metabolitos secundários já isolados da família Annonaceae permitiu a construção do banco de dados com 1860 diferentes moléculas e 5321 ocorrências botânicas, onde os alcaloides e terpenos foram as classes mais representativas.

O estudo quimiotaxonômico realizado com os diterpenos do banco de moléculas de Annonaceae conseguiu separar os subtipos de diterpenos em diferentes tribos da subfamília Annonoideae e Malmeoideae, respeitando a classificação taxonômica proposta por Guo e colaboradores, 2017.

Através da triagem virtual realizada foi possível selecionar compostos potencialmente ativos contra as formas infectantes dos protozoários *L. amazonensis* e *T. cruzi*. Para o *T. cruzi* o ácido 13-epicupressico foi o mais promissor, pois foi previsto como composto ativo no estudo *in silico* contra a forma amastigota do *T. cruzi*, além de possuir atividade *in vitro* contra a forma epimastigota. Já para *L. amazonensis* O triterpeno lupeol, apresentou a melhor atividade em ensaios biológicos *in silico* e *in vitro* para a forma promastigota, além de possuir probabilidade de potencial ativo superior a 77% contra a forma amastigota.

Na análise metabolômica utilizando análise estatística multivariada (PCA e PLS) e dados de LC-MS, para correlacionar dados espectroscópicos com atividade leishmanicida, foram identificados 16 íons responsáveis pela atividade biológica de 4 espécies de *Annona*.



ANEXOS I

Produção Científica

1. Natural Products from Annonaceae as Potential Antichagasic Agents

Journal: ChemMedChem

Impact Factor: 3.54

ChemMedChem

Research Article
doi.org/10.1002/cmdc.202200196

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Natural Products from Annonaceae as Potential Antichagasic Agents

Renata Priscila Barros de Menezes,^[a] Josean Fechine Tavares,^[a] Massuo Jorge Kato,^[b] Francisco Alex da Rocha Coelho,^[c] Airton Lucas Sousa dos Santos,^[c] Klinger Antonio da Franca Rodrigues,^[c] Zoe L. Sessions,^[d] Eugene N. Muratov,^[d] Luciana Scotti,^[a] and Marcus Tullius Scotti^{*[a]}

Chagas disease, a neglected tropical disease, is endemic in 21 Latin American countries and particularly prevalent in Brazil. Chagas disease has drawn more attention in recent years due to its expansion into non-endemic areas. The aim of this work was to computationally identify and experimentally validate the natural products from an Annonaceae family as antichagasic agents. Through the ligand-based virtual screening, we identified 57 molecules with potential activity against the epimastigote form of *T. cruzi*. Then, 16 molecules were analyzed in the

in vitro study, of which, six molecules displayed previously unknown antiepipimastigote activity. We also evaluated these six molecules for trypanocidal activity. We observed that all six molecules have potential activity against the amastigote form, but no molecules were active against the trypomastigote form. 13-Epicupressic acid seems to be the most promising, as it was predicted as an active compound in the *in silico* study against the amastigote form of *T. cruzi*, in addition to having *in vitro* activity against the epimastigote form.

2. Annonaceae Terpenoids as Potential Leishmanicidal Agents

Journal: Brazilian Journal of Pharmacognosy

Impact Factor: 2.71

Revista Brasileira de Farmacognosia

<https://doi.org/10.1007/s43450-022-00296-0>

ORIGINAL ARTICLE



Annonaceae Terpenoids as Potential Leishmanicidal Agents

Renata Priscila Barros de Menezes¹ • Josean Fechine Tavares¹ • Massuo Jorge Kato² •
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Received: 30 May 2022 / Accepted: 10 August 2022

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Abstract

Leishmaniasis is a neglected tropical disease caused by protozoa of the genus *Leishmania*. Current drug treatments for leishmaniasis are outdated and questionable due to variability in their effectiveness between different *Leishmania* species. In this perspective, we combined two predictive ligand-based virtual screening models to select potentially active molecules against *Leishmania amazonensis* from specialized metabolites of Annonaceae. To construct the predictive models, compounds with known activity for *L. amazonensis* were selected from the ChEMBL database and subdivided into *L. amazonensis* protozoal, amastigote, and promastigote datasets. Our models obtained an accuracy of 63% for the amastigote and 81% for the promastigote form, enabling ligand-based virtual screening of the Annonaceae databank. We performed a consensus analysis between the two models to select 16 substances to undergo *in vitro* testing against the promastigote form of *L. amazonensis*. Four substances, namely lupeol, morolic acid, lithocholic acid, and xylodiol, showed leishmanicidal activity. The triterpene lupeol showed the best activity in *in silico* and *in vitro* biological assays. The predicted activity against the amastigote form of *Leishmania amazonensis* exceeds 77% for lupeol, morolic acid, and lithocholic acid. The hits proposed here could serve as a promising starting point for the development of new natural products-based leishmanicidal therapeutical agents.

3. Computer-Assisted Discovery of Alkaloids with Schistomicidal Activity

Journal: Current Issues in Molecular Biology

Impact Factor: 2.976



current issues in
molecular biology



Article

Computer-Assisted Discovery of Alkaloids with Schistosomicidal Activity

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Abstract: Schistosomiasis is a chronic parasitic disease caused by trematodes of the genus *Schistosoma*; it is commonly caused by *Schistosoma mansoni*, which is transmitted by *Bioamphalaria* snails. Studies show that more than 200 million people are infected and that more than 90% of them live in Africa. Treatment with praziquantel has the best cost-benefit result on the market. However, hypersensitivity, allergy, and drug resistance are frequently presented after administration. From this perspective, ligand-based and structure-based virtual screening (VS) techniques were combined to select potentially active alkaloids against *S. mansoni* from an internal dataset (SistematX). A set of molecules with known activity against *S. mansoni* was selected from the ChEMBL database to create two different models with accuracy greater than 84%, enabling ligand-based VS of the alkaloid bank. Subsequently, structure-based VS was performed through molecular docking using four targets of the parasite. Finally, five consensus hits (i.e., five alkaloids with schistosomicidal potential), were selected. In addition, in silico evaluations of the metabolism, toxicity, and drug-like profile of these five selected alkaloids were carried out. Two of them, namely, 11,12-methylethylenedioxypropoxy and methyl-3-oxo-12-methoxy-n(1)-decarbomethoxy-14,15-didehydrochanofruticosinate, had plausible toxicity, metabolomics, and toxicity profiles. These two alkaloids could serve as starting points for the development of new schistosomicidal compounds based on natural products.

Keywords: *Schistosoma mansoni*; random forest; MuDRA; molecular docking; alkaloids



Citation: Menezes, R.P.B.d.; Viana, J.d.O.; Muratov, E.; Scotti, L.; Scotti, M.T. Computer-Assisted Discovery of Alkaloids with Schistosomicidal Activity. *Curr. Issues Mol. Biol.* **2022**, *44*, 383–408. <https://doi.org/10.3390/cimb44010028>

Academic Editor: Hidayat Hussain

Received: 16 December 2021

Accepted: 9 January 2022

Published: 15 January 2022

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1. Introduction

Neglected tropical diseases (NTDs) are a global public health concern. Most prevalent in Latin America, Africa, and Southeast Asia, NTDs generally affect the poorest populations, which are living in lower sanitary and socioeconomic conditions. According to the World Health Organization, there are 17 major parasitic infections, of which the neglected tropical diseases are schistosomiasis, leishmaniasis, trachoma, lymphatic filariasis, and Chagas diseases [1–3].

Schistosomiasis is a chronic parasitic disease caused by trematodes of the genus *Schistosoma*, where the most common causes of the disease are *Schistosoma mansoni* and *Schistosoma haematobium*, which are transmitted by *Bioamphalaria* and *Bulinus* snails, respectively. Studies show more than 200 million people are infected with *Schistosoma* and that more than 90% of them live in Africa [4,5].

Pyrazinoisoquinoline derivatives with anthelmintic activity, such as praziquantel, were discovered in 1972 [6,7]. To date, numerous studies have suggested praziquantel to be safe, efficacious, and cost-effective [8]. However, hypersensitivity and allergic reactions, as

4. Machine Learning Analysis of Essential Oils from Cuban Plants: Potential Activity against Protozoa Parasites

Journal: Molecules

Impact Factor: 4.927



Article

Machine Learning Analysis of Essential Oils from Cuban Plants: Potential Activity against Protozoa Parasites

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Citation: Barros de Menezes, R.P.; Scotti, L.; Scotti, M.T.; García, J.; González, R.; Monzote, L.; Setzer, W.N. Machine Learning Analysis of Essential Oils from Cuban Plants: Potential Activity against Protozoa Parasites. *Molecules* **2022**, *27*, 1366. <https://doi.org/10.3390/molecules27041366>

Academic Editors:
Francesca Mancianti and Valentina Virginia Ebani

Received: 16 December 2021
Accepted: 15 February 2022
Published: 17 February 2022

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Abstract: Essential oils (EOs) are a mixture of chemical compounds with a long history of use in food, cosmetics, perfumes, agricultural and pharmaceuticals industries. The main object of this study was to find chemical patterns between 45 EOs and antiprotozoal activity (antiplasmodial, antileishmanial and antitrypanosomal), using different machine learning algorithms. In the analyses, 45 samples of EOs were included, using unsupervised Self-Organizing Maps (SOM) and supervised Random Forest (RF) methodologies. In the generated map, the hit rate was higher than 70% and the results demonstrate that it is possible find chemical patterns using a supervised and unsupervised machine learning approach. A total of 20 compounds were identified (19 are terpenes and one sulfur-containing compound), which was compared with literature reports. These models can be used to investigate and screen for bioactivity of EOs that have antiprotozoal activity more effectively and with less time and financial cost.

Keywords: essential oil; Cuban plants; machine learning analysis; antiprotozoal activity

1. Introduction

An essential oil (EO) is a concentrated plant secondary metabolite composed of a mixture of volatile chemical compounds [1], with a long history of use in food, cosmetics, perfumes, agricultural and pharmaceuticals industries [2]. In the last decade, almost 5000 articles related to uses of EOs have been published, with a positive increment of more than 7% per year [3]. In this scenario, the scientific, economic, and biological importance of EOs is growing as alternatives to synthetic compounds commonly used in industry [4–6].

In particular, numerous studies demonstrated the wide pharmacological spectrum of EOs, including: antimicrobial [7,8], antifungal [9], antiparasitic [10], antiviral [10,11], insecticidal [12], anticarcinogenic [13–16], immunomodulatory [17], anti-inflammatory and antioxidant [14]. Nevertheless, the chemical composition of obtained EOs can unfortunately be different depending on the chosen method, geographical origins, the season, the type of soil and the agricultural conditions in which plants have grown. Thus, the same plant could produce different EO chemical composition profiles and therefore display different biological effects [3,8,14]. In this sense, some approaches have been used, such as the ‘chemotype concept’ with the aim to discern the bioactivity of EOs based on chemical profile. However, more complex questions have emerged due to the high complexity in the

5. Secondary Metabolites Extracted from Annonaceae and Chemotaxonomy Study of Terpenoids

Journal: Journal of Brazilian Chemical Society

Impact Factor: 2.135

<https://dx.doi.org/10.21577/0103-5053.20210097>

J. Braz. Chem. Soc., Vol. 00, No. 00, 1-10, 2021
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Article



Secondary Metabolites Extracted from Annonaceae and Chemotaxonomy Study of Terpenoids

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The Annonaceae family of plants is one of the most anatomically and structurally uniform families. Chemotaxonomy is a common practice to determine the chemical patterns within these families at different phylogenetic levels. The aim of this study was to build a dataset of all the secondary metabolites isolated within the Annonaceae family and to perform the respective chemotaxonomic analysis using self-organizing maps (SOMs). This dataset is composed of 5321 botanical occurrences and 1860 unique molecules present in all subfamilies of the Annonaceae. Diterpenes account for 366 unique compounds and 533 botanical occurrences seen in both Annonoideae and Malmeoideae subfamilies. The Annoneae, Xylopieae and Miliuseae tribes had the highest number of botanical occurrences and were therefore selected for the analysis. Molecular descriptors of the diterpenes and their respective botanical occurrences were used to generate the SOMs. These SOMs demonstrated clear and indicative tribe separations, with a match rate higher than 70%. Our results corroborate with the morphological and molecular data. These models can be used to predict the phylogenetic location of certain diterpenes and to accelerate the research of Annonaceae secondary metabolites and their biological potentials.

