UNIVERSIDADE FEDERAL DA PARAÍBA

CENTRO DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ESTUDO DA ATIVIDADE ANTIFÚNGICA *in vitro* DO ÓLEO ESSENCIAL DE *Schinus terebinthifolius* Raddi CONTRA ESPÉCIES DE *Candida* ISOLADAS DA CAVIDADE BUCAL DE USUÁRIOS DE PRÓTESE

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, da Universidade Federal da Paraíba, como parte dos requisitos para obtenção do título de Mestre em Odontologia – Área de Concentração em Ciências Odontológicas.

Orientador: Profa. Dra. Edeltrudes de Oliveira Lima

João Pessoa

2016

P438e Pérez, Ana Luíza Alves de Lima.
Estudo da atividade antifúngica *in vitro* do óleo essencial de *Schinus terebinthifolius* Raddi contra espécies de Candida isoladas da cavidade bucal de usuários de prótese / Ana Luíza Alves de Lima Pérez.- João Pessoa, 2016.
78f. : il.
Orientadora: Edeltrudes de Oliveira Lima
Dissertação (Mestrado) - UFPB/CCS
1. Odontologia. 2. Prótese dentária. 3. Candidíase bucal. 4. Plantas medicinais. 5. Fitoterapia.

UFPB/BC CDU: 616.314(043)

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ESTUDO DA ATIVIDADE ANTIFÚNGICA *IN VITRO* DO ÓLEO ESSENCIAL DE *SHINUS TEREBINTHIFOLIUS RADDI* CONTRA ESPÉCIES DE CANDIDA ISOLADAS DA CAVIDADE BUCAL DE USUÁRIOS DE PRÓTESE

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DEDICATÓRIA

Aos meus pais, Pedro Paulo e Elineide, por todo amor, dedicação e compromisso com a minha formação e por acreditarem nos meus sonhos.

AGRADECIMENTOS

À Deus, pelo dom da vida, por me fazer forte e perseverante na concretização dos meus sonhos, iluminando meus caminhos e abençoando a minha vida!

Aos meus pais, Pedro Paulo e Elineide, responsáveis pelo que sou e influenciadores eternos sobre o que serei. A eles que estão presentes em todos os momentos da minha vida com muito amor e dedicação e que, por muitas vezes, sacrificaram seus sonhos em favor dos meus e da minha irmã. Pelo constante incentivo aos estudos, e de nunca medir esforços para tal, sempre proporcionando as melhores oportunidades de crescimento moral e intelectual. São meu porto seguro, minha vida, e agradeço eternamente todo amor e dedicação a nossa família!

À minha família, especialmente minha irmã Ana Paula e minha sobrinha Ana Cecília, que estão sempre ao meu lado dividindo alegrias e preocupações, sendo fonte de amor, incentivo e confiança. Aos meus amados avós, tios e primos, pelo carinho e pelas orações. A tio Zeca pela ajuda e incentivo em todas as etapas da minha formação intelectual.

À minha orientadora, profa. Edeltrudes de Oliveira Lima pela confiança depositada em mim e pelos ensinamentos durante toda a caminhada, tendo meu carinho e admiração pela pessoa compreensiva e humana que é. Agradeço pela oportunidade, disponibilidade e confiança.

À minha amiga de graduação e mestrado, Gabriela Lacet, pelo apoio, compreensão, motivação e experiências científicas compartilhadas ao longo da minha trajetória acadêmica. Agradeço pela amizade, ajuda constante e auxílio em todos os momentos da minha vida!

Aos colegas Ana Lúcia Tavares de Oliveira, Aratã Oliveira Cortez Costa, André Parente de Brito Bezerra, Daniele de Figueredo Silva, Cássio Ilan Soares Medeiros e Camilla Pinheiro de Menezes pelo auxílio nas diversas etapas desta pesquisa, pela excelente convivência, motivação e apoio nos momentos de dúvida.

A todos os colegas e professores do PPGO/UFPB que fizeram com que alguns momentos difíceis dessa caminhada se tornassem mais prazerosos e

encantadores. Em especial, agradeço ao professor Ricardo Dias de Castro, por toda a disponibilidade, incentivo e conhecimento transmitido para a concretização deste trabalho.

Ao professor Pablo Queiroz Lopes por, gentilmente, disponibilizar o óleo essencial utilizado no estudo.

Aos professores André Ulisses Dantas Batista, Franklin Delano Soares Forte, Jozinete Vieira Pereira, Ricardo Dias de Castro, Thompson Lopes de Oliveira e Abrahão Alves de Oliveira Filho pelo interesse e disponibilidade em contribuir para o aperfeiçoamento deste trabalho.

Aos participantes da pesquisa, que gentilmente cederam seus materiais biológicos para contribuir no conhecimento científico, sendo peças principais para a realização deste estudo.

A todas as pessoas que, direta ou indiretamente, contribuíram para realização deste trabalho.

RESUMO

Introdução: A fitoterapia tem estimulado a avaliação da atividade de diferentes produtos à base de plantas para o controle de problemas bucais, visando criar novas estratégias para o controle químico das infecções da cavidade bucal, na tentativa de suprir os incovenientes e fragilidades dos produtos do mercado. **Objetivo:** isolar, identificar leveduras do gênero *Candida* da cavidade bucal de usuários de prótese (parcial e total removível) e avaliar a atividade antifúngica *in vitro* do óleo essencial extraído das folhas de *Schinus terebinthifolius* Raddi (aroeira). **Materiais e Métodos:** Foram coletados material biológico de 23 usuários de prótese e a identificação das leveduras foi realizada com base na macro e micromorfologia, provas fisiológicas e bioquímicas. Para os ensaios da atividade antifúngica de *S. terebinthifolius* Raddi foram utilizadas quatro cepas de *Candida albicans* (*C. albicans* LM-1A, *C. albicans* LM-3A, *C. albicans* LM-16B, *C. albicans* LM-19A), quatro cepas de *Candida tropicalis* (*C. tropicalis* LM-2A, *C. tropicalis* LM-7A, *C. tropicalis* LM-11A, *C. tropicalis* LM-13A) de leveduras isoladas e identificadas da coleta do material biológico e cepas padrão da coleção americana ATCC (American Type Culture Collection) *C. albicans* ATCC 76485 e *C. tropicalis* ATCC 13803. O perfil de sensibilidade aos antifúngicos sintéticos foi avaliado pela técnica de difusão em meio sólido indicada para antifungigrama. Os antifúngicos utilizados foram anfotericina B (100 µg), fluconazol (25 µg), itraconazol (10 µg), miconazol (50µg), nistatina (100 U.I.), cetoconazol (50µg). Foi determinada a concentração inibitória mínima (CIM) do óleo essencial e da nistatina (controle) pela técnica da microdiluição e calculada a concentração fungicida mínima (CFM) através do subcultivo em Agar Sabouraud Dextrose (ASD). **Resultados:** A maioria dos usuários de prótese eram do sexo feminino (78%) e a média de idade de 48 anos e dois meses. *C. albicans* e *C. tropicalis* foram as espécies mais prevalentes nas coletas realizadas nas mucosas palatinas (17,39% cada) e nas bases das próteses (21,73% cada). O antifúngico que todas as cepas testadas apresentaram-se sensíveis foi a anfotericina B, seguida da nistatina, cetoconazol e miconazol com respectivamente 90%, 80%, 50% das cepas sensíveis. A maioria das cepas (90%) foram resistentes ao fluconazol e todas foram ao itraconazol. Os valores da CIM do óleo essencial variaram entre 128µg/mL ($CIM_{60\%}$) e 256µg/mL ($CIM_{100\%}$). Para a nistatina a $CIM_{100\%}$ foi de 16µg/mL. A relação CFM/CIM do óleo essencial indica atividade fungicida frente a 80% das cepas testadas, sendo considerada fungistática diante da *C. tropicalis* LM-7A e *C. tropicalis* LM-13A. E a relação CFM/CIM da nistatina indica atividade fungicida frente a todas as cepas estudadas. **Conclusão:** As espécies mais prevalentes nos usuários de prótese foi *C. albicans* e *C. tropicalis,* com perfil de sensibilidade variado em relação aos antifúngicos sintéticos, com destaque para um padrão de resistência de algumas cepas, especialmente ao fluconazol e itraconazol. O óleo essencial de *S. terebinthifolius* Raddi apresentou atividade fungicida sobre a maioria das cepas testadas.

Palavras-chave: Prótese Dentária; Candidíase Bucal; Plantas Medicinais; Fitoterapia.

ABSTRACT

Introduction: The phytotherapy has encouraged the evaluation of different herbal products activity to control dental problems, by creating new strategies for the chemical control of oral cavity infections, in an attempt to overcome the drawbacks and weaknesses of market products. **Objective:** To isolate, identify *Candida* genus yeasts from denture wearers (removable partial dentures or complete dentures) and evaluate the *in vitro* antifungal activity of an essential oil extracted from the leaves of *Schinus terebinthifolius* Raddi. **Materials and Methods:** It was collected biological material from 23 prosthesis wearers and the identification of yeasts was based on macro and micro-morphology, physiological and biochemical tests. In the antifungal activity tests of *S. terebinthifolius* Raddi was used four strains of *Candida albicans* (*C. albicans* LM-1A, *C. albicans* LM-3A, *C. albicans* LM-16B, *C. albicans* LM-19A), four strains of *Candida tropicalis* (*C. tropicalis* LM-2A, *C. tropicalis* LM-7A, *C. tropicalis* LM-11A, *C. tropicalis* LM-13A) of yeasts isolated and identified by biological material collect and standard strains of the american collection ATCC (American Type Culture Collection): *C. albicans* ATCC 76485 and *C. tropicalis* ATCC 13803. The sensitivity profile to synthetic antifungal was evaluated by diffusion technique in solid medium suitable for antifungal susceptibility test. The antifungal agents used were amphotericin B (100 µg), fluconazole (25 µg), itraconazole (10 µg), miconazole (50 µg), nystatin (100 I.U.), ketoconazole (50 µg). It was determined the minimum inhibitory concentration (MIC) of essential oil by the microdilution technique and calculated the minimum fungicidal concentration (MFC) by subculture on Sabouraud Dextrose Agar (SDA). **Results:** Most of the denture wearers were female (78%) and the average age was 48 years and two months. *C. albicans* and *C. tropicalis* were the most prevalent species in samples taken in the palatal mucosa (17.39% each) and the bases of prostheses (21.73% each). The antifungal that all strains tested were sensitive to was amphotericin B followed by nystatin, ketoconazole and miconazole with respectively 90%, 80%, 50% of the susceptible strains. Most strains (90%) were resistant to fluconazole and all were to itraconazole. The MIC values varied from 128µg/mL to 256µg/mL, being determined the $MIC_{100\%}$ as 256µg/mL. For nystatin, the $MIC_{100\%}$ was 16µg/mL. The MFC/MIC ratio indicates fungicidal activity against 80% of the strains tested and is considered fungistatic against *C. tropicalis* LM-7A and *C. tropicalis* LM-13A. For nystatin, indicated fungicidal activity against all tested strains. **Conclusion:** The most prevalent species in denture wearers were *C. albicans* and *C. tropicalis*, with a varied susceptibility profile compared to synthetic antifungal, highlighting a pattern of resistance of some strains, particularly to fluconazole and itraconazole. The essential oil of *S. terebinthifolius* Raddi showed fungicidal activity against most of the strains tested.

Keywords: Dental Prosthesis; Candidiasis, Oral; Plants, Medicinal; Phytotherapy.

LISTA DE ABREVIATURAS E SIGLAS

ASD - Ágar Sabouraud Dextrose

ATCC - American Type Culture Collection

C. albicans - *Candida albicans*

C. krusei - *Candida krusei*

C. parapsilosis - *Candida parapsilosis*

C. tropicalis - *Candida tropicalis*

CG - Cromatografia Gasosa

CG-EM - Cromatografia Gasosa acoplada a Espectrometria de Massa

CIM - Concentração Inibitória Mínima

CFM - Concentração Fungicida Mínima

CSD - Caldo Sabouraud Dextrose

IR - Índice de Retenção

LM - Laboratório de Micologia

OMS - Organização Mundial da Saúde

RENISUS - Relação Nacional de Plantas Medicinais de Interesse ao SUS

SB Brasil 2010 - Pesquisa Nacional de Saúde Bucal

SIDA - Síndrome da Imunodeficiência Adquirida

SUS - Sistema Único de Saúde

TCLE - Termo de Consentimento Livre e Esclarecido

UFC - Unidades formadoras de colônias

UFC/mL - Unidades formadoras de colônias por mililitro

SUMÁRIO

1 INTRODUÇÃO

O gênero *Candida* é caracterizado como estruturas leveduriformes, sendo considerado patógenos oportunista, pois são encontrados no corpo humano em uma relação comensal, sendo a maior causa de infecção fúngica nos seres humanos (MONGE et al., 2006). Normalmente, nos estados patológicos a espécie mais prevalente é a *Candida albicans,* entretanto, outras espécies como *C. tropicalis, C. parapsilosis, C. glabrata, C. guilliererermondii* e *C. krusei*, são encontradas (AVRELLA; GOULART, 2008; FAVALESSA; MARTINS; HAHN, 2010; MÍMICA et al., 2009; OBEROI et al., 2012; RUKAYADI et al., 2011). Além de fazerem parte da microbiota humana, as leveduras do gênero *Candida* podem ser encontradas em vários ambientes como solo, água, alimentos e microbiota de animais por possuírem facilidade de adaptação (GIOLO; SVIDZINSKI, 2010).

O aparecimento de candidíase é favorecida por uma série de fatores sistêmicos como: câncer; antibioticoterapia prolongada; xerostomia; desnutrição; idade (especialmente idosos e crianças); diabetes; Síndrome da Imunodeficiência Adquirida (SIDA) e gravidez. Os fatores locais para o surgimento de candidíase bucal são: fumo; doenças preexistentes na mucosa bucal; higiene precária e uso de prótese dentária. A existência de fatores sistêmicos e/ou locais favorecem o desequilíbrio da relação micro-organismo/hospedeiro, condição onde as defesas do indivíduo ficam comprometidas, permitindo o crescimento desordenado do fungo e a invasão de tecidos, características da doença infecciosa oportunista. É importante deixar claro que somente a presença do fungo não garante o desenvolvimento da infecção (DE ROSSI et al., 2011; FAVALESSA; MARTINS; HAHN, 2010; GABLER et al., 2008; LOTFI-KAMRAN et al., 2009; WINGETER et al., 2007; OLIVEIRA et al., 2006).

Candidíase é uma infecção humana de natureza fúngica que tornou-se preocupante devido à sua frequência e gravidade das suas complicações em pacientes imunocomprometidos (RUKAYADI et al., 2011). Quando essa infecção é encontrada na mucosa bucal é chamada de candidíase bucal e quando acomete a área do palato em usuários de prótese pode está associada a estomatite protética (PEREIRA-CENCI, 2008).

As características clínicas da estomatite protética podem surgir em diferentes graus de inflamação da mucosa palatina que está em contato com a

base da prótese superior, que vão desde petéquias a inflamação generalizada com hiperplasia papilar (PELLIZZARO et al., 2012). A classificação da estomatite protética baseia-se em critérios clínicos e divide-se em três tipos que podem apresentar-se associados. Tipo I: inflamação localizada, puntiforme ou pontos de hiperemia, que se manifesta por discretas áreas de inflamação focal do palato, localizado e limitado aos ductos das glândulas salivares palatinas menores; Tipo II (eritema difuso): observa-se eritema generalizado abrangendo parte ou toda a área coberta pela prótese; Tipo III (hiperplasia papilar do palato): caracteriza-se pela presença de nódulos ou placas (geralmente localizados na parte central do palato) e eritema da mucosa de suporte da prótese (NEWTON, 1962).

Embora saibamos que a inflamação da mucosa tenha diferentes e diversas causas, a capacidade da *Candida* spp. aderir à resina da base da prótese e formar biofilmes tem sido considerado um dos principais fatores responsáveis pelo desenvolvimento desta patologia (PELLIZZARO et al., 2012).

Em um estudo sobre a saúde bucal de um grupo representativo de idosos não institucionalizados com mais de 70 anos nos estados da Nova Inglaterra foi visto que 36,7% dos participantes eram edêntulos e não havia relação entre o edentulismo e os níveis de educação e renda. E 89,9% dos indivíduos edêntulos usavam próteses totais superiores e inferiores (MARCUS et al., 1996).

Segundo o Inquérito Comunitário Canadense de Saúde, 24% dos indivíduos com 15 anos ou mais velhos relataram uso de próteses em 2003. No geral, o uso de prótese foi mais prevalente entre as mulheres do que os homens, principalmente entre os idosos. Em 2003, cerca de 9% da população de edêntulos relataram que não usavam dentaduras (MILLAR; LOCKER, 2005).

Em uma revisão da literatura sobre a prevalência e incidência de edentulismo e perda de dentes em países europeus foi visto que a incidência de perda dentária nesse continente é baixa e que o número de dentes perdidos aumenta com a idade, havendo uma tendência para a diminuição da incidência nas últimas décadas. Entretanto, a meta da Organização Mundial da Saúde (OMS) de possuir pelo menos 20 dentes na idade de 80 anos ainda não foi atendida em todos os países (MÜLLER; NAHARRO; CARLSSON, 2007).

Já os dados epidemiológicos da Pesquisa Nacional de Saúde Bucal (SB Brasil 2010) mostram que, no Brasil 0,5% dos indivíduos de 15 a 19 anos,

25,8% de 35 a 44 anos e 71,9% de 65 a 74 anos fazem uso de algum tipo de prótese removível superior, sendo o percentual de necessidade de algum tipo de prótese de 13,7%, 68,7%, 92,6% para as mesmas faixas etárias (BRASIL, 2012). No estudo de Colussi e Freitas (2002) que objetivou analisar os estudos epidemiológicos sobre a saúde bucal dos idosos no Brasil a partir de 1988 até 2001, foi observado que a prevalência do edentulismo ficou em 68% e somente 3,9% dos idosos não necessitavam nem usavam qualquer tipo de prótese. No estudo de Crispim et al. (2009), no estado de Santa Catarina, dos 196 participantes com idade superior a 60 anos foi encontrado que 74,0% usavam algum tipo de prótese dentária superior e 42,9% inferior e foi constatado alto percentual de necessidade de próteses (63,3% arco superior; 82,1% arco inferior) para esta população.

Tendo em vista o grande número de usuários e indivíduos com necessidade do uso de próteses torna-se evidente a possibilidade de envolvimento de uma considerável parcela da população com essa patologia, incluindo a necessidade de tratamento e conscientização dos cuidados com a higiene bucal.

Em uma revisão da literatura dos 24 artigos mais recentes sobre o tratamento da candidíase bucal, Garcia-Cuesta, Sarrion-Pérez e Bagán (2014) encontraram que a condução da terapêutica depende do tipo da candidíase e a virulência da infecção, sendo a nistatina e a anfotericina B os fármacos mais utilizados localmente e que suspensão oral de fluconazol está demonstrando ser uma droga muito eficaz no tratamento da candidíase bucal. Verificou-se que fluconazol é a droga de escolha para o tratamento sistémico da candidíase bucal devido às boas propriedades antifúngicas, a elevada aceitação do paciente e da eficácia em comparação com outras drogas antifúngicas. Para os casos em que o fluconazol não mostra-se eficaz, deve-se avaliar e distinguir outras opções como itraconazol ou cetoconazol.

Diante das dificuldades encontradas no tratamento de infecções fúngicas, como por exemplo a resistência adquirida pelos agentes etiológicos à ação dos antifúngicos, reduzido número de medicamentos disponíveis, interações medicamentosas ou toxicidade torna-se necessária a busca por novas alternativas terapêuticas advindas da utilização empírica e cultural de diferentes povos na tentativa de descoberta de alguma terapia que supra essas situações

(ARAÚJO et al., 2004; KLAN et al., 2012; CASTILHO et al., 2007). Sendo assim, a etnobotânica e a etnofarmacologia têm demonstrado ser importantes ferramentas na busca por substâncias naturais de ação terapêutica (ALBUQUERQUE; HANAZAKI, 2006).