Keywords: Annonaceae, secondary metabolites, diterpenes, chemotaxonomy, self-organization maps

6. Exploring Secondary Metabolites Database of Apocynaceae, Menispermaceae, and Annonaceae to Select Potential Anti-HCV Compound

Journal: Current Topics in Medicinal Chemistry

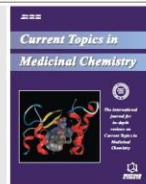
Impact Factor: 3.570

Current Topics in Medicinal Chemistry, 2019, 19, 900-913

RESEARCH ARTICLE



Exploring Secondary Metabolites Database of Apocynaceae, Menispermaceae, and Annonaceae to Select Potential Anti-HCV Compounds



Renata P.C. Barros¹, Luciana Scotti¹ and Marcus T. Scotti^{1,*}

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Abstract: Background: Hepatitis C is a disease that constitutes a serious global health problem, is often asymptomatic and difficult to diagnose and about 60-80% of infected patients develop chronic diseases over time. As there is no vaccine against hepatitis C virus (HCV), developing new cheap treatments is a big challenge.

Objective: The search for new drugs from natural products has been outstanding in recent years. The aim of this study was to combine structure-based and ligand-based virtual screening (VS) techniques to select potentially active molecules against four HCV target proteins from in-house secondary metabolite dataset (SistematX).

Materials and Methods: From the ChEMBL database, we selected four sets of 1199, 355, 290 and 237 chemical structures with inhibitory activity against different targets of HCV to create random forest models with an accuracy value higher than 82% for cross-validation and test sets. Afterward, a ligand-based virtual screen of the entire 1848 secondary metabolites database stored in SistematX was performed. In addition, a structure-based virtual screening was also performed for the same set of secondary metabolites using molecular docking.

Results: Finally, using consensus analyses approach combining ligand-based and structure-based VS, three alkaloids were selected as potential anti-HCV compounds.

Conclusion: The selected structures are a starting point for further studies in order to develop new anti-HCV compounds based on natural products.

Keywords: Hepatitis C virus, Alkaloids, Terpenes, Ligand-based virtual screening, Structure-based virtual screening, Molecular dynamics.

Received: November 14, 2018
Revised: March 27, 2019
Accepted: March 27, 2019

DOI:
10.2174/1568026619666190510094228



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7. Virtual Screening of Secondary Metabolites of the Genus *Solanum* With Potential Antimicrobial Activity

Journal: Brazilian Journal of Pharmacognosy

Impact Factor: 2.71



Brazilian Journal
of Pharmacognosy

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Original Article

Virtual screening of secondary metabolites of the genus *Solanum* with potential antimicrobial activity

Renata Priscila Costa Barros^a, Emidio Vasconcelos Leitão da Cunha^b, Raïssa Mayer Ramalho Catão^b, Luciana Scotti^a, Maria Sallett Rocha Souza^a, Amanda Amona Queiroz Brás^a, Marcus Tullius Scotti^{a,*}

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ARTICLE INFO

Article history:

Received 20 April 2018

Accepted 11 August 2018

Available online xxx

Keywords:

Rutin

Virtual Screening

Random Forest

Antibacterial activity

ABSTRACT

Infectious diseases are a health problem today and have high mortality rates with a wide diversity of potentially pathogenic microorganisms. Research that is based either on the search for new drugs from plants or on the improvement of phytotherapeutics is prominent and continues to play an important role nowadays. From this perspective, use of *in silico* studies to carry out investigations of new molecules potentially active for methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* using an in-house database with 421 different secondary metabolites selected from the literature from *Solanum* genus was performed. We also realized an *in vitro* study with strains of *S. aureus* and *E. coli* and compared the results. Two databases from ChEMBL were selected, the first one with activity against methicillin-resistant *S. aureus* and another against *E. coli*. The compounds were classified according to the pIC₅₀ values to generate and validate the model using a "Random Forest". The "Random Forest" prediction model for methicillin-resistant *S. aureus* obtained an accuracy of 81%, area under the Receiver Operating Characteristic curve of 0.885, selecting eight molecules with an active potential above 60%. The prediction model for *E. coli* obtained an accuracy rate of 88%, area under the Receiver Operating Characteristic curve of 0.932, selecting four molecules with potential probability above 84%. Rutin proved to be potentially active in the *in silico* study for *S. aureus* and *E. coli*. Microbiological tests have shown that rutin has activity only for *E. coli*. An interaction study with strains of *S. aureus* ATCC 25923, a standard strain sensitive to all antibiotics, and SAM-01, a multidrug-resistant strain, was designed. There was interaction only between rutin and oxacillin, one of the three antibiotics studied in the interaction, for the strain SAM-01, reducing the resistance of this strain.

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8. Chapter 7: Use of Machine Learning and Classical QSAR Methods in Computational Ecotoxicology

Book: Ecotoxicological QSARs

Editor: Kunal Roy

Publisher: Springer

ISBN: Chapter – 978-1-0716-0149-5

Book – 978-1-0716-0150-1



Chapter 7

Use of Machine Learning and Classical QSAR Methods in Computational Ecotoxicology

Renata P. C. Barros, Natália F. Sousa, Luciana Scotti, and Marcus T. Scotti

Abstract

In recent years, there has been an increase in concern about environmental pollution and human health, especially in the areas of manufacturing, storage, distribution, and release of hazardous substances after use. Several researchers have been dedicating studies to develop methods to identify and assess the toxicity of chemicals. Quantitative structure-activity relationship (QSAR) modeling has evolved a lot in recent years and also developed in the area of ecotoxicology. In the course of this evolution, there was the application of machine learning techniques in QSAR studies. The use of ML algorithms is a great approach for assessing toxicity to generate predictive models involving QSAR. Several studies are being conducted not only comparing ML techniques but applying them to generate potentially predictive models and excellent performances.

Key words Ecotoxicology, Machine learning, Quantitative structure-activity relationship (QSAR)

9. Chapter 24: The Use and Evolution of Web Tools for Aquatic Toxicology Studies

Book: Chemometrics and Cheminformatics in Aquatic Toxicology

Editor: Kunal Roy

Publisher: Wiley

ISBN: 978-1-119-68159-5

24

The Use and Evolution of Web Tools for Aquatic Toxicology Studies

Renata P. B. Menezes¹, Natália F. Sousa¹, Luana de Moraes e Silva², Luciana Scotti¹, Wilton Silva Lopes², and Marcus T. Scotti¹

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24.1 Introduction

The presence of chemicals in the aquatic environment is a global concern because many chemicals are continuously released into the environment, either through direct discharge into water or from terrestrial runoff and atmospheric deposition, which eventually reaches aquatic matrices. Therefore, the chemicals in the water represent a highly complex mixture of various contaminants, including industrial chemicals, surfactants, ionic liquids, pharmaceuticals, personal care products, steroid hormones, pesticides, and other compounds [1]. Variations in the physical and chemical profiles may result in the formation of compounds in the aquatic environment that may be hazardous to human health [2–4].

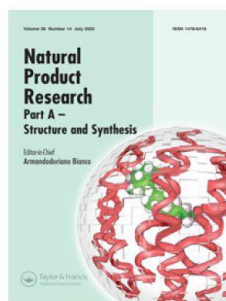
Determining the effects of compounds in aquatic ecosystems is of paramount importance for the assessment of hazards and the development of environmental regulations. Although the amount of available experimental data is growing, the number and diversity of contaminants can greatly complicate comprehensive risk assessments, requiring complex experimental analyses, which can be time-consuming and expensive [5, 6]. Therefore, experimental evaluations are not suitable for the screening of a wide variety of compounds. This limits current knowledge regarding the effects of these chemicals in complex effluents.

Artigos Publicados em Parcerias

1. Four Diterpenes Identified *in silico* Were Isolated from Hyptidinae and Demonstrated *in vitro* Activity Against *Mycobacterium tuberculosis*

Journal: Natural Product Research

Impact Factor: 2.488



Natural Product Research
Formerly Natural Product Letters



ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/gnpl20>

Four diterpenes identified *in silico* were isolated from Hyptidinae and demonstrated *in vitro* activity against *Mycobacterium tuberculosis*

Andreza Barbosa Silva Cavalcanti, Mayara dos Santos Maia, Pedro Thiago Ramalho de Figueiredo, Renata Priscila Barros de Menezes, Alex France Messias Monteiro, Roseana Araújo Ramos Meireles, Gabriela Cristina Soares Rodrigues, Ana Rita Rodrigues de Almeida Silva, Jociano da Silva Lins, Laísa Vilar Cordeiro, Valnês S. Rodrigues Junior, Ana Paula O. T. Castelo Branco, Maria de Fátima Agra, Zoe L. Sessions, Eugene N. Muratov, Luciana Scotti, Marcelo Sobral da Silva, Vicente Carlos de Oliveira Costa, Josean Fachine Tavares & Marcus Tullius Scotti

To cite this article: Andreza Barbosa Silva Cavalcanti, Mayara dos Santos Maia, Pedro Thiago Ramalho de Figueiredo, Renata Priscila Barros de Menezes, Alex France Messias Monteiro, Roseana Araújo Ramos Meireles, Gabriela Cristina Soares Rodrigues, Ana Rita Rodrigues de Almeida Silva, Jociano da Silva Lins, Laísa Vilar Cordeiro, Valnês S. Rodrigues Junior, Ana Paula O. T. Castelo Branco, Maria de Fátima Agra, Zoe L. Sessions, Eugene N. Muratov, Luciana Scotti, Marcelo Sobral da Silva, Vicente Carlos de Oliveira Costa, Josean Fachine Tavares & Marcus Tullius Scotti (2022): Four diterpenes identified *in silico* were isolated from Hyptidinae and demonstrated *in vitro* activity against *Mycobacterium tuberculosis*, Natural Product Research, DOI: [10.1080/14786419.2022.2096604](https://doi.org/10.1080/14786419.2022.2096604)

To link to this article: <https://doi.org/10.1080/14786419.2022.2096604>

2. *Sidastrum paniculatum* (L.) Fryxell (Malvaceae): A Promising Source of Bioactive Sulfated Flavonoids Against *Aedes aegypti* L

Journal: Frontiers in Pharmacology

Impact Factor: 5.988



ORIGINAL RESEARCH
published: 07 January 2022
doi: 10.3389/fphar.2021.760156



Sidastrum paniculatum (L.) Fryxell (Malvaceae): A Promising Source of Bioactive Sulfated Flavonoids Against *Aedes aegypti* L

Sany D. G. Marques^{1,2}, Diégina A. Fernandes^{1,2}, Yanna C. F. Teles³, Renata. P. B. Menezes¹, Mayara S. Maia¹, Marcus T. Scotti^{1,4}, Maria F. Agra⁵, Tania M. S. Silva⁶ and Maria de Fátima Vanderlei de Souza^{1,2*}

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OPEN ACCESS

Edited by:

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Universidade Lusófona, Portugal

Reviewed by:

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Specialty section:

This article was submitted to
Ethnopharmacology,
a section of the journal
Frontiers in Pharmacology

Received: 17 August 2021

Accepted: 20 December 2021

Published: 07 January 2022

Citation:

Marques SDG, Fernandes DA, Teles YCF, Menezes RPB, Maia MS, Scotti MT, Agra MF, Silva TMS and de Souza MdFV (2022) *Sidastrum paniculatum* (L.) Fryxell (Malvaceae): A Promising Source of Bioactive Sulfated Flavonoids Against *Aedes aegypti* L. *Front. Pharmacol.* 12:760156. doi: 10.3389/fphar.2021.760156

Aedes aegypti L. is known as the most relevant vector mosquito for viruses such as yellow fever, chikungunya, dengue, and Zika, especially in places with unplanned urbanization, and erratic water supply. Plants used in folk medicine have become a useful source of active compounds with the potential to control the dissemination of *Ae. aegypti*. Compounds isolated from Malvaceae *sensu lato* have been previously reported as larvicides, repellents, and insecticides. Recent studies have demonstrated the anti *Ae. aegypti* activity of sulfated flavonoids, an uncommon type of flavonoid derivatives. This research reports the phytochemical investigation of *Sidastrum paniculatum* (L.) Fryxell, a Malvaceae species with the potential against *Ae. aegypti*. Chromatographic procedures resulted in the isolation of the compounds: stearic acid (1), N-trans-feruloyltyramine (2), acacetin (3), apigenin (4), tiliroside (5), along with the sulfated flavonoids: wissadulin (6), 7,4'-di-O-methyl-8-O-sulfate flavone (7), yannin (8), beltraonin (9a), 7-O-sulfate isoscutellarein (paniculatumin) (9b), and condadin (10). This is the first report of compound 7-O-sulfate isoscutellarein (9b). The structures were elucidated by spectroscopic analysis (NMR, LC-HRMS and FT-IR). The sulfated flavonoids identified were submitted to a ligand-based and structure-based virtual screening against two targets: 1YIY (from adult *Ae. aegypti*) and 1PZ4 (from *Ae. aegypti* larvae). The results indicated that when the O-sulfate group is bearing the position 7, the structures are potentially active in 1PZ4 protein. On the other hand, flavonoids with the O-sulfate group bearing position 8 were showed to be more likely to bind to the 1YIY protein. Our findings indicated that *S. paniculatum* is a promising source of sulfated flavonoids with potential against *Ae. aegypti*.