Apesar da medicina moderna ser bem desenvolvida na maior parte do mundo, a OMS reconhece que a medicina tradicional é essencial para a atenção primária de grande parte dos países em desenvolvimento, tendo em vista que 80% desta população utilizam práticas tradicionais nos seus cuidados básicos de saúde e 85% destes utilizam plantas ou preparações destas (BRASIL, 2006).

O Brasil por apresentar extensa biodiversidade, em torno de 15 a 20% do total mundial, tem grande potencial para fabricação de fitoterápicos e outros medicamentos, visto que as plantas são a matéria-prima desse processo. Além dessa utilização as plantas são também aproveitadas em práticas populares e tradicionais como remédios caseiros e comunitários, processo conhecido como medicina tradicional. Sem contar com a rica diversidade cultural e étnica do Brasil que resultou em um acúmulo considerável de conhecimentos e tecnologias tradicionais, passados de geração a geração, entre os quais se destaca o vasto acervo de conhecimentos sobre manejo e uso de plantas medicinais (ALBUQUERQUE; HANAZAKI, 2006; BRASIL, 2006).

Assim, a Política Nacional de Práticas Integrativas e Complementares no Sistema Único de Saúde (SUS), pactuada na Comissão Intergestores Tripartite, aprovada pelo Conselho Nacional de Saúde no ano de 2005 e publicada por meio de Portaria GM nº 971, de 03 de maio de 2006, propõe a inclusão das plantas medicinais e fitoterapia, homeopatia, medicina tradicional chinesa/acupuntura e termalismo social/crenoterapia como opções terapêuticas no sistema público de saúde. Essa política traz dentre suas diretrizes para plantas medicinais e fitoterapia a elaboração da Relação Nacional de Plantas Medicinais e de Fitoterápicos; e o provimento do acesso à plantas medicinais e fitoterápicos aos usuários do SUS (BRASIL, 2006). Sendo o *Schinus terebinthifolius*, planta utilizada no presente estudo, uma das 71 espécies de plantas com potencial medicinal incluídas na Relação Nacional de Plantas Medicinais de Interesse ao SUS (RENISUS).

Schinus terebinthifolius Raddi *(*aroeira*)* é uma planta utilizada popularmente para diversos problemas de caráter inflamatórios, dentre os quais

os de origem bucal. É nativa da região nordeste do Brasil e apresenta várias propriedades farmacológicas, com atividade antimicrobiana, antiinflamatória e cicatrizante, as quais estão relacionadas aos taninos, flavonóides e triterpenos que encontram-se em diferentes partes da planta (LIMA et al., 2006).

Em uma pesquisa que objetivou realizar um estudo etnobotânico sobre a indicação de plantas medicinais para tratamentos de patologias bucais, bem como investigar sobre o uso de plantas medicinais entre usuários de serviços odontológicos em João Pessoa, Brasil, foi observado que o *Schinus terebinthifolius* Raddi foi a segunda planta mais vendida pelos raizeiros para uso odontológico conhecida pela atividade cicatrizante e anti-inflamatória, e que 27% dos usuários de serviços odontológicos do município utilizam a aroeira para patologias bucais, sendo indicada para inflamação e a forma de uso a infusão (SANTOS et al., 2009).

A atividade anti-inflamatória de um anti-séptico bucal de *S. terebinthifolius* foi comprovada na redução da inflamação gengival em um ensaio clínico triplo-cego randomizado (FREIRES et al., 2013).

Avaliando o potencial genotóxico de um extrato de *S. terebinthifolius, in vitro*, Carvalho et al. (2009) observaram que, embora o extrato não tenha sido capaz e causar uma ruptura direta na estrutura do DNA, ele mostrou potencial para causar dano oxidativo ao DNA, bem como mutação bacteriana. Além disso, Varela-Barca et al. (2007) mostrou que as frações de flavonóide encontrado nas cascas do caule de *S. terebinthifolius* foram capazes de quebrar ligações fosfodiéster do DNA, gerando lesões que pode potencialmente levar a mutações. Ainda, Veiga Júnior et al. (2005) relataram que dermatite alérgica por contato era um efeito colateral do uso da aroeira.

Nota-se, inclusive, que no Brasil, as plantas medicinais da flora nativa são utilizadas com pouca ou nenhuma comprovação de suas propriedades farmacológicas, sendo, muitas vezes, empregadas para fins medicinais diferentes daqueles utilizados pelos primeiros consumidores. Logo, a toxicidade de plantas medicinais passa a ser um problema sério de saúde pública, além disso, os efeitos adversos, possíveis adulterações e nível tóxico, bem como a interação com outras drogas podem ocorrer. As pesquisas realizadas para avaliação do uso seguro de plantas medicinais e fitoterápicos no Brasil ainda são incipientes, assim como o controle da comercialização pelos órgãos oficiais em feiras livres,

mercados públicos ou lojas de produtos naturais. Assim, grande parte dos consumidores de plantas medicinais sentem-se encorajados por acreditarem que estes remédios, por serem naturais, são inerentemente seguros (VEIGA JÚNIOR et al., 2005).

Assim, muitos estudos almejam aliar a extensa biodiversidade brasileira, os conhecimentos populares das comunidades e práticas culturais com o conhecimento científico para buscar produzir evidências para aplicabilidade dos produtos naturais, visando criar novas estratégias de controle químico das infecções da cavidade bucal por *Candida*, bem como suprir os inconvenientes e fragilidades dos produtos do mercado. Nesse sentido, o objetivo deste estudo foi isolar, identificar leveduras do gênero *Candida* da cavidade bucal dos usuários de prótese (parcial e total removível) em uma comunidade e avaliar a atividade antifúngica *in vitro* do óleo essencial extraído das folhas de *Schinus terebinthifolius* Raddi (aroeira).

2 OBJETIVOS

2.1 Objetivo Geral:

Isolar, identificar leveduras do gênero *Candida* da cavidade bucal de usuários de prótese (parcial e total removível) em uma comunidade e avaliar a atividade antifúngica *in vitro* do óleo essencial extraído das folhas de *Schinus terebinthifolius* Raddi (aroeira).

2.2 Objetivos Específicos:

- Isolar leveduras do gênero *Candida* da cavidade bucal de usuários de prótese (parcial e total removível) da Associação Afrocultural Bessen Dan/Santa Rita;
- Identificar as leveduras do gênero *Candida* com base na macro e micromorfologia, provas fisiológicas e bioquímicas;
- Avaliar o comportamento de sensibilidade ou resistência das leveduras identificadas frente antifúngicos comercialmente disponíveis;
- Determinar a Concentração Inibitória Mínima (CIM) do óleo essencial extraído das folhas de *Schinus terebinthifolius* Raddi sobre as cepas de *Candida* provenientes da coleta da cavidade bucal de usuários de prótese;
- Determinar a Concentração Fungicida Mínima (CFM) do óleo essencial extraído das folhas de *Schinus terebinthifolius* Raddi sobre as cepas de *Candida* provenientes da coleta da cavidade bucal de usuários de prótese;

3 MATERIAIS E MÉTODOS

3.1 Delineamento do Estudo:

Realizou-se um estudo epidemiológico transversal e laboratorial, no qual foi adotada uma abordagem indutiva, com procedimento comparativo estatístico e técnicas de observação direta extensiva - Formulário e documentação direta - Pesquisa de Laboratório (LAKATOS; MARCONI, 2010).

3.2 Locais da Pesquisa:

O local destinado à realização das coletas do material biológico foi a Associação Afrocultural Bessen Dan/Santa Rita que foi fundada e ativa desde 18/08/2010, tem natureza de associação privada e desenvolve atividades associativas ligadas à cultura, arte, educação, de recreação, lazer, esportivas e produção de espetáculos de dança.

Os estudos de investigação da atividade antifúngica e procedimentos laboratoriais foram desenvolvidos no Laboratório de Micologia do Departamento de Ciências Farmacêuticas (DCF), do Centro de Ciências da Saúde (CCS), da Universidade Federal da Paraíba (UFPB).

3.3 Universo e Amostra:

O universo foi composto pelos 125 indivíduos cadastrados na Associação Afrocultural Bessen Dan/Santa Rita da grande João Pessoa-PB.

A amostra, definida por conveniência, foi composta por 23 indivíduos da comunidade que atendiam aos seguintes critérios de inclusão: fazer uso de prótese dentária parcial ou total removível, ter idade superior a 18 anos, aceitar participar voluntariamente do estudo e não ter utilizado antimicrobianos nos últimos três meses que antecederam a realização dos exames.

3.4 Aspectos Éticos:

Aos participantes da pesquisa, foram garantidos os princípios da Autonomia, Beneficência, Não-Maleficência e Justiça. A participação de cada um dos componentes da amostra foi de forma voluntária, sendo garantido o direito de desistir do estudo, em qualquer tempo, sem que essa decisão o prejudicasse. O projeto vinculado ao estudo foi aprovado pelo Comitê de Ética em Pesquisa do

Centro de Ciências da Saúde da Universidade Federal da Paraíba (CAAE: 34309614.3.0000.5188).

Os indivíduos participantes deste estudo foram apresentados ao Termo de Consentimento Livre e Esclarecido (TCLE - Apêndice) e esclarecidos sobre os aspectos éticos do estudo. Mediante concordância com as condições do estudo, os voluntários realizaram impressão datiloscópica ou assinaram o TCLE.

3.5 Coleta dos Dados:

Os procedimentos de preenchimento do formulário e coleta do material biológico foram realizados por um único pesquisador. As informações coletadas foram registradas em um formulário (Apêndice), direcionado para cada participante do estudo. O formulário contemplava perguntas sobre dados pessoais; o acesso a serviços de saúde bucal; identificação de produtos naturais utilizados em infecções orais; as condições de higiene e uso de próteses dentárias; presença de doenças sistêmicas e uso de medicamento.

3.6 Coleta do material biológico, Isolamento e identificação das espécies de *Candida*:

O período de coleta das amostras foi de agosto de 2014 a setembro de 2015. Para coleta das amostras do material biológico foram utilizados dois swabs estéreis. O primeiro foi umedecido em solução fisiológica esterilizada e aplicado no palato duro do paciente com movimentos de vai e vêm (fricção), por 30 segundos. Depois, o mesmo foi inserido em um tubo de ensaio com Caldo Sabouraud Dextrose (CSD) (Difco Laboratories Ltda. USA/France) para o transporte até o laboratório. O segundo swab foi friccionado em toda base da prótese e imerso em outro tubo com CSD (SIDRIM; ROCHA, 2004; NEGRONI; GUELFAND, 1999).

A identificação das leveduras foi realizada com base na macro e micromorfologia, provas fisiologias e bioquímicas, seguindo os critérios estabelecidos por Looder (1970); Hoog e Guarro (1995); Kurtzmann e Fell (1998); Sidrim e Rocha (2004).

O material biológico coletado foi inoculado em placas descartáveis 15x90 mm (Dispopetri), contendo Ágar Sabouraud Dextrose (ASD) (Difco Laboratories Ltda. USA/France), adicionado de cloranfenicol a 100 µg/mL (Sigma

Chemichal Corporation, St Louis, MO, USA). Decorrido o período de 48h em estufa bacteriológica a 35±2ºC as colônias crescidas e com aspectos de fungos leveduriformes foram isoladas em CHROMOagar-Candida (Difco Laboratories Ltda. USA/France) (Lote: 4104408; Validade: 31/03/2016) e após a verificação do crescimento nas placas as colônias foram avaliadas quanto à coloração e ao morfotipo. Após esta avaliação, a identificação presuntiva foi realizada. A coloração verde claro a médio da colônia indicava presença da *C.albicans*, a coloração azul escuro a azul metálico indicava *C. tropicalis,* a rosa claro com bordas esbranquiçadas indicava *C. krusei e* as outras leveduras podiam aparecer tanto na sua cor natural (creme) ou cor de malva claro/escuro (exemplo *C. glabrata* e outras espécies), segundo os critérios estabelecidos pelo fabricante. Os meios de culturas foram preparados de acordo com as instruções do fabricante. Os cultivos foram incubados a temperatura de 35±2ºC por 48 h.

Outros testes foram realizados para a identificação da espécie de *Candida* como:

1) Prova do Tubo Germinativo:

A prova do tubo germinativo é um teste que caracteriza rápida e presuntivamente a levedura da espécie *Candida albicans*. A técnica baseia-se, fundamentalmente, na semeadura de um pequeno inóculo dessa levedura em soro (SIDRIM; ROCHA, 2004).

Retirou-se uma alíquota de uma colônia da levedura com uma alça de platina calibrada (0,001mL) e, em seguida, emulsionou-se de forma asséptica em 0,5 mL de soro. Incubou-se a 35º C por um período de 1,5-2 horas em banho-maria. Finalizando este período, foi removida uma gota da suspensão e montada uma preparação do tipo lâmina-lamínula, para observação microscópica. O tubo germinativo, quando o teste é positivo, aparece como filamento fino e cilíndrico, originado do blastoconídio da levedura, no qual não se observa nenhuma zona de constricção, quer na base ou ao longo de sua extensão (Figura 1) (TASCHDJIAN et al., 1960; SIDRIM; ROCHA, 2004).

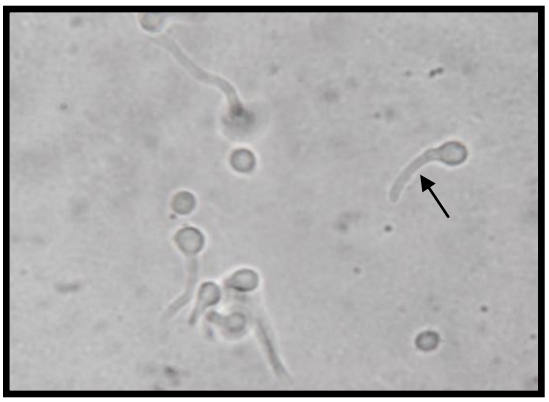

Figura 1: Teste positivo para tubo germinativo. Presença de filamento fino e cilíndrico (seta) originado do blastoconídio da levedura. (Fonte: portal.anvisa.gov.br/.../14-identificacao_leveduras_interesse_medico.ppt....)

2) Microcultivo de Leveduras:

Esta técnica baseia-se no princípio de que leveduras, quando incubadas em um meio com Tween-80 apresentam a capacidade de filamentar, formando pseudo-hifas e/ou hifas verdadeiras. Assim, pelas características morfológicas diferenciadas das estruturas filamentosas, pode-se sugerir a espécie de levedura implicada na identificação (Figura 2) (SIDRIM; ROCHA, 2004).

Colocou-se 3 mL de Ágar Fubá, ainda líquido, sobre a lâmina do microcultivo, com pipeta estéril. Esperou-se o meio solidificar e, com alça de platina, foi apreendida uma alíquota da colônia de levedura e semeada em estria no Ágar Fubá e, em seguida, coberta com lamínula estéril. Após 2 a 3 dias à 28-30ºC a preparação foi observada em microscópio com aumento de 40x (DALMAU, 1929; LODDER, 1970; SIDRIM, ROCHA, 2004).

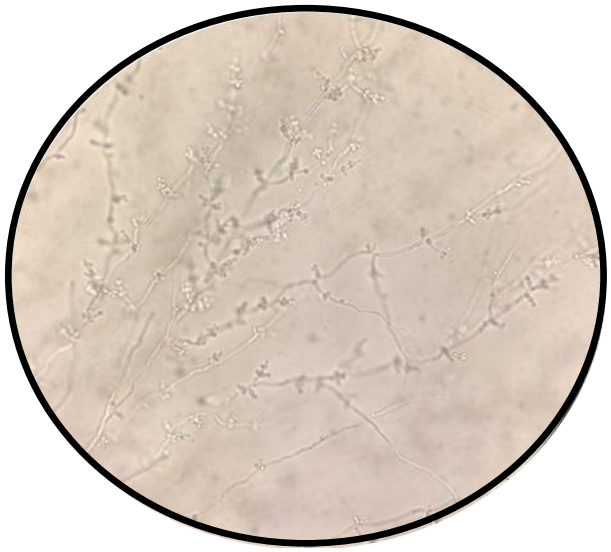

Figura 2: Microcultivo de *C. tropicalis*. Aumento: 40x.

3) Auxanograma (Assimilação de Carboidratos e Nitrogênio):

3.1) Assimilação de Carboidratos:

Esta técnica baseia-se na capacidade que as leveduras apresentam de utilizar determinado carboidrato como única fonte de carbono, para sua viabilidade celular. Desta forma utiliza-se um meio basal destituído de qualquer fonte de carbono (sem o qual a célula fúngica não pode crescer) e a levedura que se deseja identificar é semeada. Em seguida, é adicionado ao cultivo um carboidrato e observada a capacidade de utilização deste como fonte de carbono. Quando o carboidrato é assimilado pela levedura observou-se crescimento desta ao redor da fonte de carbono (SIDRIM; ROCHA, 2004).

Preparou-se o meio Yeast Nitrogen Base de acordo com as normas preconizadas pelo fabricante e simultaneamente preparou-se uma suspensão de leveduras com turvação equivalente ao tubo 0,5 da escala de MacFarland. Uma alíquota de 1 mL desta suspensão de leveduras foi adicionada a 20 mL de meio basal fundido e resfriado, sendo transferida para uma placa de Petri e homogeneizada suavemente. Após a solidificação do meio, discos impregnados com açúcares foram posicionados em locais previamente demarcados. Incubou-se à 28-30ºC por 24-48 horas e foram observadas regiões opacas ao redor dos açúcares assimilados (Figura 3) (SIDRIM; ROCHA, 2004).

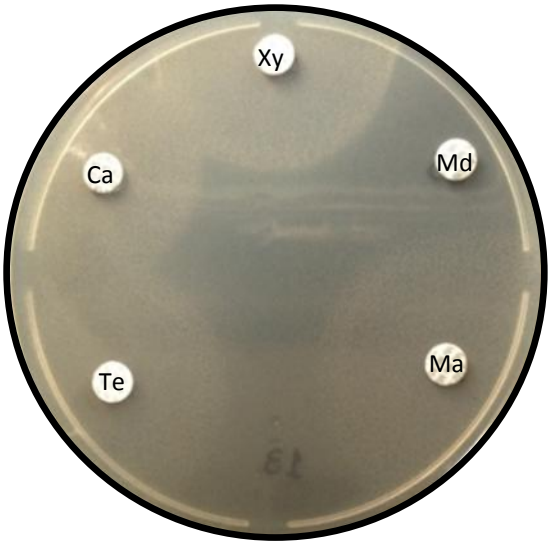

Figura 3: Crescimento da levedura (regiões opacas) ao redor dos açúcares assimilados. Xy: Xylose; Ca: Cellobiose; Te: Trehalose; Ma: Maltose; Md: Melibiose.

3.2) Assimilação de Nitrogênio:

A assimilação de nitrogênio demonstra a capacidade que algumas leveduras apresentam de assimilar nitrato de potássio (nitrogênio inorgânico), como única fonte de nitrogênio utilizado na sua viabilidade biológica (SIDRIM; ROCHA, 2004).

Preparou-se o meio Yeast Carbon Base, de acordo com as indicações do fabricante. E em 20 mL deste meio resfriado foi acrescentado 1mL da suspensão de levedura previamente preparada (com turvação correspondente ao tubo 0,5 da escala de MacFarland). Após a homogeneização e solidificação do meio, foram distribuídas discos impregnados de compostos nitrogenados (nitrato de potássio e peptona). A peptona foi empregada como controle da viabilidade do inóculo, visto que todas as leveduras a utilizam como fonte nitrogenada. Incubou-se à 28-30ºC por 24-48 horas e foram observadas regiões opacas ao redor dos compostos nitrogenados assimilados (Figura 4) (SIDRIM; ROCHA, 2004).