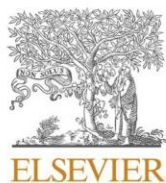
Keywords: sulfated flavonoids, virtual screening, neglected tropical diseases, *Aedes aegypti* L, *Sidastrum paniculatum* (L.) Fryxell

3. Lupanes and Friedelanes, The First Chemical Constituents of the Aerial Parts of *Maytenus Erythroxylon* Reissek

Journal: Phytochemistry Letters

Impact Factor: 2.02

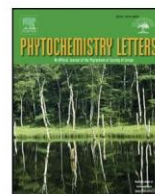
Phytochemistry Letters 45 (2021) 19–24



Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol



Lupanes and friedelanes, the first chemical constituents of the aerial parts of *Maytenus erythroxylon* Reissek

Pedro T.R. de Figueiredo*, Emille W.R. Silva, Laísa V. Cordeiro, Renata P.C. Barros, Edeltrudes Lima, Marcus T. Scotti, Marcelo S. da Silva, Josean F. Tavares, Vicente C. de O. Costa

Program of Natural and Synthetic Bioactive Products (PgPNSB), Health Sciences Center, Federal University of Paraíba, Campus I, Cidade Universitária, João Pessoa, PB, Brazil

ARTICLE INFO

Keywords:

Celastraceae

Maytenus erythroxylon

Triterpenes

In silico

Antimicrobial potential

ABSTRACT

The first phytochemical study of the aerial parts of *Maytenus erythroxylon* Reissek resulted in the isolation of five triterpenes, two with a lupane skeleton: 29-norlupan-3,20-dione (**1**) and 30-hydroxy-20 (29) -lupen-3-one (**2**) and three with friedelane skeleton: 3,4-secofriedelan-3-oic acid (**3**), 3-oxo-30-hydroxyfriedelane (**4**) and 3 β -Hydroxyfriedelane (**5**), where the compound (**1**) is reported for the first time in the *Maytenus* genus. The structures were identified through the analysis of uni and two-dimensional NMR spectral data. The isolated phases and compounds were subjected to *in silico* and *in vitro* techniques to evaluate the microbiological potential against microorganisms that cause intestinal disorders, showing that only the compound (**3**) was able to inhibit *Candida* spp. strains.

4. Natural Products as Potential Agents Against SARS-CoV and SARS-CoV-2

Journal: Current Medicinal Chemistry

Impact Factor: 4.53

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Current Medicinal Chemistry, 2021, 28, 1-0

1

REVIEW ARTICLE

Natural Products as Potential Agents Against SARS-CoV and SARS-CoV-2

Joanda Paolla Raimundo e Silva¹, Chonny Alexander Herrera Acevedo¹, Thalisson Amorim de Souza¹, Renata Priscila Barros de Menezes¹, Zoe L. Sessions², Lucas Silva Abreu¹, Samuel Paulo Cibulski¹, Luciana Scotti¹, Marcelo Sobral da Silva¹, Eugene N. Muratov², Marcus Tullius Scotti¹ and Josean Fechine Tavares^{1,*}

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Abstract: Background: Natural products are useful agents for the discovery of new lead-compounds and effective drugs to combat coronaviruses (CoV).

Objective: The present work provides an overview of natural substances, plant extracts, and essential oils as potential anti-SARS-CoV agents. In addition, this work evaluates their drug-like properties which are essential in the selection of compounds in order to accelerate the drug development process.

ARTICLE HISTORY

Received: August 18, 2020
Revised: December 06, 2020
Accepted: December 15, 2020

DOI:
10.2174/0929867328666210125113938

Methods: The search was carried out using PubMed, ScienceDirect and SciFinder. Articles addressing plant-based natural products as potential SARS-CoV or SARS-CoV-2 agents within the last seventeen years were analyzed and selected. The descriptors for Chemometrics analysis were obtained in alvaDesc and the principal component analysis (PCA) was carried out in SIMCA version 13.0.

Results: Based on *in vitro* assays and computational analyses, this review covers twenty-nine medicinal plant species and more than 300 isolated substances as potential anti-coronavirus agents. Among them, flavonoids and terpenes are the most promising compound classes. *In silico* analyses of drug-like properties corroborate these findings and indicate promising candidates for *in vitro* and *in vivo* studies to validate their activity.

Conclusion: This paper highlights the role of ethnopharmacology in drug discovery and suggests the use of integrative (*in silico*/ *in vitro*) and chemocentric approaches to strengthen current studies and guide future research in the field of antiviral agents.

Keywords: Natural products, drug discovery, SARS-CoV, SARS-CoV-2, COVID-19, *in silico*, *in vitro*.

5. Recent Studies on Neglected Drug Design

Journal: Current Topics in Medicinal Chemistry

Impact Factor: 3.57

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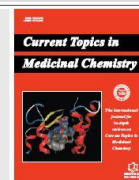
1943

Current Topics in Medicinal Chemistry, 2021, 21, 1943-1974

REVIEW ARTICLE



Recent Studies on Neglected Drug Design



Natália Ferreira de Sousa¹, Luciana Scotti^{1,2,*}, Gabriela Cristina Soares Rodrigues¹, Érika Paiva de Moura¹, Renata Priscila Barros Costa Barros¹, Zoe L. Sessions³, Eugene N. Muratov³ and Marcus Tullius Scotti²

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Abstract: Background: Neglected diseases require special attention when looking for new therapeutic alternatives, as these are diseases of extreme complexity and severity that affect populations belonging to lower social classes who lack access to basic rights, such as sanitation.

Introduction: Among the alternatives available for obtaining new drugs is Medicinal Chemistry, which is responsible for the discovery, identification, invention, and preparation of prototypes. In this perspective, the present work aims to make a bibliographic review on the recent studies of Medicinal Chemistry applied to neglected diseases.

Methods: A literature review was carried out by searching the “Web of Sciences” database, including recent articles published on the Neglected Drug Design.

Results: In general, it was noticed that the most studied neglected diseases corresponded to Chagas disease and leishmaniasis, with studies on organic synthesis, optimization of structures, and molecular hybrids being the most used strategies. It is also worth mentioning the growing number of computationally developed studies, providing speed and optimization of costs in the procurement process.

Conclusion: The CADD approach and organic synthesis studies, when applied in the area of Medicinal Chemistry, have proven to be important in the research and discovery of drugs for Neglected Diseases, both in terms of planning the experimental methodology used to obtain it and in the selection of compounds with higher activity potential.

Keywords: Neglected diseases, Drug discovery, Medicinal chemistry, Computer-aided drug design (CADD), Ligand-based drug design (LBDD), Structure-based drug design (SBDD), Organic synthesis.

ARTICLE HISTORY

Received: April 20, 2021

Revised: August 16, 2021

Accepted: August 20, 2021

DOI:

10.2174/1568026621666210920155939



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6. Computer-Assisted Design of Thiophene-Indole Hybrids as Leishmanial Agents

Journal: Current Topics in Medicinal Chemistry

Impact Factor: 3.57

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1700

Current Topics in Medicinal Chemistry, 2020, 20, 1700-1715

RESEARCH ARTICLE

Computer-Assisted Design of Thiophene-Indole Hybrids as Leishmanial Agents

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Abstract: Background: Chemoinformatics has several applications in the field of drug design, helping to identify new compounds against a range of ailments. Among these are Leishmaniasis, effective treatments for which are currently limited.

Objective: To construct new indole 2-aminothiophene molecules using computational tools and to test their effectiveness against *Leishmania amazonensis* (sp.).

Methods: Based on the chemical structure of thiophene-indol hybrids, we built regression models and performed molecular docking, and used these data as bases for design of 92 new molecules with predicted pIC₅₀ and molecular docking. Among these, six compounds were selected for the synthesis and to perform biological assays (leishmanicidal activity and cytotoxicity).

Results: The prediction models and docking allowed inference of characteristics that could have positive influences on the leishmanicidal activity of the planned compounds. Six compounds were synthesized, one-third of which showed promising antileishmanial activities, with IC₅₀ ranging from 2.16 and 2.97 μM (against promastigote forms) and 0.9 and 1.71 μM (against amastigote forms), with selectivity indexes (SI) of 52 and 75.

Conclusion: These results demonstrate the ability of Quantitative Structure-Activity Relationship (QSAR)-based rational drug design to predict molecules with promising leishmanicidal potential, and confirming the potential of thiophene-indole hybrids as potential new leishmanial agents.

ARTICLE HISTORY

Received: October 21, 2019
Revised: December 01, 2019
Accepted: December 15, 2019

DOI:
10.2174/1568026620666200616142120

Keywords: QSAR, drug design, 2-amino-thiophene, indole, leishmaniasis, *Leishmania amazonensis*.

7. Computer-assisted Discovery of Compounds with Insecticidal Activity Against *Musca domestica* and *Myrthima separata*

Journal: Food and Chemical Toxicology

Impact Factor: 5.572



Contents lists available at [ScienceDirect](#)

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox



Computer-assisted discovery of compounds with insecticidal activity against *Musca domestica* and *Mythimna separata*

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Andreza Barbosa Silva Cavalcanti^a, **Renata Priscila Costa Barros^a**, Luciana Scotti^a,
Carlos L. Cespedes-Acuña^b, Eugene N. Muratov^{a,c}, Marcus Tullius Scotti^{a,*}

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ARTICLE INFO

Keywords:

Insecticidal activity
QSAR
Docking
Computational hits

ABSTRACT

Pesticides are used to control and combat insects and pests in the agricultural sector, households, and public health programs. The frequent and disorderly use of these pesticides may lead to variety of undesired effects. Therefore, natural products have many advantages over to synthetic compounds to be used as insecticides. The goal of this study was to find natural products with insecticidal potential against *Musca domestica* and *Mythimna separata*. To achieve this goal, we developed predictive QSAR models using MuDRA, PLS, and RF approaches and performed virtual screening of 117 natural products. As a result of QSAR modeling, we formulated the recommendations regarding physico-chemical characteristics for promising compounds active against *Musca domestica* and *Mythimna separata*. Homology models were successfully built for both species and molecular docking of QSAR hits vs known insecticides allowed us to prioritize twenty-two compounds against *Musca domestica* and six against *Mythimna separata*. Our results suggest that pimarane diterpenes, abietanes diterpenes, dimeric diterpenes and scopadulane diterpenes obtained from aerial parts of species of the genus *Calceolaria* (Calceolariaceae: Scrophulariaceae) can be considered as potential insecticidal.

8. Ligand and Structure-based Virtual Screening of Lamiaceae Diterpenes with Potential Activity Against a Novel Coronavirus (2019-nCoV)

Journal: Current Topics in Medicinal Chemistry

Impact Factor: 3.57

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Current Topics in Medicinal Chemistry, 2020, 20, 1-20

1

RESEARCH ARTICLE

Ligand and Structure-based Virtual Screening of Lamiaceae Diterpenes with Potential Activity against a Novel Coronavirus (2019-nCoV)

Gabriela Cristina Soares Rodrigues¹, Mayara dos Santos Maia¹, **Renata Priscila Barros de Menezes¹**, Andreza Barbosa Silva Cavalcanti¹, Natália Ferreira de Sousa¹, Érika Paiva de Moura¹, Alex France Messias Monteiro¹, Luciana Scotti¹ and Marcus Tullius Scotti^{1,*}

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Abstract: Background: The emergence of a new coronavirus (CoV), named 2019-nCoV, as an outbreak originated in the city of Wuhan, China, has resulted in the death of more than 3,400 people this year alone and has caused worldwide an alarming situation, particularly following previous CoV epidemics, including the Severe Acute Respiratory Syndrome (SARS) in 2003 and the Middle East Respiratory Syndrome (MERS) in 2012. Currently, no exists for infections caused by CoVs; however, some natural products may represent potential treatment resources, such as those that contain diterpenes.

Objective: This study aimed to use computational methods to perform a virtual screening (VS) of candidate diterpenes with the potential to act as CoV inhibitors.

Methods: 1,955 diterpenes, derived from the Nepetoideae subfamily (Lamiaceae), were selected using the SistematX tool (<https://sistematx.ufpb.br>), which were used to make predictions. From the ChEMBL database, 3 sets of chemical structures were selected for the construction of predictive models.

Results: The chemical structures of molecules with known activity against SARS CoV, two of which were tested for activity against specific viral proteins and one of which was tested for activity against the virus itself, were classified according to their pIC₅₀ values [-log IC₅₀ (mol/l)].