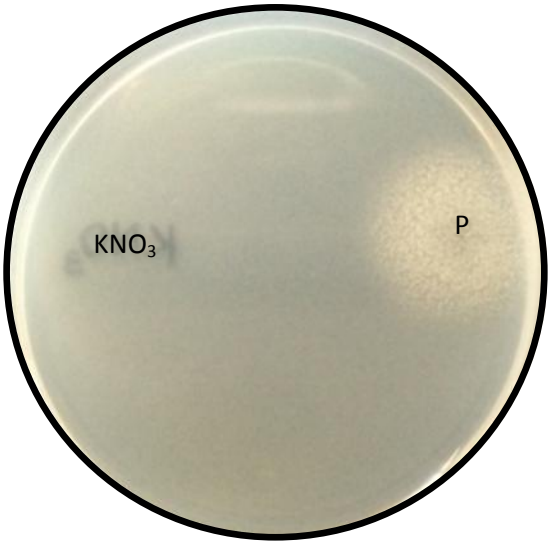

Figura 4: Crescimento da levedura (região opaca) ao redor da peptona (P) e ausência de crescimento ao redor do KNO_3.

4) Fermentação de Carboidratos:

A capacidade de uma levedura fermentar determinado carboidrato está diretamente ligada à habilidade desta de possuir sistemas enzimáticos eficientes, capazes de permitir, em baixas tensões de oxigênio, degradar açúcares para produção de energia, formando, entre outros metabólitos, etanol e gás carbônico. Assim, utiliza-se também na identificação das leveduras essa característica fenotípica, onde se investiga a habilidade que uma determinada levedura possui de fermentar um açúcar, através da demonstração da produção de CO_2 (SIDRIM; ROCHA, 2004).

Preparou-se o meio basal para fermentação e distribuiu-se em tubos de ensaio, contendo um tubo de Durham invertido em seu interior, em alíquotas de 3 mL. Em seguida foram adicionado ao meio 1,5mL da solução de açúcares (6% em água destilada), mantendo sempre a relação de ½ da solução de açúcares para meio basal, 0,2 mL da suspensão de levedura (turvação correspondente ao tubo 0,5 da escala de MacFarland). Incubou-se os tubos em estufa bacteriológica à 37ºC por 10-14 dias e observou-se a produção de CO_2 através da visualização de bolhas (Figura 5) (SIDRIM; ROCHA, 2004).

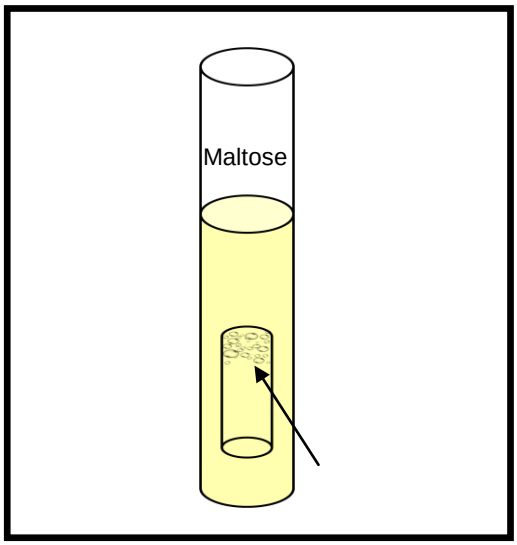

Figura 5: Esquema da **v**isualização da formação de bolhas (seta) dentro do tubo (produção de CO_2).

3.7 Avaliação da atividade antifúngica do óleo essencial de *Schinus terebinthifolius* Raddi:

3.7.1 Produtos natural - Óleo essencial das folhas de *Schinus terebinthifolius* Raddi

O óleo essencial utilizado para a avaliação da atividade antifúngica foi extraído das folhas frescas de *Schinus terebinthifolius* Raddi, que foi obtido pelo Professor Pablo Queiroz Lopes do Departamento de Ciências Farmacêuticas da Universidade Federal da Paraíba. Essas folhas foram coletadas em abril-julho 2013 a partir do jardim do Laboratório Rabelo na cidade de Cabedelo-PB (Latitude: 6° 58' 49" Sul Longitude: 34° 49' 49" Oeste) e um espécime de vouchers (JPB 85943 e 85944) foi depositada no Herbário Lauro Pires Xavier da Universidade Federal da Paraíba. A identificação dos componentes do óleo essencial foi realizada pelo técnico Sócrates Golzio do Santos por cromatografia gasosa acoplada a espectrometria de massa para detecção ultra CG-EM. Este produto natural foi selecionado de acordo com as respostas obtidas pelo formulário do estudo.

Este óleo essencial foi extraído por arraste de vapor de água utilizando o extrator D2 Mini Linax. As folhas foram transferidas para o reservatório de destilação a vapor, que apresenta ciclo contínuo de água vinda diretamente do

sistema de água. Esta água não estava em contato com a amostra, o vapor de água passa através do reservatório onde estão as folhas riscadas e arrasta para baixo os constituintes voláteis. As amostras foram armazenadas em frascos de vidro sob refrigeração para evitar eventuais perdas de matérias voláteis. Diferentes tempos de extração (1 a 6 horas) foram utilizadas para estimar o melhor rendimento (TAVARES et al., 2005).

A identificação dos componentes do óleo foi efectuada por cromatografia gasosa acoplada a espectrometria de massa para detecção em Ultra CG-EM, o amostrador automático foi um injector série COA-20is (Shimadzu), o cromatógrafo de fase gasosa era um GC-2010 Plus (Shimadzu), o espectrômetro de massa foi um GCMS-QP2010 Ultra (Shimadzu) e o íon detector (MS, modelo 4000) (Shimadzu), com espectrometria de massa por ionização de elétrons - (EI-MS 70 eV). A análise foi desenvolvida em Rtx®-5MS (Restek, Bellefonte, PA, EUA) em coluna capilar (Shimadzu), 30m de comprimento, 0,25mm de espessura e 0,25mm de diâmetro interno com hélio a 1 mL/min como gás portador. Programa de temperatura: injector a 250ºC, coluna-forno de 60 a 240ºC em 3ºC/min. O índice de retenção (IR) foi calculado para todos os constituintes voláteis utilizando uma série homóloga de n-alcanos, variando entre C8 e C40, usando uma equação linear de temperatura programada (VAN DEN DOOL; KRATZ, 1963).

3.7.2 Antifúngico Sintético Padrão

Para controle da atividade antifúngica foi utilizada nistatina (Sigma Chemichal Corporation, St Louis, MO, USA) nas concentrações 1024µg/mL até 1µg/mL.

3.7.3 Micro-organismos

Para os ensaios de atividade antifúngica de *Schinus terebinthifolius* Raddi foram utilizadas quatro cepas de *Candida albicans* (*C. albicans* LM-1A, *C. albicans* LM-3A, *C. albicans* LM-16B, *C. albicans* LM-19A), quatro cepas de *Candida tropicalis* (*C. tropicalis* LM-2A, *C. tropicalis* LM-7A, *C. tropicalis* LM-11A, *C. tropicalis* LM-13A) de leveduras isoladas e identificadas da coleta do material biológico e cepas padrão da coleção americana ATCC (American Type Culture Collection) *C. albicans* ATCC 76485 e *C. tropicalis* ATCC 13803.

3.7.4 Inóculo

Para o procedimento de preparação do inóculo das leveduras, os isolados foram cultivados em meio ASD inclinado a 35±2ºC por 24h (overnight). Foram preparadas suspensões dos micro-organismos em tubos contendo 5 mL de solução salina 0,9% estéril (Farmax - Distribuidor Ltd., Amaral, Divinópolis, MG, Brasil). Em seguida, essas suspensões foram agitadas por 2 minutos com auxílio do aparelho Vortex (Fanem Ltd., Guarulhos, SP, Brasil). Após agitação, cada suspensão teve sua turbidez comparada e ajustada àquela apresentada pela suspensão de sulfato de bário do tubo 0,5 da escala McFarland, a qual corresponde a um inóculo de aproximadamente 10^6UFC/mL. Em seguida, essa suspensão foi diluída com água destilada numa proporção de 1:10 resultando em um inóculo contendo aproximadamente 10^5UFC/mL, que foi utilizado nos ensaios (CLSI, 2002; SAHIN et al., 2004; HADACECK; GREEGER, 2000; CLEELAND; SQUIRES, 1991)

3.7.5 Perfil de sensibilidade das cepas fúngicas frente a antifúngicos

A metodologia utilizada para traçar o perfil de sensibilidade das leveduras utilizadas foi difusão em meio sólido indicada para antifungigrama pelo fabricante dos discos impregnados de antifúngicos (Centro de Controle e Produtos para Diagnósticos Ltda - CECON, São Paulo, SP, Brasil). Semeou-se a suspensão das leveduras em swab estéril em uma placa descartável 15x90mm, contendo ASD. Em seguida, pressionou-se levemente os discos contendo os antifúngicos anfotericina B (100 µg), fluconazol (25 µg), itraconazol (10 µg), miconazol (50µg), nistatina (100 U.I.), cetoconazol (50µg) (Centro de Controle e Produtos para Diagnósticos Ltda - CECON, São Paulo, SP, Brasil) sobre o meio de cultura e incubou-se em estufa bacteriológica por 48h a 35±2ºC. Os halos de inibição foram medidas com régua em duas diagonais e a média correspondendo ao resultado expresso em milímetros (mm) e comparado com a interpretação dos resultados do fabricante.