Conclusion: In the consensus analysis approach, combining both ligand- and structure-based VSs, 19 compounds were selected as potential CoV inhibitors, including isotanshinone IIA (01), tanshinlactone (02), isocryptotanshinone (03), and tanshinlactone (04), which did not present toxicity within the evaluated parameters.

ARTICLE HISTORY

Received: February 20, 2020
Revised: April 15, 2020
Accepted: April 20, 2020

DOI:
10.2174/1568026620666200716114546

Keywords: Coronavirus, Virtual screening, Natural products database, Diterpenes, Consensus analysis, Ligand.

9. Pyrimidine Derivatives: QSAR Studies of Larvicidal Activity Against *Aedes aegypti*

Journal: Journal of the Brazilian Chemical Society

Impact Factor: 2.135

Artigo foi Capa da Revista



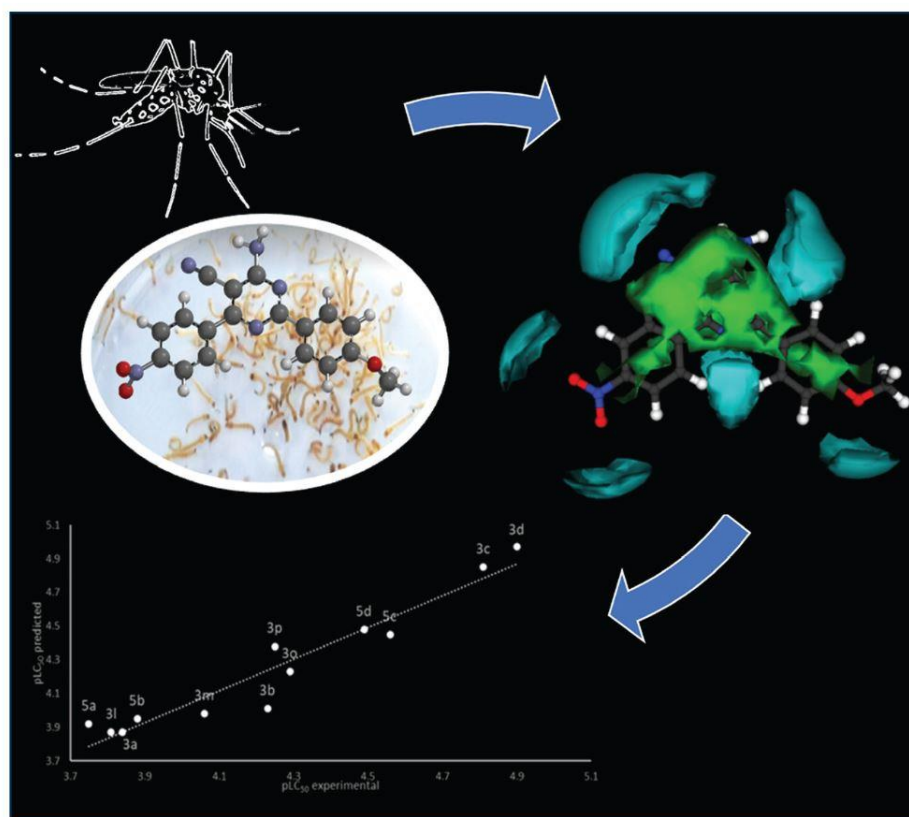
Journal of the Brazilian Chemical Society

JBCS

Vol. 31, No. 7

July, 2020

ISSN 0103-5053
ISSN online 1678-4790



Pyrimidine Derivatives: QSAR Studies of Larvicidal Activity against *Aedes aegypti*

Zenaide S. Monte, Daniela M. A. F. Navarro, Júlio C. R. O. F. Aguiar, Jessica S. Nascimento, Marcus T. Scotti, Luciana Scotti, Renata P. C. Barros, Aline C. S. Santos, Valéria R. A. Pereira, Emerson P. S. Falcão and Sebastião J. Melo

10. Virtual Screening and Assessment of Anticancer Potential of Selenium-Based Compounds Against HL-60 and MCF7 Cells

Journal: Future Medicinal Chemistry

Impact Factor: 4.767

Research Article

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Future
**Medicinal
Chemistry**

Virtual screening and assessment of anticancer potential of selenium-based compounds against HL-60 and MCF7 cells

Ericka Fernanda Ferreira de Queiroz¹, José Alixandre de Sousa Luis², Diego de Sousa Dantas³, Lúcia Cristina Pereira Arruda⁴, Ellen Cordeiro Bento da Silva¹, João Manoel de Sousa Silva², Helivaldo Diogenes da Silva Souza⁵, Renata Priscila Costa Barros⁶, Luciana Amaral de Mascena Costa¹, Petrônio Figueiras de Athayde Filho⁵, Luciana Scotti⁶, Marcus Tullius Scotti^{*,6} & Manoel Adrião Gomes Filho¹

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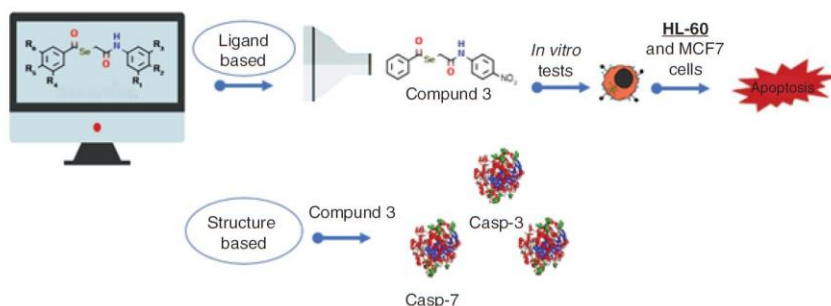
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Aim: Selenium-based compounds have antitumor potential. We used a ligand-based virtual screening analysis to identify selenoglycolicamides with potential antitumor activity. **Results & Conclusion:** Compounds **3**, **6**, **7** and **8** were selected for *in vitro* cytotoxicity tests against various cell lines, according to spectrophotometry results. Compound **3** presented the best cytotoxicity results against a promyelocytic leukemia line (HL-60) and was able to induce cell death at a frequency similar to that observed for doxorubicin. The docking study showed that compound **3** has good interaction energies with the targets caspase-3, 7 and 8, which are components of the apoptotic pathway. These results suggested that selenium has significant pharmacological potential for the selective targeting of tumor cells, inducing molecular and cellular events that culminate in tumor cell death.

Graphical abstract:



First draft submitted: 13 April 2020; Accepted for publication: 9 September 2020; Published online: 27 November 2020

Keywords: anticancer activity • HL-60 cells • MCF7 cells • organoselenium • selenoglycolicamides • virtual screening

newlands
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11. Virtual Screening of Natural Products Database

Journal: Mini-Reviews in Medicinal Chemistry

Impact Factor: 3.737

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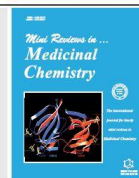
2657

Mini-Reviews in Medicinal Chemistry, 2021, 21, 2657-2730

REVIEW ARTICLE



Virtual Screening of Natural Products Database



José Alixandre de Sousa Luis^{1,2}, Renata Priscila Costa Barros², Natália Ferreira de Sousa², Eugene Muratov³, Luciana Scotti² and Marcus Tullius Scotti^{2,*}

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ARTICLE HISTORY

Received: January 24, 2020
Revised: February 03, 2020
Accepted: February 19, 2020

DOI:
10.2174/1389557520666200730161549



CrossMark

Abstract: Constant research on natural products has generated, over time, a large number of compounds with the potential to be evaluated in several biological tests and subsequently have been cataloged in databases that allow other researchers to perform virtual screenings of activity in various biological systems. This considerably reduces the time for the development of new drugs. This review describes the main databases of natural products available for searching bioactive compounds. It also describes the main features of virtual screening strategies for the identification of molecules with the potential to be used as new drugs. In addition, a search was made in the Web of Science database, using the search term "Virtual screening of natural products databases" from 2003 to 2018. The search criterion resulted in 230 articles, which had their abstracts evaluated with pertinence to the criteria required for this work, which are: a) be a research article; b) performing a virtual screening on databases of natural products or containing natural products; and c) works that identified drug candidate molecules. Based on these criteria, the bibliographic review on the topic was excluded. After this analysis, 104 works were selected for this review. We selected relevant papers describing the potential drug candidates that were distributed in 15 classes, of which the anticancer, antibacterial and anti-inflammatory hits were the most abundant. The works showing efforts to search for new molecules against various other diseases in distinct biological systems were also described. In this way, this work shows an overview of several methodologies and we hope they can help and inspire the development of new research to improve people's quality of life.

Keywords: Natural products, natural products databases, virtual screening, ligand-based, structure-based, drug candidates.

12. Virtual Screening of Secondary Metabolites of the Family Velloziaceae J. Agardh with Potential Antimicrobial Activity

Journal: Journal of the Brazilian Chemical Society

Impact Factor: 2.135

<http://dx.doi.org/10.21577/0103-5053.20200112>

J. Braz. Chem. Soc., Vol. 00, No. 00, 1-6, 2020

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Article



Virtual Screening of Secondary Metabolites of the Family Velloziaceae J. Agardh with Potential Antimicrobial Activity

Anderson A. V. Pinheiro,^{1a} Renata P. C. Barros,^a Edileuza B. de Assis,^a
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The objective of this work was to carry out a bibliographic survey of secondary metabolites isolated from the Velloziaceae family, creating a bank of compounds. After the bank was created, four prediction models for potentially active compounds against pathogenic microorganisms (*Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* sp.) were obtained trying to identify which metabolites would be more active against the strains. Four sets of compounds with known activity for microorganisms were selected for the construction of predictive models from the ChEMBL database. Another bank with 163 unique molecules isolated from the Velloziaceae family was built. The Volsurf+ v.1.0.7 software obtained the molecular descriptors and Knime 3.5 generated the *in silico* model. The performances of the internal and external tests were also analyzed. The study contributed through the virtual screening of a bank of metabolites to select several compounds with potential antimicrobial activity, highlighting the biflavonoid amentoflavone which showed potential activity against the four strains.

Keywords: amentoflavone, virtual screening, antibacterial activity, antifungal activity, Velloziaceae

13. Computer-Aided Chemotaxonomy and Bioprospecting Study of Diterpenes of the Lamiaceae Family

Journal: Molecules

Impact Factor: 4.927



Article

Computer-Aided Chemotaxonomy and Bioprospecting Study of Diterpenes of the Lamiaceae Family

Andreza Barbosa Silva Cavalcanti, Renata Priscila Costa Barros, Vicente Carlos de Oliveira Costa, Marcelo Sobral da Silva, Josean Fechine Tavares , Luciana Scotti  and Marcus Tullius Scotti * 

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Received: 4 October 2019; Accepted: 27 October 2019; Published: 30 October 2019



Abstract: Lamiaceae is one of the largest families of angiosperms and is classified into 12 subfamilies that are composed of 295 genera and 7775 species. It presents a variety of secondary metabolites such as diterpenes that are commonly found in their species, and some of them are known to be chemotaxonomic markers. The aim of this work was to construct a database of diterpenes and to use it to perform a chemotaxonomic analysis among the subfamilies of Lamiaceae, using molecular descriptors and self-organizing maps (SOMs). The 4115 different diterpenes corresponding to 6386 botanical occurrences, which are distributed in eight subfamilies, 66 genera, 639 different species and 4880 geographical locations, were added to SistematX. Molecular descriptors of diterpenes and their respective botanical occurrences were used to generate the SOMs. In all obtained maps, a match rate higher than 80% was observed, demonstrating a separation of the Lamiaceae subfamilies, corroborating with the morphological and molecular data proposed by Li et al. Therefore, through this chemotaxonomic study, we can predict the localization of a diterpene in a subfamily and assist in the search for secondary metabolites with specific structural characteristics, such as compounds with potential biological activity.

Keywords: Lamiaceae; database; diterpenes; chemotaxonomic; SOMs

14. *In Silico* Studies for Bacterystic Evaluation Against *Staphylococcus aureus* of 2-Naphthoic Acid Analogues

Journal: Current Topics in Medicinal Chemistry

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Current Topics in Medicinal Chemistry, 2020, 20, 293-304

RESEARCH ARTICLE



In Silico Studies for Bacterystic Evaluation against *Staphylococcus aureus* of 2-Naphthoic Acid Analogues



Alex France Messias Monteiro¹, Marcus Tullius Scotti^{1,*}, Alejandro Speck-Planche²,
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Abstract: Background: *Staphylococcus aureus* is a gram-positive spherical bacterium commonly present in nasal fossae and in the skin of healthy people; however, in high quantities, it can lead to complications that compromise health. The pathologies involved include simple infections, such as folliculitis, acne, and delay in the process of wound healing, as well as serious infections in the CNS, meninges, lung, heart, and other areas.

Aim: This research aims to propose a series of molecules derived from 2-naphthoic acid as a bioactive in the fight against *S. aureus* bacteria through in silico studies using molecular modeling tools.

Methods: A virtual screening of analogues was done in consideration of the results that showed activity according to the prediction model performed in the KNIME Analytics Platform 3.6, violations of the Lipinski rule, absorption rate, cytotoxicity risks, energy of binder-receptor interaction through molecular docking, and the stability of the best profile ligands in the active site of the proteins used (PDB ID 4DXD and 4WVG).

Results: Seven of the 48 analogues analyzed showed promising results for bactericidal action against *S. aureus*.

Conclusion: It is possible to conclude that ten of the 48 compounds derived from 2-naphthoic acid presented activity based on the prediction model generated, of which seven presented no toxicity and up to one violation to the Lipinski rule.

ARTICLE HISTORY

Received: April 02, 2019
Revised: September 01, 2019
Accepted: September 10, 2019

DOI:
10.2174/1568026619666191206111742



CrossMark

Keywords: *Staphylococcus aureus*, 2-Naphthoic Acid Analogues, KNIME Analytics Platform, Lipinski rule, Cytotoxicity risks, Phenolic compounds.

15. Larvicidal Compounds Extracted from *Helicteres velutina* k. Schum (Sterculiaceae) Evaluated Against *Aedes aegypti* L.

Journal: Molecules

Impact Factor: 4.972



Article

Larvicidal Compounds Extracted from *Helicteres velutina* K. Schum (Sterculiaceae) Evaluated against *Aedes aegypti* L.

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Academic Editors: Valeria Patricia Sülsen and Albertina Moglioni

Received: 23 April 2019; Accepted: 17 June 2019; Published: 22 June 2019



Abstract: *Helicteres velutina* K. Schum (Sterculiaceae), a member of Malvaceae *sensu lato*, is a Brazilian endemic plant that has been used by the indigenous tribe Pankarare as an insect repellent. A previous study has reported the isolation of terpenoids, flavonoids and pheophytins, in addition to the larvicidal activity of crude *H. velutina* extracts derived from the aerial components (leaves, branches/twigs, and flowers). The present study reports the biomonitoring of the effects of fractions and isolated compounds derived from *H. velutina* against *A. aegypti* fourth instar larvae. A crude ethanol extract was submitted to liquid–liquid extraction with hexane, dichloromethane, ethyl acetate and *n*-butanol to obtain their respective fractions. Larvicidal evaluations of the fractions were performed, and the hexane and dichloromethane fractions exhibited greater activities than the other fractions, with LC₅₀ (50% lethal concentration) values of 3.88 and 5.80 mg/mL, respectively. The phytochemical study of these fractions resulted in the isolation and identification of 17 compounds. The molecules were subjected to a virtual screening protocol, and five molecules presented potential larvicidal activity after analyses of their applicability domains. When molecular docking was analysed, only three of these compounds showed an ability to bind with sterol carrier protein-2 (1PZ4), a protein found in the larval intestine. The compounds tiliroside and 7,4'-di-*O*-methyl-8-*O*-sulphate flavone showed in vitro larvicidal activity, with LC₅₀ values of 0.275 mg/mL after 72 h and 0.182 mg/mL after 24 h of exposure, respectively. This is the first study to demonstrate the larvicidal activity of sulphated flavonoids against *A. aegypti*. Our results showed that the presence of the OSO₃H group attached to C-8 of the flavonoid was crucial to the larvicidal activity. This research supports the traditional use of *H. velutina* as an alternative insecticide for the control of *A. aegypti*, which is a vector for severe arboviruses, such as dengue and chikungunya.

Keywords: *Helicteres velutina*; in silico study; larvicidal activity; 7,4'-di-*O*-methyl-8-*O*-sulphate flavone; *Aedes aegypti*

16. Virtual Screening based on Ligand and Structure with *in vitro* Assessment of Neolignans Against *Trypanosoma cruzi*

Journal: Journal of the Brazilian Chemical Society

Impact Factor: 2.135

<https://dx.doi.org/10.21577/0103-5053.20220113>

J. Braz. Chem. Soc., Vol. 00, No. 00, 1-20, 2022
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Article

Virtual Screening Based on Ligand and Structure with *in vitro* Assessment of Neolignans against *Trypanosoma cruzi*

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Gabriela C. S. Rodrigues,^a Renata P. B. Menezes,^a Marcelo S. Silva,^b Josean F. Tavares,^b
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Chagas disease, caused by the parasite *Trypanosoma cruzi*, occurs most commonly in Latin America. As the treatment is highly toxic and ineffective in the chronic phase of the disease, alternative treatments are needed. Through quantitative structure-activity relationship modeling (QSAR) analysis using ligand-based and structure-based virtual screening methods, we predicted the trypanocidal potential of 47 neolignans against three targets, the enzymes cruzain, trypanothione reductase, and sterol 14- α demethylase. A combined analysis allowed for the selection of potent inhibitors against *Trypanosoma cruzi*. Of these compounds, two were isolated and shown to inhibit the growth of epimastigotes at concentrations of 9.64 and 8.72 μ M, and trypomastigote forms at 4.88 and 2.73 μ M. Therefore, the compounds (2*R*, 3*R*)-2,3-dihydro-2-(4-methoxyphenyl)-3-methyl-5-(*E*)-propenylbenzofuran (**46**) and ottomentosa (**47**) may be a good option of growth inhibitors for the parasite stages and warrant additional study.

Keywords: Chagas disease, *Trypanosoma cruzi*, neolignane, virtual screening, molecular docking, molecular dynamics simulations

17. Diterpenos Ent-Abietanos de *Euphorbia phosphorea* (Euphorbiaceae)

Journal: Química Nova

Impact Factor: 0.961

Quim. Nova, Vol. XY, No. 00, 1-6, 200_

<http://dx.doi.org/10.21577/0100-4042.20170932>

DITERPENOS ENT-ABIETANOS DE *Euphorbia phosphorea* (EUPHORBIACEAE)

Roseana A. R. Meireles^a, Lucas S. Abreu^b, Joanda Paolla R. e Silva^a, Andreza B. S. Cavalcanti^a, Renata Priscila B. de Menezes^a, Gabriela Cristina S. Rodrigues^a, Valnês S. Rodrigues Junior^{c,d}, José Iranildo M. de Melo^e, Massuo Jorge Kato^f, Vicente Carlos de O. Costa^a, Marcus Tullius Scotti^a e Josean F. Tavares^{a,*} 

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Recebido em 11/12/2021; aceito em 06/06/2022; publicado na web em 19/08/2022

ENT-ABIETANE DITERPENES FROM *Euphorbia phosphorea* (EUPHORBIACEAE). Phytochemical study of the roots of *Euphorbia phosphorea* Mart. (Euphorbiaceae) was carried out through chromatographic techniques, resulting in the isolation of a new *ent*-abietane diterpene named 11 β ,12 β -dihydroxy-*ent*-abieta-8(14),13(15)-dien-16,12 α -olide (**1**), and of nine known *ent*-abietane diterpenes jolkinolide A (**2**), jolkinolide E (**3**), euphorin H (**4**), euphopilolide (**5**) jolkinolide F (**6**), *ent*-12-hydroxy-12[*R*]-abieta-8(14),13(15)-dien-16,12-olide (**7**), *ent*-11 α -hydroxyabieta-8(14),13(15)-dien-16,12 α -olide (**8**), 17-hydroxyjolkinolide B (**9**) and caudicifolin (**10**). The structures of all compounds were established using spectroscopic techniques such as 1D and 2D NMR, and the structure of the compound **1** was established also with MS, IR and ECD. All compounds were submitted to an *in silico* study through of a predictive model and then submitted to *in vitro* tests against *Mycobacterium tuberculosis* and *M. smegmatis* for evaluation of their antimycobacterial activity. Compounds **5** and **9** showed mycobacterial growth inhibition with MIC values of 62.5 μ M against *M. tuberculosis* and *M. smegmatis*, respectively.

Keywords: Euphorbiaceae; *Euphorbia*; *ent*-abietane diterpenes; tuberculosis; *Mycobacterium*.

18. MolPredictX: in House Collection of QSAR Models from Federal University of Paraiba

Journal: Molecular Informatics

Impact Factor: 6.376

Research Article

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molecular
informatics

doi.org/10.1002/minf.202200133

MolPredictX: Online Biological Activity Predictions by Machine Learning Models

Marcus Tullius Scotti,^[a] Chonny Herrera-Acevedo,^[a, b] Renata Priscila Barros de Menezes,^[a] Holli-Joi Martin,^[c] Eugene N. Muratov,^[c] Ávilla Ítalo de Souza Silva,^[a] Emmanuella Faustino Albuquerque,^[a] Lucas Ferreira Calado,^[a] Ericsson Coy-Barrera,^[d] and Luciana Scotti.^[a]

Abstract: Here we report the development of MolPredictX, an innovate and freely accessible web interface for biological activity predictions of query molecules. MolPredictX utilizes in-house QSAR models to provide 27 qualitative predictions (active or inactive), and quantitative probabilities for bioactivity against parasitic (*Trypanosoma* and *Leishmania*), viral (Dengue, Sars-CoV and Hepatitis C),

pathogenic yeast (*Candida albicans*), bacterial (*Salmonella enterica* and *Escherichia coli*), and Alzheimer disease enzymes. In this article, we introduce the methodology and usability of this webtool, highlighting its potential role in the development of new drugs against a variety of diseases. MolPredictX is undergoing continuous development and is freely available at <https://www.molpredictx.ufpb.br/>.

Keywords: MolPredictX · Online web-based tool · biological activity · Qualitative prediction · Drug development

1 Introduction

Quantitative Structure Activity Relationship (QSAR) modeling is a cheminformatics method that establishes a relationship between a molecules biological activity and its structure.^[1–5] Studies by Hammet in 1937 and 1940^[6,7] initiated our understanding of molecular properties of organic compounds by establishing a linear relationship between varying substituent groups and the impact on biological properties. Subsequently, this relationship became known as the Hammet equation.

Decades later, Hansch and Fujita laid the foundations for QSAR/QSPR studies.^[8,9] In their pioneering study in 1962, Hansch and Fujita demonstrated that biological activity could be linearly correlated with different physicochemical parameters, related to hydrophobic, steric and electronic effects.^[8,9] Providing scientific support for the modeling of a biochemical property.

Over 60 years later, these models are still being developed^[8–10] using the principle that the molecular structure of a compound contains information about physical, chemical, and biological properties; in other words, similar chemical structures will have similar properties.^[5,10] Machine learning-based QSAR models are based on this same principle^[10] and have been extremely useful in to guide synthesis efforts and aid in early drug design.^[11,12] The use of these models also save time and decrease the costs associated with experimental procedures.^[10,13–20] However, like all computational methods, it is extremely important that QSAR models are properly validated before being used to interpret and predict the biological responses of compounds.^[21]

In recent years, several web servers have emerged to develop and apply Quantitative Structure Activity Relation-

ship (QSAR) models using the Predictive Model Markup Language (PMML) format. Chembench, developed by Capuzzi, et al., in 2017^[22] is an integrated cheminformatics portal that automatically executes the complete QSAR process. Chembench uses five available machine learning algorithms (random forest (RF), support vector machine (SVM), k-nearest neighbors (kNN), genetic algorithm (GA) and simulated annealing (SA)) to perform ligand-based virtual screening. In the Chembench portal, it is also possible to use the QSAR MuDRA model, developed by Alves et al., 2018,^[23] which is conceptually related to the well-known kNN approach, but uses different types of chemical descriptors simultaneously for similarity assessment, providing a powerful alternative to consensus QSAR modeling.