3.7.6 Determinação da Concentração Inibitória Mínima (CIM)

A determinação da CIM do óleo essencial foi realizada pela técnica de microdiluição, utilizando placas contendo 96 cavidades (Kasvi, Itália) e em duplicata (CLEELAND; SQUIRES, 1991; ELLOF, 1998; HADACEK; GREEGER,

2000; KONEMAN et al., 2001; SAHIN et al., 2004). Em cada orifício da placa, foram adicionados 100 µL do meio líquido CSD duplamente concentrado. Posteriormente, 100 µL da emulsão do óleo essencial, também duplamente concentrado, foram dispensados nas cavidades da primeira linha da placa, que foram diluídos seriadamente, à partir da retirada de uma alíquota de 100 µL da cavidade mais concentrada para a cavidade sucessora, obtendo-se concentrações de 1024 µg/mL até 8 µg/mL, de modo que na primeira linha da placa encontravam-se a maior concentração e na última, a menor concentração. Por fim, foram adicionados 10 µL do inóculo das leveduras nas cavidades, onde cada coluna da placa referiu-se a uma cepa fúngica, especificamente. Foram feitos controles para a viabilidade das cepas fúngicas, com nistatina (Sigma Chemichal Corporation, St Louis, MO, USA) e do meio líquido nas mesmas condições do ensaio. As placas foram seladas e incubadas a 35±2ºC por 24-48h. Definiu-se a CIM para o óleo essencial e nistatina como a menor concentração capaz de inibir visualmente o crescimento fúngico verificado nos orifícios, quando comparado com o crescimento controle. Os ensaios foram realizados em duplicata.

Utilizou-se como critérios para determinação da CIM os seguintes valores: 50 até 500µg/mL são considerados com forte/ótima atividade antimicrobiana; 600 até 1500µg/mL possuem atividade moderada; acima de 1500µg/mL, atividade fraca ou produto inativo (SARTORATTO et al., 2004; HOUGHTON et al., 2007).

3.7.7 Determinação da Concentração Fungicida Mínima (CFM)

Após determinação da CIM, 1 µL da concentração correspondente à inibitória e as duas concentrações imediatamente mais concentradas foram subcultivadas em placas descartáveis 15x90mm, contendo ASD. Após 24-48h de incubação a 35±2ºC, as leituras das CFMs foram realizadas, sendo considerada CFM a menor concentração da droga que impediu o crescimento visível do subcultivo (ERNST et al., 1999; ESPINEL-INGROFF et al., 2002).

A relação CFM/CIM foi calculada de forma a determinar se a substância possui uma atividade fungistática (CFM/CIM ≥ 4) ou fungicida (CFM/CIM <4) (SIDDIQUI et al., 2013).

3.8 Análise dos dados:

As amostras das leveduras isoladas e identificadas da coleta do material biológico foram codificadas com as letras LM (Laboratório de Micologia) seguida do número correspondente a sequência da coleta realizada.

Os dados foram analisados por estatística descritiva, a partir da qual foram construídos tabelas para subsidiar a abordagem comparativa e estatística.

4 CAPÍTULO 1

O manuscrito a seguir foi submetido para publicação no periódico “Planta Medica”, cuja classificação QUALIS/CAPES na área Odontologia é B1.

Isolation and identification of *Candida* species from the oral cavity of denture users and *in vitro* susceptibility to *Schinus terebinthifolius* Raddi

Abstract

The present study aimed to isolate and identify *Candida* yeasts from denture users and evaluate their *in vitro* susceptibility to the essential oil extract of *Schinus terebinthifolius* Raddi leaves. Biological material was collected from 23 denture users, and yeasts were identified based on macro- and micromorphological assessments and on physiological and biochemical assays. The antifungal activity of *S. terebinthifolius* Raddi was assessed with different strains of *Candida albicans* (*C. albicans* ATCC 76485, LM-1A, LM-3A, LM-16B and LM-19A) and *C. tropicalis* (*C. tropicalis* ATCC 13803, LM-2A, LM-7A, LM-11A and LM-13A). The susceptibility profile to synthetic antifungals was determined via the disk-diffusion method to establish an antifungigram. The minimum inhibitory concentration (MIC) of the essential oil was determined by microdilution, whereas the minimum fungicidal concentration (MFC) was calculated by means of subculture in Sabouraud dextrose agar (SDA). The majority of denture users were women (78 %), and the mean age was 48 years and two months. *C. albicans* and *C. tropicalis* were the most prevalent species in samples collected from the palatal mucosa and denture bases. All of the studied strains were susceptible to amphotericin B; however, none were susceptible to itraconazole, and most were resistant to fluconazole. MIC values of the essential oil ranged from 128 µg/mL to 256 µg/mL, and the $MIC_{100\%}$ was 256 µg/mL. The $MIC_{100\%}$ found for nystatin was 16 µg/mL. The MFC/MI Cratio indicated fungicidal activity against all tested strains for nystatin and against most of them, except for *C. tropicalis* LM-7A and *C. tropicalis* LM-13A, when it was used the essential oil; the activity against those strains was fungistatic. In conclusion, the most prevalent species present in

denture users were *C. albicans* and *C. tropicalis,* and the susceptibility profile varied with the synthetic antifungal agent, highlighting a resistance pattern in some strains, especially to fluconazole and itraconazole. The essential oil of *S. terebinthifolius* Raddi exhibited fungicidal activity against most of the tested strains.
Keywords: Denture; Oral Candidiasis; Medicinal Plants; Fungal Resistance.

Introduction

Yeasts from the *Candida* genus are frequently found as commensals of several human organs, present in the normal microbiota of the skin, oral mucosa, gastrointestinal tract and vagina [1]. If microbiological imbalance occurs, colonization becomes pathological; in the oral cavity, this condition is called candidiasis. Characteristically, candidiasis is an opportunistic fungal infection triggered by changes in the oral microbiota, systemic diseases or compromised patient immunity. *Candida albicans* is the most prevalent species and exhibits the highest pathogenicity among the strains involved in oral candidiasis [2]. Other species, including *C. tropicalis*, *C. krusei*, *C. parapsilosis, C. guilliermondii* and *C. glabrata*, are also involved in the course of disease, and eventually become representative in clinical findings, making them important in its etiology [3].

The occurrence of oral candidiasis in the palate of denture users is associated most often with denture stomatitis. The clinical characteristics of this condition correspond to different degrees of inflammation of the palatal mucosa, which is in contact with the upper denture base. Manifestations range from petechiae to systemic inflammation with papillary hyperplasia. The ability of *Candida* spp. to adhere to the denture base resin and form biofilms has been considered one of the main factors responsible for the development of pathology [4].

Current research suggests that the prevalence of edentulous individuals and denture users, especially among the elderly, is significant in developing and developed countries. These groups represent a relevant fraction of the population in need of assistance in the prevention and development of oral candidiasis [5-7].

The treatment of human fungal infections has become difficult due to increasing antifungal resistance, low numbers of available drugs, therapeutic

limitations, inefficacy, toxicity, severe neutropenia, drug interactions, and insufficient bioavailability of the available synthetic antifungals. This situation justifies the need for the discovery of new therapeutic alternatives [8,9]. In this context, the potential of natural resources, such as plant extracts, including those from *Schinus terebinthifolius*, is worthy of further study.

S. terebinthifolius Raddi *(*Brazilian pepper tree*)* is a plant popularly used to treat several inflammatory conditions, including those affecting the oral cavity. This plant is native to the northeast of Brazil and exhibits several pharmacological properties, such as antimicrobial, anti-inflammatory and wound healing properties, which are related to the tannins, flavonoids and triterpenes present in several parts of the plant [10].

According to the literature, the alcohol extract from the bast of *S. terebinthifolius* Raddi has a positive impact and seems promising as a natural local antiseptic against highly severe peritonitis in rats [11]. The anti-inflammatory activity of an *S. terebinthifolius* mouth wash was demonstrated in a randomized triple-blind clinical trial that observed reduced gingival inflammation levels [12]. Further, a study in rats suggested that the oral administration of dry *S. terebinthifolius* bark extract did not exhibit toxicity [13].

Considering the significant number of denture users, the associated potential for the development of oral candidiasis, and the popular knowledge and use of the Brazilian peppertree in the treatment of inflammatory processes of the oral cavity, the present study aimed to isolate and identify *Candida* yeasts from denture users of a local community and evaluate their *in vitro* susceptibility to essential oil extracted from *S. terebinthifolius* Raddi leaves.

Results

Of the 23 denture users who participated in sample collection, 78 % (*n*=18) were women. The age of the participants ranged from 32 years and two months to 77 years and three months, with a mean age of 48 years and two months.

Table 1 shows the prevalence of *Candida* species in samples from the palatal mucosa and denture base.

Table 1: Prevalence of *Candida* species in samples from the palatal mucosa and denture base.

	Palatal mucosa		Denture base	
	N	**%**	**N**	**%**
C. albicans	4	17.39	5	21.73
C. tropicalis	3	13.04	4	17.39
C. parapsilosis	1	4.35	1	4.35
C. krusei	0	0	1	4.35
C. tropicalis + *C. parapsilosis*	1	4.35	1	4.35
Absent	14	60.87	11	47.83
TOTAL	**23**	**100**	**23**	**100**

Table 2 exhibits the diameters of the antifungal-impregnated disk inhibition zone for amphotericin B (100 µg), fluconazole (25 µg), itraconazole (10 µg), miconazole (50 µg), nystatin (100 IU) and ketoconazole (50 µg) against the isolated yeasts and the interpretation of the results according to the criteria of the disk manufacturer and the behavior of the yeasts studied in the presence of the essential oil of *S. terebinthifolius* Raddi in the concentration 128µg/mL.

Data on the antifungal activity of the essential oil of *S. terebinthifolius* Raddi and nystatin against the clinical *C. albicans* (*C. albicans* LM-1A, *C. albicans* LM-3A, *C. albicans* LM-16B and *C. albicans* LM-19A) and *C. tropicalis* (*C. tropicalis* LM-2A, *C. tropicalis* LM-7A, *C. tropicalis* LM-11A and *C. tropicalis* LM-13A) strains and the laboratory *Candida* strains (*C. albicans* ATCC-76485 and *C. tropicalis* ATCC-13803) are listed in Table 3.

Table 2: Diameters of the antifungal-impregnated disk inhibition zone (mm) against the studied yeasts and the interpretation of results according to the criteria of the disk manufacturer and behavior of the yeasts studied in the presence of the essential oil of *S. terebinthifolius* Raddi in the concentration 128 µg/mL.

YEAST	**ANTIFUNGAL**						**ESSENTIAL OIL**
	Anphotericin B (100 µg) **Inhibition zone (Interpretation)**	Fluconazole (25 µg) **Inhibition zone (Interpretation)**	Itraconazole (10 µg) **Inhibition zone (Interpretation)**	Miconazole (50 µg) **Inhibition zone (Interpretation)**	Nystatin (100 IU) **Inhibition zone (Interpretation)**	Ketoconazole (50 µg) **Inhibition zone (Interpretation)**	*S. terebinthifolius*Raddi (128µg/mL) **Presence or absence of yeast (Interpretation)**
***C. albicans* LM-1A**	18 mm (S)	0 mm (R)	0 mm (R)	20 mm (I)	20 mm (S)	20 mm (I)	Presence (R)
***C. albicans* LM-3A**	13 mm (S)	20 mm (S)	15 mm (I)	30 mm (S)	28 mm (S)	33 mm (S)	Presence (R)
***C. albicans* LM-16B**	19 mm (S)	15 mm (I)	13 mm (I)	30 mm (S)	24 mm (S)	25 mm (S)	Absence (S)
***C. albicans* LM-19A**	16 mm (S)	14 mm (R)	12 mm (I)	28 mm (S)	26 mm (S)	28 mm (S)	Absence (S)
***C. albicans* ATCC-76485**	12 mm (S)	12 mm (R)	10 mm (R)	16 mm (I)	15 mm (S)	30 mm (S)	Absence (S)
***C. tropicalis* LM- 2A**	15 mm (S)	0 mm (R)	0 mm (R)	10 mm (I)	0 mm (R)	25 mm (S)	Absence (S)
***C. tropicalis* LM-7A**	18 mm (S)	0 mm (R)	0 mm (R)	20 mm (I)	30 mm (S)	30 mm (S)	Absence (S)
***C. tropicalis* LM-11A**	15 mm (S)	10 mm (R)	14 mm (I)	26 mm (S)	22 mm (S)	25 mm (S)	Presence (R)
***C. tropicalis* LM-13A**	14 mm (S)	0 mm (R)	0 mm (R)	30 mm (S)	23 mm (S)	15 mm (I)	Presence (R)
***C. tropicalis* ATCC-13803**	14 mm (S)	15 mm (I)	16 mm (I)	20 mm (I)	15 mm (S)	28 mm (S)	Absence (S)
TOTAL (%) susceptible strains	**100%**	**10%**	**0%**	**50%**	**90%**	**80%**	**60%**

Source: Centro de Controle e Produtos para Diagnósticos Ltda - CECON, São Paulo, SP, Brazil.

S - susceptible; **I** - intermediate; **R**- resistant

Table 3: Antifungal activity of the essential oil of *S. terebinthifolius* Raddi and nystatin against the studied strains

YEAST	ESSENTIAL OIL *S. terebinthifolius* Raddi				NYSTATIN			
	MIC (µg/mL)	MFC (µg/mL)	MFC/ MIC	Antifungal activity	MIC (µg/mL)	MFC (µg/mL)	MFC/ MIC	Antifungal activity
***C. albicans* LM-1A**	256	256	1	Fungicidal	8	16	2	Fungicidal
***C. albicans* LM-3A**	256	256	1	Fungicidal	8	16	2	Fungicidal
***C. albicans* LM-16B**	128	256	2	Fungicidal	16	16	1	Fungicidal
***C. albicans* LM-19A**	128	256	2	Fungicidal	8	16	2	Fungicidal
***C. albicans* ATCC-76485**	128	128	1	Fungicidal	16	32	2	Fungicidal
***C. tropicalis* LM-2A**	128	128	1	Fungicidal	16	32	2	Fungicidal
***C. tropicalis* LM-7A**	128	> 512	> 4	Fungistatic	8	8	1	Fungicidal
***C. tropicalis* LM-11A**	256	512	2	Fungicidal	8	8	1	Fungicidal
***C. tropicalis* LM-13A**	256	> 512	≥ 4	Fungistatic	8	8	1	Fungicidal
***C. tropicalis* ATCC-13803**	128	128	1	Fungicidal	16	32	2	Fungicidal

$MIC_{60\%}$essential oil= 128 µg/mL; $MIC_{60\%}$ nystatin= 8 µg/mL

Discussion

Epidemiological studies focusing on candidiasis in denture users are of great importance and necessary to evaluate the prevalence of disease. The identification of isolated species, virulence factors and the analysis of the susceptibility profile towards antifungal agents are essential phases in the development of new research and the discovery of new therapeutic proposals for the treatment of candidiasis [14].

The literature states that denture stomatitis is a pathological condition that affects approximately two-thirds of all denture users, of which the majority are women and the elderly. Furthermore, most of the patients do not report symptoms, whereas others exhibit symptoms that render denture use impossible, such as pain, swelling, dry mouth, halitosis and/or bleeding [15]. However, in this study, more than half the sample of denture users (54.3 %) tested negative for *Candida*,

which is can closely associated with denture stomatitis. The majority of the participants of the present study were women (78%), which is in agreement with the literature. This situation might be explained by the higher prevalence of female denture users who seek help due to possible symptoms that arise and render denture use impossible, which ultimately impacts esthetics and function. Furthermore, women historically seek health care services more often than men [16].

In the present study, *C. albicans* and *C. tropicalis* were isolated in equal amounts(*n*=4 each; 40 % each), but other studies have reported that the majority of strains isolated from denture users are *C. albicans* [3,17].

Was found 39.13% of positive tests for *Candida* in the palatal mucosa of the participants and 52.17% in the denture base. In view of the higher number of positive findings in the denture base in the present study, it is important to note that not only is it relevant to treat patients with denture stomatitis, but denture hygiene and exchange periods are essential factors in the prevention of reinfection. According to Yildirim-Bicer *et al*. [18], the cleansing process must be properly performed to prevent infections and lesions of the oral mucosa, and it is important to keep the dentures submerged in water and cleansing solution overnight. Specifically, sodium hypochlorite diluted in water is among the most common and easily accessible solutions.

As for the susceptibility profile of the isolated yeast strains, responses varied depending on the tested antifungal agent. The best-performing antifungal was amphotericin B, tow hich all tested strains exhibited susceptibility, followed by nystatin with 90 % susceptible strains, ketoconazole with 80 %, miconazole with 50 %, fluconazole with 10 %, and itraconazole, tow hich no strain was susceptible. According to Khan *et al*. [19], *Candida* species exhibit resistance to some synthetic antifungal drugs. This finding was confirmed in the present study and demonstrates the need to search for alternative and/or complementary therapies in the treatment of infections involving these microorganisms.

In recent years, medicinal plants have been the focus at a global level and have gained considerable popularity given that these natural products provide an unparalleled source of chemical diversity for the discovery of significant biologically active molecules [20].

Currently, *S. terebinthifolius* Raddi has been the object of many studies due to its broad empiric use for different health problems, including oral health problems [11, 12, 13, 21]. Vieira *et al*. [22] have found that the antimicrobial activity of this plant towards *Streptococcus mutans* was similar to that of 0.12 % chlorhexidine, which was used as the control. The essential oil of *S. terebinthifolius* Raddi has shown antibacterial activity against hospital-borne strains [23] and antimicrobial activity against *Enterococcus faecalis* [24].

Essential oils are composed of different substances in different proportions, which can differ quantitatively depending on the method of analysis and the plant tissue being studied [25]. Additionally, it is still not possible to narrow down the specific compound responsible for the observed effect, however, it is suggested that the main compound has its importance in this process.

The main component of the essential oil of the present study was the monoterpene limonene (63.96 %). In a study by Gundidza *et al*. [26] on the essential oil of fresh *S. terebinthifolius* leaves collected in sub-Saharan Africa, the main constituents included sabinene, α-pinene, α-phellandrene, β-pinene, terpinen-4-ol, trans-β-ocimene, andmyrcene, and the essential oil exhibited 49.8 % inhibition of *C. albicans*, evidently, local conditions have an influence on the chemical composition of essential oils.

D-limonene is an isomer of limonene and is one of the most common terpenes in nature. It is a major constituent in many citrus oils (orange, lemon, tangerine, lime and grapefruit). D-limonene is considered to have relatively low toxicity. It has been tested for carcinogenicity in mice and rats. In humans, it showed low toxicity after single or repeated administration for up to one year, is an excellent solvent of cholesterol, has been used to relieve heartburn, has well established chemo preventive activity against many kinds of cancers [27]. But it is not found in the literature studies on their possible antifungal activity.

The $MIC_{60\%}$of the essential oil of *S. terebinthifolius* Raddi against the tested strains was 128 µg/mL, and the MFC/MIC ratio reflected fungicidal activity against 80 % of the strains. The different MIC values of the essential oil against the tested strains might be explained by the fact that these were clinical strains, possibly exhibiting different genetic profiles and mechanisms of acquired resistance. The present study corroborates a study from the literature in which a tincture of *S.*

terebinthifolius exhibited antifungal activity against a laboratory *Candida* strain, with a MIC of 312.5 µg/mL [28].

Possible explanations for the mechanisms underlying the antifungal activity of the essential oil include interference with cell wall biosynthesis and increases in the permeability of the yeast cell membrane to ions [29]. No studies describing the mode of action of the essential oil of *S. terebinthifolius* against *Candida* species were found in the literature.

With respect to the toxicity of *S. terebinthifolius*, Lima *et al*. [13] have shown that the oral administration of dry *S. terebinthifolius* bark extract for 45 days induced no toxic effects in Wistar rats of both sexes. Furthermore, no significant changes were observed in the biochemical and hematological indices or in the anatomical and histopathological characteristics.

Given its popular use as a medicinal plant, *S. terebinthifolius* has exhibited great potential in the development of novel plant-based products [30], and can be used for treatment or even as an adjuvant agent in the oral hygiene process.

The present investigation is a pioneer study on the antifungal activity of the essential oil of *Schinus terebinthifolius* Raddi against *Candida* species isolated from denture users, including some strains resistant to synthetic antifungals. The exhibited results reflect a high potential for follow-up studies addressing the kinetics of the inhibition of fungal growth, the mode of action of the substance, changes in fungal micromorphology, antibiofilm activity, and the cytotoxicity and antimicrobial action of the isolated compounds.