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19. Targeted Isolation of Anti-inflammatory Lignans from *Justicia aequilabris* by Molecular Networking Approach

Journal: Journal of Natural Products

Impact Factor: 4.803



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Article

Targeted Isolation of Anti-inflammatory Lignans from *Justicia aequilabris* by Molecular Networking Approach

Joanda P. R. e Silva, Laiane C. O. Pereira, Lucas S. Abreu, Francisca S. V. Lins, Thalisson A. de Souza, Renan F. do Espírito-Santo, **Renata P. C. Barros**, Cristiane F. Villarreal, José I. M. de Melo, Marcus T. Scotti, Vicente C. de O. Costa, Lucas H. Martorano, Fernando M. dos Santos Jr., Raimundo Braz Filho, Marcelo S. da Silva, and Josean F. Tavares*



Cite This: <https://doi.org/10.1021/acs.jnatprod.2c00478>



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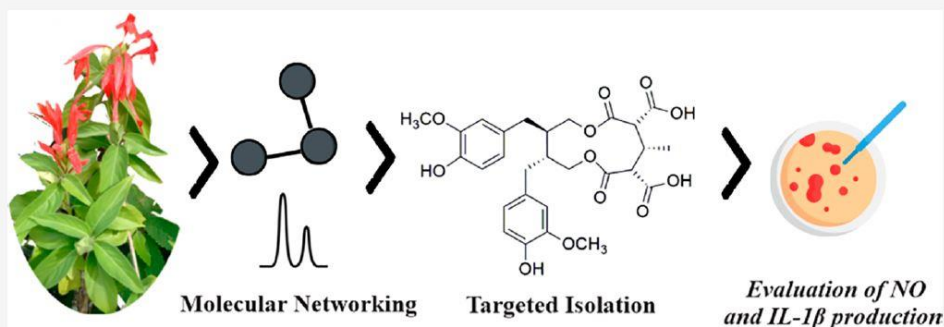
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Supporting Information



ABSTRACT: Herein, the isolation of secondary metabolites from the aerial parts of *Justicia aequilabris* guided by HPLC-MSⁿ and molecular networking analyses is reported. Twenty-two known compounds were dereplicated. Three new lignans (aequilabrine A–C (**1–3**)) and three known compounds (lariciresinol-4'-O- β -glucose (**4**), roseoside (**5**), and allantoin (**6**)) were obtained. The anti-inflammatory activity of compounds **1–3** was evaluated *in vitro* by inhibiting the nitric oxide production (NO) and pro-inflammatory activity on the cytokine IL-1 β . Compounds **2** and **3** showed significant inhibitory activity against NO production, with IC₅₀ values of 9.1 and 7.3 μ M, respectively. The maximum inhibition of IL-1 β production was 23.5% (**1**), 27.3% (**2**), and 32.5% (**3**).

20. Patente

Natureza Patente: Patente de Invenção (PI)

Título da Invenção ou Modelo de Utilidade: Formulação Nutracêutica, seu Processo de Obtenção e seu Uso

Depositante: Universidade Estadual da Paraíba

Inventores: Edja Maria Melo de Brito Costa, Joanilda Paolla Raimundo e Silva, Joanda Paolla Raimundo e Silva, Josean Fechine Tavares, Marcus Tullius Scotti, Renata Priscila Barros de Menezes

Número de Processo: BR 10 2021 021115 6



Pedido nacional de Invenção, Modelo de Utilidade, Certificado de Adição de Invenção e entrada na fase nacional do PCT

Número do Processo: BR 10 2021 021115 6

Dados do Depositante (71)

Depositante 1 de 2

Nome ou Razão Social: UNIVERSIDADE ESTADUAL DA PARAIBA

Tipo de Pessoa: Pessoa Jurídica

CPF/CNPJ: 12671814000137

Nacionalidade: Brasileira

Qualificação Jurídica: Instituição de Ensino e Pesquisa

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Artigos Submetidos em Revistas

1. Discovery of Novel Acetylcholinesterase Inhibitors Using the Integration of Machine Learning and other *In Silico* Screening Approaches

Authors: Mohd Imran Khan, Park Taehwan, Yunseong Cho, Marcus Scotti, Renata Priscila Barros De Menezes, Fohad Mabood Husain, Suliman Alomar, Mohammad Hassan Baig and Jaejune Dong

Journal: Frontiers in Neuroscience, section Neurogenomics

Impact Factor: 5.152

Status: In process



ANEXOS II

Eventos e Congressos

1. Ligand-Based and Structure-based virtual screening for the discovery of natural larvicidal against *Aedes aegypti*

Authors: Renata Priscila Barros de Menezes, Chonny Herrera Acevedo, Luciana Scotti, Marcus Tullius Scotti

DOI: 10.3390/mol2net-07-11743

Conference: MOL2NET'21, Conference on Molecular, Biomedical & Computational Sciences and Engineering, 7th ed. . Congress CHEMBIOINFO-07: EJIBCE & Chem-Bioinfo. Congress Coimbra, Portugal-München, Germany-Ch. Hill, USA, 2021

MOL2NET, 2021, 7, ISSN: 2624-5078

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MOL2NET, International Conference Series on Multidisciplinary Sciences

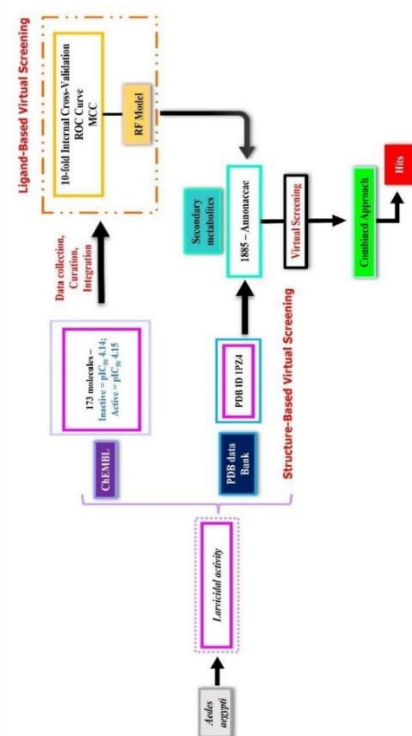
<http://sciforum.net/conference/mol2net-03>

Ligand-Based and Structure-based virtual screening for the discovery of natural larvicidal against *Aedes aegypti*

<Renata Priscila Barros de Menezes> (renatabarros@lft.ufpb.br)^a, <Chonny Herrera Acevedo> (chonny622@gmail.com)^a, <Luciana Scotti> (luciana.scotti@gmail.com)^a
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^a < Post-Graduate Program in Natural Synthetic Bioactive Products, Federal university of Paraiba >

Graphical Abstract



Abstract. The *Aedes aegypti* mosquito belongs to the order Diptera and is one of the main vectors of transmission of etiological agents that cause several diseases. This mosquito can transmit diseases such as dengue, yellow fever, Zika, chikungunya, among others. The aim of this study was combining structure-based and ligand-based virtual screening (VS) techniques to select potentially larvicidal active molecules against *Ae. aegypti* from in-house secondary metabolite dataset (SistematX). From the ChEMBL database, we selected a set of 161 chemical structures with larvicidal activity against *Ae. aegypti* to create random forest models with an accuracy value higher than 82% for cross-validation and test sets. Afterward, the ligand-based virtual screen selected 38 secondary metabolites. In addition, a structure-based virtual screening was also performed for the 38 molecules selected. Finally, using consensus analyzes approach combining ligand-based and structure-based VS, five molecules were selected as potential larvicidal against *Ae. aegypti*.

Keywords: Virtual Screening; Secondary Metabolites; Annonaceae; Larvicidal activity; *Aedes aegypti*

2. *In Silico* Identification of Potential Sesquiterpene Lactones from Sistemax Database against *Schistosoma mansoni*

Authors: Chonny Herrera-Acevedo, Renata Priscila Barros de Menezes, Luciana Scotti, Ericsson Coy-Barrera, Marcus Tullius Scotti

DOI: 10.3390/mol2net-07-11836

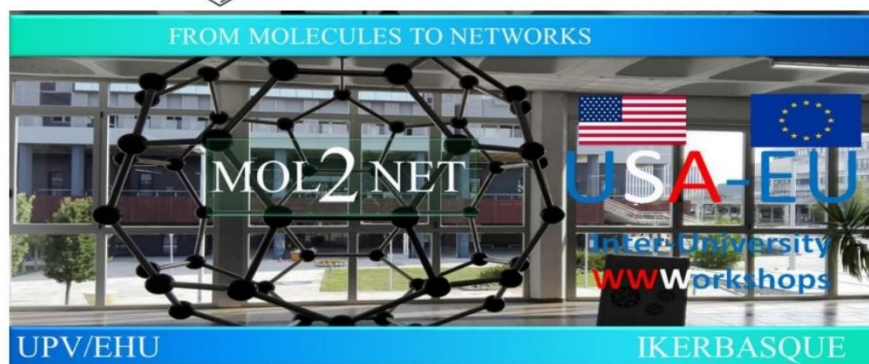
Conference: MOL2NET'21, Conference on Molecular, Biomedical & Computational Sciences and Engineering, 7th ed. Congress CHEMBIOINFO-07: EJIBCE & Chem-Bioinfo. Congress Coimbra, Portugal-München, Germany-Ch. Hill, USA, 2021

MOL2NET, 2021, 7, ISSN: 2624-5078
<https://mol2net-07.sciforum.net/>

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MOL2NET, International Conference
Series on Multidisciplinary Sciences



In silico Identification of Potential Sesquiterpene Lactones from Sistemax Database against *Schistosoma mansoni*

Chonny Herrera-Acevedo ^{a,b}, Renata Priscila Barros de Menezes ^a, Luciana Scotti ^a,
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Nueva Granada, Cajicá 250247, Colombia.

Graphical Abstract	Abstract. Schistosomiasis is an acute and chronic parasitic disease, caused by blood flukes (trematode worms) of the genus <i>Schistosoma</i> . For 2019, the World Health Organization estimated that close to 240 million people required preventive treatment against this disease, mainly poor communities without access to safe drinking water and adequate sanitation. Similarly, to others Neglected Tropical Diseases (NTDs) the treatments against this disease are limited, and new chemotherapies are necessary to the control

3. Ligand-Based and Structure-Based virtual screening for the discovery of natural inhibitor the Hepatitis C Virus

Authors: Renata Priscila Costa Barros, Marcus Tullius Scotti

DOI: 10.3390/mol2net-04-05873

Conference: MOL2NET 2018, International Conference on Multidisciplinary Sciences, 4th edition

MOL2NET, 2018, 4, <http://sciforum.net/conference/mol2net-04>

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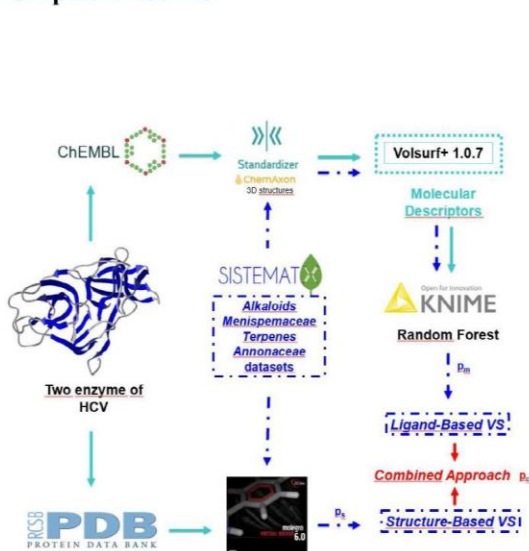
MOL2NET, International Conference Series on Multidisciplinary Sciences

Ligand-Based and Structure-Based virtual screening for the discovery of natural inhibitor the Hepatitis C Virus

<Renata Priscila Costa Barros> (renatabarros@ltf.ufpb.br)^a, <Marcus Tullius Scotti> (mtscotti@gmail.com)^a

^a < Post-Graduate Program in Natural Synthetic Bioactive Products, Federal university of Paraiba >

Graphical Abstract



Abstract. Hepatitis C is a disease that constitutes a serious global health problem, is often asymptomatic and difficult to diagnose, about 60-80% of infected patients develop chronic diseases over time. As there is no vaccine against hepatitis C virus (HCV), developing new cheap treatments is a big challenge. The search for new drugs from natural products has been outstanding in recent years. The aim of this study was combining structure-based and ligand-based virtual screening (VS) techniques to select potentially active molecules against two HCV target proteins from in-house secondary metabolite dataset (SistematX). From the ChEMBL database, we selected two sets of 1199 and 237 chemical structures with inhibitory activity against different targets of HCV to create random forest models with an accuracy value higher than 72% for cross-validation and test sets. Afterward, a ligand-based virtual screen of the entire 1378 secondary metabolites database stored in SistematoX was performed. In addition, a structure-based virtual screening was also performed for the same set of secondary metabolites using molecular docking. Finally, using consensus analyzes approach combining ligand-based and structure-based VS, two alkaloids and one triterpene were selected as potential anti-HCV compounds.

4. Proposition *in silico* of Benzoic Analogs against *Staphylococcus aureus*

Authors: Alex France Messias Monteiro, Jéssika de Oliveira Viana, Renata Priscila Costa Barros, Luciana Scotti, Marcus Tullius Scotti

DOI: 10.3390/mol2net-04-05875

Conference: MOL2NET 2018, International Conference on Multidisciplinary Sciences, 4th edition

MOL2NET, 2018, 4, <http://sciforum.net/conference/mol2net-04>

1



MOL2NET, International Conference Series on Multidisciplinary Sciences

PROPOSITION *IN SILICO* OF BENZOIC ANALOGS AGAINST *Staphylococcus aureus*

Alex France Messias Monteiro^{a*}, Jéssika de Oliveira Viana^a, Renata Priscila Costa Barros^a, Marcus Tullius Scotti^a, Luciana Scotti^{a,b}

^a Federal University of Paraíba, Health Sci. Center, 50670-910, João Pessoa, PB, Brazil;

^b Teaching and Research Management - University Hospital, Federal University of Paraíba, João Pessoa, PB, Brazil;

Graphical Abstract	Abstract
48 Analogues 15 Controls OSIRIS DataWarrior %ABS (109-0.345xTPSA) PDB PROTEIN DATA BANK DM (4DXD and 4WVG)	The microorganism <i>Staphylococcus aureus</i> is a gram-positive spherical bacteria present in the healthy human body, commonly found in the nostrils and skin. It is responsible for a number of infections such as inflammation in the follicles, contribution to the onset of acne, inflammation in the meninges, cardiovascular inflammation, lung inflammation and others. This work aims to propose new benzoic <i>in silico</i> bioactives to combat <i>Staphylococcus aureus</i>. To a prediction model of biological activity developed in the KNIME Analytics Platform 3.6, where the molecules that showed activity in the model were imported into OSIRIS DataWarrior 4.7.3 to predict the risks of cytotoxicity, the calculation of the absorption rate (% ABS). For the molecules approved by the virtual screening already commented, the calculation of the energies of interaction between receptor and binder by molecular docking and the analysis of the interactions with the residues of amino acids were carried out, comparing with the interactions of the drugs used as control in this research. As conclusion of this work it was possible to propose bioactive molecules against <i>S. aureus</i> based on the obtained computational data.

5. Application of Self-Organizing Maps generated from Molecular Descriptors of diterpenoids in Chemotaxonomy Studies of Lamiaceae Family

Authors: Andreza Cavalcanti, Marcelo Silva, Vicente Costa, **Renata Barros**, Luciana Scotti, Josean Tavares, Marcus Scotti

DOI: 10.3390/mol2net-04-05534

Conference: International Conference on Multidisciplinary Sciences, 4th edition in MOL2NET 2018, International Conference on Multidisciplinary Sciences, 4th edition session WRSAMC-03: Workshop in Medicinal Chemistry, UFPB, Paraiba, Brasil, 2018

MOL2NET, 2018, pages 1- 4

<http://sciforum.net/conference/mol2net-02/wrsamc>

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SciForum
MOL2NET

Application of Self-Organizing Maps generated from Molecular Descriptors of diterpenoids in Chemotaxonomy Studies of Lamiaceae Family

Andreza Cavalcanti ^{1,*}, Marcelo Silva ¹, Vicente Costa ¹, **Renata Barros** ¹, Luciana Scotti ¹, Josean Tavares ¹ and Marcus Scotti ¹

¹ Program of Natural and Synthetic Bioactive Products (PgPNSB), Health Sciences Center, Federal University of Paraíba, João Pessoa-PB, Brazil; E-mail: andreza.jp.pb@gmail.com

* Author to whom correspondence should be addressed; E-Mail: andreza.jp.pb@gmail.com; Tel.: 55-83-98713-6155.

Received: / Accepted: / Published:

Abstract: Lamiaceae is the largest family-level clade of the order Lamiales and comprises approximately 295 genera and 7775 species, presenting cosmopolitan distribution. It is estimated that in Brazil there are 36 genus and 490 species. Lamiaceae is classified into 10 subfamilies that present a large variety of secondary metabolites, among them diterpenes are commonly reported for this family. These diterpenes can be used in the chemotaxonomy of this family, because they have stable and quite diversified structures, being found in several species of the Lamiaceae family. Thus, the objective of this study is to classify two subfamilies of Lamiaceae based on the identification of diterpenes and their respective botanical occurrences available in our internal database (www.sistemax.ufpb.br), using descriptors calculated by DRAGON 7.0 software. The 3551 botanical occurrences and their 119 descriptors obtained from molecular fragments were used as input data in SOM Toolbox 2.0 (Matlab) to generate a self-organizing map (SOM), allowing to classify two subfamilies: Lamioideae (L) and Scutellarioideae (S). Therefore, the results obtained by the chemotaxonomic study corroborate with the phylogenetic classification based on the DNA that was proposed by Li et al., 2016.

Certificado



I Simpósio Paraibano de Estudos Químicos de Produtos Naturais

Certificamos que o trabalho **Application of Self-Organizing Maps generated from Molecular Descriptors of diterpenoids in Chemotaxonomy Studies of Lamiaceae Family** de autoria de **Andreza Barbosa Silva Cavalcanti, Marcelo Sobral da Silva, Vicente Carlos de Oliveira Costa, **Renata P. Barros**, Luciana Scotti, Josean Fachine tavares, Marcus Tullius Scotti** foi apresentado na modalidade Painel no I Simpósio Paraibano de Estudos Químicos de Produtos Naturais, realizado na Universidade Federal da Paraíba – João Pessoa, PB.

11 de outubro de 2018.

Ana Rita Rodrigues A. Silva

Ana Rita Rodrigues de Almeida Silva
Coordenadora Discente

Laisla Rangel Peixoto

Laisla Rangel Peixoto
Vice-coordenadora Discente

Realização:



6. Virtual Screening of 1,2-Diazoles Compounds for Chagas Disease: A Prediction Model

Authors: Sousa, N. F.; Barros, R. P. C.; Luís, J. A. S.; Scotti, L.; Scotti, M. T.

DOI: 10.3390/mol2net-04-05528

Conference: International Conference on Multidisciplinary Sciences, 4th edition in MOL2NET 2018, International Conference on Multidisciplinary Sciences, 4th edition session WRSAMC-03: Workshop in Medicinal Chemistry, UFPB, Paraíba, Brasil, 2018

MOL2NET, 2018, 4, <http://sciforum.net/conference/mol2net-04>

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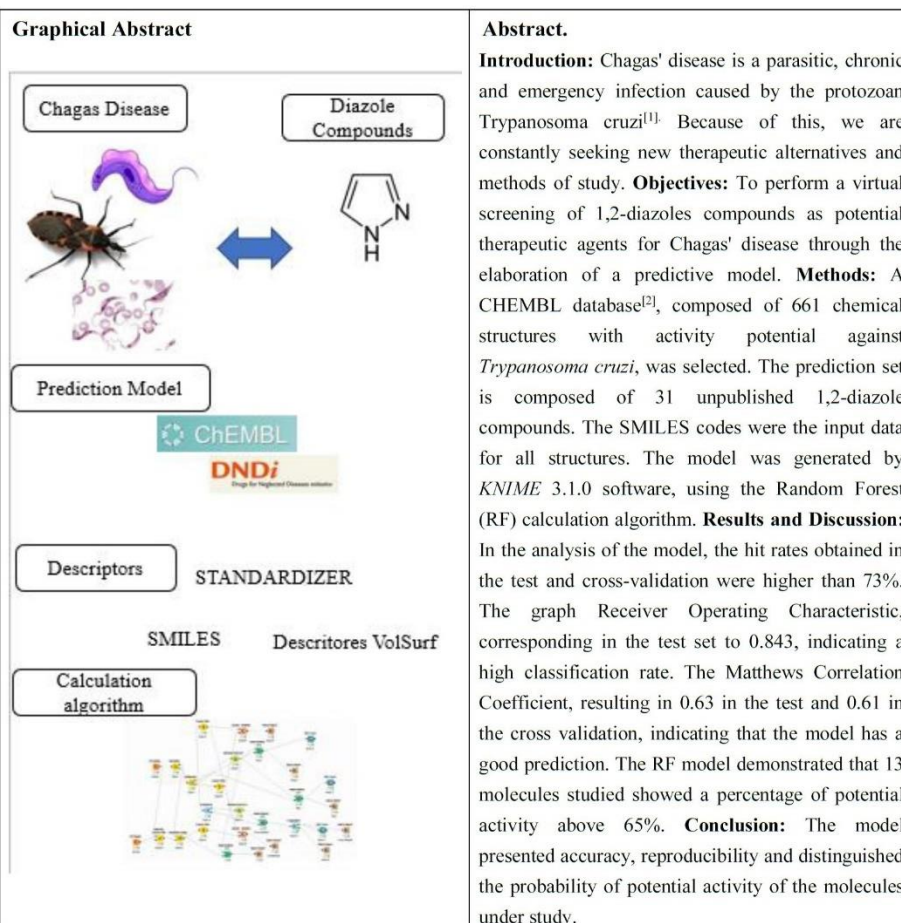
MOL2NET, International Conference Series on Multidisciplinary Sciences

Virtual Screening of 1,2-Diazoles Compounds for Chagas Disease: A Prediction Model

SOUSA, N.F.^a; BARROS, R.P.C.^a; LUÍS, J.A.S.^{a,b}; SCOTTI, L.^a; SCOTTI, M.T.^a

^aPost-Graduate Program in Natural and Synthetic Bioactive Products, Federal University of Paraíba, 58051-900 João Pessoa, PB, Brazil;

^bCenter of Education and Health, Federal University of Campina Grande. 58175-000 Cuité, PB, Brazil.
e-mail: nataliafsousa@ltf.ufpb.br



Certificado



I Simpósio Paraibano de Estudos Químicos de Produtos Naturais

Certificamos que o trabalho **Virtual Screening of 1,2-Diazoles Compounds for Chagas Disease: A Prediction Model** de autoria **Natália Ferreira de Sousa, Renata Priscila Costa Barros, José Alixandre de Sousa Luis, Luciana Scotti, Marcus Tullius Scotti** foi apresentado na modalidade Painel no I Simpósio Paraibano de Estudos Químicos de Produtos Naturais, realizado na Universidade Federal da Paraíba – João Pessoa, PB.

11 de outubro de 2018.

Vicente Carlos de Oliveira Costa
Vice-coordenador Geral

Ana Rita Rodrigues de Almeida Silva
Coordenadora Discente

Realização:



7. Participação como membro da Comissão Avaliadora no I Simpósio Paraibano de Estudos Químicos de Produtos Naturais.

Data: Outubro de 2018



8. Certificate of Attendance

Congress: Drug Discovery for Neglected Diseases International Congress 2018 and 4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

Local: Faculdade de Farmácia e Bioquímica da Universidade de Buenos Aires, Buenos Aires - Argentina



INSTITUTO DE LA QUÍMICA Y METABOLISMO DEL FÁRMACO



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CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS
FACULTAD DE FARMACIA Y BIOQUÍMICA – UNIVERSIDAD DE BUENOS AIRES



DRUG DISCOVERY FOR NEGLECTED DISEASES INTERNATIONAL CONGRESS 2018

4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

4th – 6th December 2018

Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires
Ciudad Autónoma de Buenos Aires, Argentina

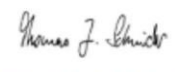
CERTIFICATE OF ATTENDANCE

This is to certify that **Renata Priscila Costa Barros**

has attended the Drug Discovery for Neglected Diseases International Congress 2018 held on 4th – 6th December 2018, at Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.


Albertina Moglioni PhD.
President of DDNDIC 2018


Valeria P. Sülsen PhD.
Vicepresident of DDNDIC 2018
Local Organizer 4th Scientific Meeting of ResNet NPND


Thomas J. Schmidt Dr. rer. nat
Coordinator of ResNet NPND

9. Poster presentation

Congress: Drug Discovery for Neglected Diseases International Congress 2018 and 4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

Title: Ligand-Based and Structure-Based Virtual Screening for the Discovery of Natural Inhibitor the DENV-2

Authors: Barros R. P. C. and Scotti M. T.



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DRUG DISCOVERY FOR NEGLECTED DISEASES INTERNATIONAL CONGRESS 2018

4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

4th – 6th December 2018

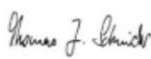
Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires
Ciudad Autónoma de Buenos Aires, Argentina

CERTIFICATE OF POSTER PRESENTATION

This is to certify that the paper entitled Ligand-based and structure-based virtual screening for the discovery of natural inhibitor the DENV-2
by Barros R. P. C. and Scotti M. T. has been presented in the Drug Discovery for
Neglected Diseases International Congress 2018 held on 4th–6th December 2018, at Facultad de Farmacia
y Bioquímica – Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.


Albertina Moglioni PhD.
President of DDNDIC 2018


Valeria P. Sülsen PhD.
Vicepresident of DDNDIC 2018
Local Organizer 4th Scientific Meeting of ResNet NPND


Thomas J. Schmidt Dr. rer. nat
Coordinator of ResNet NPND

10. Poster presentation

Congress: Drug Discovery for Neglected Diseases International Congress 2018 and 4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

Title: Phytochemical study of *Helicteres velutina* K. Schum (Sterculiaceae) biomonitoring by the tests against *Aedes aegypti* L. (Diptera: Culicidae)

Authors: D. Araújo Fernandes, L. H. Guimarães, M. D. Leite Ferreira, **R. P. Costa Barros**, M da Silva Oliveira, J. Batista Lima, F. da Cruz Nunes, M. Tullius Scotti, A. de Souza Conceição, M. de F. Vanderlei de Souza



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FACULTAD DE FARMACIA Y BIOQUÍMICA – UNIVERSIDAD DE BUENOS AIRES

DRUG DISCOVERY FOR NEGLECTED DISEASES INTERNATIONAL CONGRESS 2018

4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

4th – 6th December 2018

Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires
Ciudad Autónoma de Buenos Aires, Argentina

CERTIFICATE OF POSTER PRESENTATION

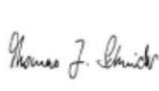
This is to certify that the paper entitled *Phytochemical study of Helicteres velutina* K. Schum (Sterculiaceae) biomonitoring by the tests against *Aedes aegypti* L. (Diptera: Culicidae)

by **D. Araújo Fernandes, L. H. Guimarães de Oliveira, M. D. Leite Ferreira, R. P. Costa Barros, M. da Silva Oliveira, J. Batista Lima, F. da Cruz Nunes, M. Tullius Scotti, A. de Souza Conceição, M. de F. Vanderlei de Souza.** has been presented in the Drug Discovery for

Neglected Diseases International Congress 2018 held on 4th–6th December 2018, at Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.


Albertina Moglioni PhD.
President of DDNDIC 2018


Valeria P. Sülsen PhD.
Vicepresident of DDNDIC 2018
Local Organizer 4th Scientific Meeting of ResNet NPND


Thomas J. Schmidt Dr. rer. nat
Coordinator of ResNet NPND

11. Poster presentation

Congress: Drug Discovery for Neglected Diseases International Congress 2018 and 4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

Title: The use of Oxadiazoles derivatives in Chagas's Disease: Elaboration of a Classification Model

Authors: Natália Ferreira de Souza, Renata Priscila Costa Barros, José Alixandre de Souza Luís, Marcus Tullius Scotti



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CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS
FACULTAD DE FARMACIA Y BIOQUÍMICA – UNIVERSIDAD DE BUENOS AIRES

DRUG DISCOVERY FOR NEGLECTED DISEASES INTERNATIONAL CONGRESS 2018

4th Scientific Meeting of the Research Network Natural Products against Neglected Diseases

4th – 6th December 2018

Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires
Ciudad Autónoma de Buenos Aires, Argentina

CERTIFICATE OF POSTER PRESENTATION

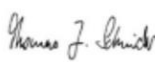
This is to certify that the paper entitled **The use of oxadiazoles derivatives in Chagas's disease: elaboration of a classification model**

by **Natália Ferreira de Sousa, Renata Priscila Costa Barros, José Alixandre de Sousa Luís, Marcus Tullius Scotti**

has been presented in the Drug Discovery for Neglected Diseases International Congress 2018 held on 4th–6th December 2018, at Facultad de Farmacia y Bioquímica – Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina.


Albertina Moglioni PhD.
President of DDNDIC 2018


Valeria P. Sülsen PhD.
Vicepresident of DDNDIC 2018
Local Organizer 4th Scientific Meeting of ResNet NPND


Thomas J. Schmidt Dr. rer. nat
Coordinator of ResNet NPND

12. Poster presentation

Congress: XX Simpósio Brasileiro de Química Teórica

Title: Prospecção Baseada no Ligante de Derivados Imidazóis como Agentes Terapêuticos Potenciais para a Doença de Chagas

Authors: Natália Ferreira de Souza, Renata Priscila Costa Barros, Mayara dos Santos Maia, Gabriela Cristina Soares Rodrigues, Luciana Scotti, Eugene Muratov, Marcus Tullius Scotti



XX SIMPÓSIO BRASILEIRO DE QUÍMICA TEÓRICA 2019

CERTIFICADO

Certificamos que o trabalho “*Prospecção baseada no ligante de derivados Imidazóis como agentes terapêuticos potenciais para a Doença de Chagas*”, do(s) autor(es) **Natália Ferreira de Sousa, Renata Priscila Costa Barros, Mayara dos Santos Maia, Gabriela Cristina Soares Rodrigues, Luciana Scotti, Eugene Muratov, Marcus Tullius Scotti**, foi apresentado em forma de Pôster no XX Simpósio Brasileiro de Química Teórica, realizado no período de 10 a 14 de novembro de 2019 Centro de Convenções da Paraíba.

João Pessoa-PB, 14 de novembro de 2019.


Profª Elizete Ventura do Monte
Coordenadora Geral do Evento

Apoio:



Patrocínio:



13. Poster presentation

Congress: XX Simpósio Brasileiro de Química Teórica

Title: Computer-Assisted Discovery of Compounds with Insecticidal Activity against *Musca domestica*

Authors: Gabriela Cristina Soares Rodrigues, Mayara dos Santos Maia, Andreza Barbosa Silva Cavalcanti, **Renata Priscila Costa Barros**, Carlos L. Céspedes, Eugene Muratov, Marcus Tullius Scotti



XX SIMPÓSIO BRASILEIRO DE QUÍMICA TEÓRICA 2019

CERTIFICADO

Certificamos que o trabalho “Computer-assisted discovery of compounds with insecticidal activity against *Musca domestica*”, do(s) autor(es) **Gabriela Cristina Soares Rodrigues, Mayara dos Santos Maia, Andreza Barbosa Silva Cavalcanti, Renata Priscila Costa Barros, Natália Ferreira de Sousa, Luciana Scotti, Carlos L. Céspedes, Eugene N. Muratov, Marcus Tullius Scotti**, foi apresentado em forma de Pôster no XX Simpósio Brasileiro de Química Teórica, realizado no período de 10 a 14 de novembro de 2019 Centro de Convenções da Paraíba.

João Pessoa-PB, 14 de novembro de 2019.


Profª Elizete Ventura do Monte
Coordenadora Geral do Evento

Apoio:



Patrocínio:



14. Poster presentation

Congress: 59º Congresso Brasileiro de Química

Title: A Utilização de Derivados Tioglicosídeos na *Leishmania infatum*: Uma Triagem Virtual

Authors: de Sousa N. F., Maia, M. S., **Barros, R. P. C.**, Souza, H. D. S., Scotti, L., Scotti, M. T.



ASSOCIAÇÃO BRASILEIRA DE QUÍMICA

Certificado

Certificamos que **de Sousa, N.F.; Maia, M.S.; Barros, R.P.C.; Souza, H.D.S.; Scotti, L.; Scotti, M.T.** apresentou(aram) o trabalho "A UTILIZAÇÃO DE DERIVADOS TIOGLICOSÍDEOS NA LEISHMANIA INFANTUM: UMA TRIAGEM VIRTUAL" no **59º Congresso Brasileiro de Química**, realizado de 05/11/2019 à 08/11/2019, no Centro de Eventos do Tambaú Hotel, em João Pessoa / PB.

João Pessoa, 08 de Novembro de 2019


Marta Célia Dantas Silva
Presidente do 59º CBQ

15. Poster presentation

Congress: VI Encontro de Química da UFPB

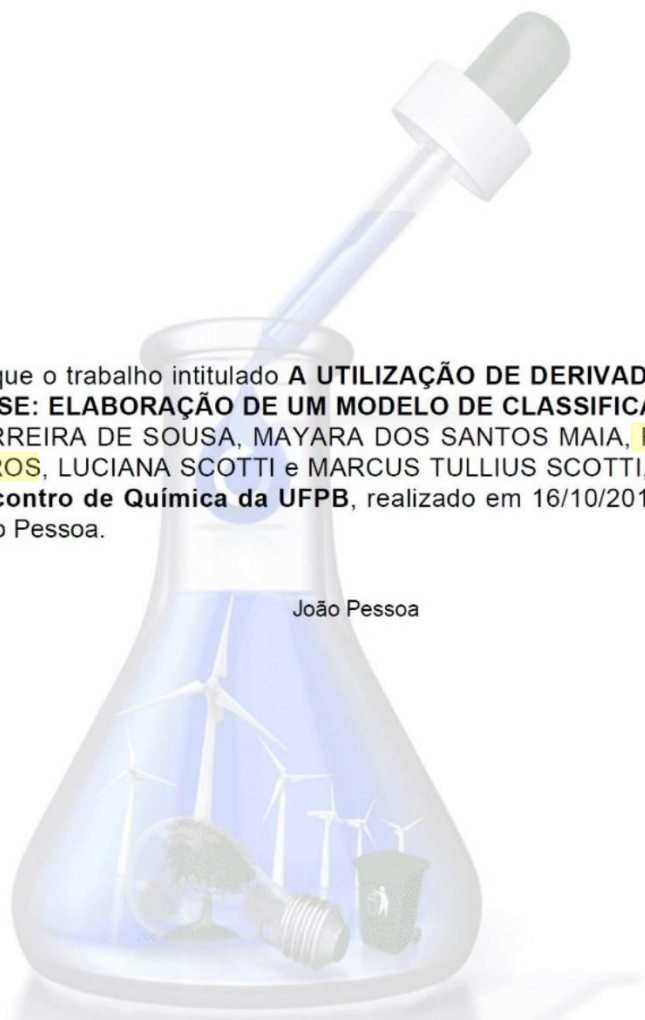
Title: A Utilização de Derivados Imidazóis na Leishmaniose: Elaboração de um Modelo de Classificação

Authors: Natália Ferreira de Sousa, Mayara dos Santos Maia, Renata Priscila Costa Barros, Luciana Scotti, Marcus Tullius Scotti

Verifique o código de autenticidade 106398.8419709.870477.8.0174998243049308339 em <https://www.even3.com.br/documentos>

Certificamos que o trabalho intitulado **A UTILIZAÇÃO DE DERIVADOS IMIDAZOIS NA LEISHMANIOSE: ELABORAÇÃO DE UM MODELO DE CLASSIFICAÇÃO** de autoria de NATÁLIA FERREIRA DE SOUSA, MAYARA DOS SANTOS MAIA, RENATA PRISCILA COSTA BARROS, LUCIANA SCOTTI e MARCUS TULLIUS SCOTTI, foi apresentado no evento **VI Encontro de Química da UFPB**, realizado em 16/10/2019 a 18/10/2019, na cidade de João Pessoa.

João Pessoa



VI Encontro de QUÍMICA da UFPB
II Workshop de ENSINO de Química da UFPB
I Workshop de PESQUISA em Química da UFPB

16. Poster presentation

Congress: 9th BrazMedChem Brazilian Symposium on Medicinal Chemistry

Title: Computational Approaches for Discovery of Alkaloids with Schistosomicidal activity

Authors: Renata Priscila Costa Barros, Jéssika de Oliveira Viana, Luciana Scotti, Marcus Tullius Scotti



17. Oral presentation

Event: I Escola de Inverno em Ciências Farmacêuticas

Title: Secondary Metabolites Extracted from Annonaceae and Chemotaxonomy Study of Terpenoids (Resultado do meu projeto de pesquisa)

Author: Renata Priscila Barros de Menezes



Universidade do Estado da Bahia

Departamento de Ciências da Vida - Campus I

Programa de Pós-Graduação em Ciências Farmacêuticas

Certificado

Certificamos que **RENATA PRISCILA BARROS DE MENEZES** participou da I Escola De Inverno em Ciências Farmacêuticas, promovida pelo PPGFARMA/UNEB em parceria com o Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos da Universidade Federal da Paraíba (PPgPNSB/UFPB), apresentando os resultados do seu projeto de pesquisa (carga-horária 2 horas).

Salvador, 14 de Agosto de 2021


Aníbal de Freitas Santos Júnior
Coordenação Geral do Evento

Certificado registrado sob o número 402893946.39.20210814

18. Minicurso

Title: Análise de dados de metabolômica explorando ferramentas do GNPS: dos princípios básicos a ferramentas *in silico* de anotação

Workload: 5 hours

Organization: Sociedade Brasileira de Química de Produtos Naturais



Sociedade Brasileira de Química
Divisão de Produtos Naturais

Certificado

Certificamos que

Renata Priscila Barros de Menezes

Participou como ouvinte do minicurso intitulado “Análise de dados de metabolômica explorando ferramentas do GNPS: dos princípios básicos a ferramentas *in silico* de anotação” proferido pelas Dra. Anelize Bauermeister, Naydja Moralles Maimone, Danielle Rocha Pinto Bastos e Tiago Leão nos dias 28 e 29 de abril de 2021 das 15:00 h até as 17:30 h perfazendo um total de 05 horas.



Prof. Dr. Warley de Souza Borges
Matr. SIAPE: 1817281
DQUI – CCE / UFES

Prof. Dr. Warley de Souza Borges
Diretor da Divisão de Produtos Naturais da SBQ