Materials and Methods

Area and samples

Denture users were recruited from a local community of the Municipality of João Pessoa, Paraíba State, Brazil. From a population of 125 subjects, 23 were selected to represent the sample according to the following inclusion criteria: uses dentures; is above 18 years of age; accepts voluntary participation in the study; and has not used antimicrobials in the past three months prior to the exams. This research project was approved by the research ethics committee of Center of Health Sciences of the Federal University of Paraiba (Universidade Federal da Paraíba) (CAAE: 34309614.3.0000.5188).

Collection of biological material and yeast isolation and identification

Biological material samples were collected with two sterile swabs. Specifically, one swab was moistened with sterile saline solution and swabbed (friction) along the patient's hard palate for 30 sec, whereas the other swab was rubbed all over the base of the samepatient's denture. Each swab was then inserted into a test tube containing Sabouraud dextrose broth (SDB; Difco Laboratories Ltd., USA/FRANCE) for transport to the laboratory [31, 32].

Yeasts were identified based on macro- and micromorphology and via physiological and biochemical assays, following the criteria established byLooder [33], Hoog and Guarro [34], Kurtzmann and Fell [35], and Sidrim and Rocha [31].

Sample material was inoculated into 15x90 mm disposable plates (Dispopetri) containing Sabouraud dextrose agar (SDA; Difco Laboratories Ltd., USA/FRANCE) supplemented with 100 µg/mL chloramphenicol (Sigma Chemical Corporation, St. Louis, MO, USA). After 48 h incubation at 35 ºC± 2 ºC in a bacteriological incubator, grown colonies with a yeasty appearance were isolated with CHROMagar-Candida (Difco Laboratories Ltda. USA/France; lot: 4104408; expiration date: 03/31/2016). After assessing growth in the plates, colonies were evaluated with respect to color and morphotype for presumptive identification. Light to medium green colonies indicated the presence of *C. albicans*, the colors dark blue to metallic blue indicated *C. tropicalis*, the light rose with a whitish border indicate *C. krusei* and other yeasts could appear both in its natural color (cream) or light/dark mauve (e.g. *C. glabrata* and other species), according to the criteria established by the manufacturer. Culture media was prepared according to the manufacturer's instructions. Cultures were incubated for 48 h at35 ºC±2 ºC.

Further tests for the identification of *Candida* species included the germ tube test [36,31], yeast microculture (*Candida* spp. filamentation in agar-corn flour with Tween-80) [37,33,31], auxanography (carbohydrate and nitrogen assimilation) [31], and carbohydrate fermentation [31].

Evaluation of the antifungal activity of *S. terebinthifolius* Raddi

Strains of *C. albicans* (*C. albicans* LM-1A, *C. albicans* LM-3A, *C. albicans* LM-16B and *C. albicans* LM-19A) and *C. tropicalis* (*C. tropicalis* LM-2A, *C. tropicalis* LM-7A, *C. tropicalis* LM-11A and *C. tropicalis* LM-13A) were isolated and identified from collected samples. These strains, along with two laboratory strains

from the American Type Culture Collection (ATCC; *C. albicans* ATCC 76485 and *C. tropicalis* ATCC 13803), were used in the assays.

The essential oil of *S. terebinthifolius* Raddi leaves was used to assess the antifungal activity against *Candida* species due to its known anti-microbial and anti-inflammatory activity [10,12] and because of its empiric use by some of the participants of the study who utilized traditional Brazilian medicine.

The above essential oil was provided by Professor Pablo Queiroz Lopes from the Department of Pharmaceutical Sciences of the Federal University of Paraíba. *S. terebinthifolius* Raddi leaves were collected from April to June of 2013 in the garden of the Rabelo Laboratory in the city of Cabedelo, Paraíba State (Latitude: 6° 58' 49'' South, Longitude: 34° 49' 49'' West). One specimen voucher (JPB 85943 and 85944) was deposited at the Lauro Pires Xavier Herbarium of the Federal University of Paraíba, Brazil. Essential oil components were identified by Professor Sócrates Golzio dos Santos by means of gas chromatography coupled with mass spectrophotometry (ultra GC-MS) [38]. The major components found in the composition of the essential oil of *S. terebinthifolius* Raddi leaves were limonene (63.96%), α-pinene (16.38%), p-cymene (4.87%), α-phellandrene (4.44%), β-elemene (1.98%), phtalic acid (1.67%) and myrcene (1.3%).

For the preparation of yeast inoculant, isolates were first cultivated in SDA for 24 h (overnight) at 35 ºC±2 ºC. Yeast suspensions were then prepared in sterile 0.9 % saline solution (Farmax – Distribuidor Ltda., Amaral, Divinópolis, MG, Brazil) and turbidity was adjusted to 0.5 turbidity units on the McFarland scale by comparison with the barium sulfate standard to obtain an inoculum of approximately 10^6 CFU/mL. For the assays, suspensions were diluted with distilled water to obtain an inoculum containing approximately 10^5 CFU/mL [40-43].

1) Antifungal susceptibility profiles of yeast strains

The susceptibility profiles of the studied yeast strains were determined by means of the disk-diffusion method to establish the antifungigram according to the manufacturer's instructions (Centro de Controle e Produtos para Diagnósticos Ltda - CECON, São Paulo, SP, Brazil). Sterile swab-yeast suspensions were seeded onto 15x90 mm disposable plates containing SDA. Disks impregnated with the antifungals amphotericin B (100 µg), fluconazole (25 µg), itraconazole (10 µg), miconazole(50 µg), nystatin (100 IU) and ketoconazole (50 µg) (Centro de

Controle e Produtos para Diagnósticos Ltda - CECON, São Paulo, SP, Brazil) were then lightly pressed onto the culture medium, and plates were incubated in a bacteriological incubator for 48 h at 35 ºC±2 ºC. Inhibition zones were measured with a ruler by two diagonals and the average corresponding to results expressed in millimeters (mm), and were compared with the interpretations specified by the manufacturer, as seen in Box 1.

Box 1: Interpretation of antifungal inhibition zones

Antifungal	Inhibition zone (mm)	Interpretation
Amphotericin B (100 µg)	> 10	Susceptible
	≤ 10	Intermediate or resistant
Fluconazole (25 µg)	≥ 19	Susceptible
	18-15	Intermediate
	≤ 14	Resistant
Itraconazole (10 µg)	≥ 20	Susceptible
	19-12	Intermediate
	≤ 11	Resistant
Miconazole(50µg)	> 20	Susceptible
	20-10	Intermediate
	<10	Resistant
Nystatin (100 IU)	>10	Susceptible
	≤ 10	Resistant
Ketoconazole (50µg)	> 20	Susceptible
	20-10	Intermediate
	< 10	Resistant

Source: Centro de Controle e Produtos para Diagnósticos Ltda - CECON, São Paulo, SP, Brazil

2) Determination of MIC

The MIC of the essential oil was determined in duplicate by microdilution using plates 96 U-bottom wells [41,44,45]. A volume of 100 µL of 2X SDB was added to each well. For serial dilutions,100 µL of 2X product solution was added to each well of the first row; then,100 µL was removed from each of these wells and homogenized in the wells of the following row. This procedure was repeated for all

rows to generate decreasing concentrations ranging from 1024 µg/mL to 8 µg/mL. Finally, 10 µL inoculum was added to each well, in which each column represented one yeast strain. Microorganism control was carried out in the absence of substance, as well as culture medium sterility control without insertion of the inoculum and antifungal control was run with nystatin concentrations from 1024 µg/ml to 1 µg/ml. Plates were sealed and incubated for up to 48 h at 35 ºC±2 ºC. The MIC of the essential oil was defined as the lowest concentration capable of visually inhibiting fungal growth in the wells by comparison with the growth control. All assays were performed in duplicate and the result was considered the highest concentration found.

3) Determination of MFC

After the determination of the MIC, 1 µL subcultures were grown in the presence of the MIC, as well as two of the immediately higher concentrations, in disposable 15x90 mm plates containing SDA for 24 h at35 ºC±2 ºC. The MFC was determined as the lowest concentration of the drug capable of inhibiting visible growth of the subculture[46,47].

The MFC/MIC ratio was calculated to determine whether the substance was fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC<4) [48].

Conclusion

The most prevalent species isolated from denture users were *Candida albicans* and *C. tropicalis,* with varied susceptibility profiles with respect to synthetic antifungals. A resistance pattern was observed for some strains, especially to fluconazole and itraconazole. The essential oil of *S. terebinthifolius* Raddi exhibited fungicidal activity against most of the tested strains.

Acknowledgments

The authors thank the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico– CNPq) for financial support, and Professor Pablo Queiroz Lopes from the

Department of Pharmaceutical Sciences of the Federal University of Paraíba for providing the essential oil used in the present study.

Conflicts of interest

The authors declare no conflicts of interest.

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5 CONSIDERAÇÕES GERAIS

Este estudo propôs isolar, identificar leveduras do gênero *Candida* da cavidade bucal dos usuários de prótese (parcial e total removível) e avaliar a atividade antifúngica *in vitro* do óleo essencial extraído das folhas de *Schinus terebinthifolius* Raddi (aroeira) a partir do uso popular dessa planta em uma comunidade em vulnerabilidade social.

A maioria dos usuários de prótese dentária deste estudo não apresentaram *Candida* (54,3%) no exame de coleta de material biológico realizado, indicando que, provavelmente, não havia presença de estomatite protética e confrontando com os dados da literatura que mostra que a estomatite protética é uma condição patológica que afeta cerca de dois terços dos usuários de prótese, sendo a maioria mulheres e idosos (PENHA et al., 2000; MONROY et al., 2005; SILVA; MARTINS-FILHO; PIVA, 2011).

A idade média dos usuários de prótese participantes foi de 48 anos e 2 meses, sendo justificado pelos dados epidemiológicos encontrados na literatura que apontam para um aumento considerável no quesito uso e necessidade de prótese com o avançar da idade (MILLAR; LOCKER, 2005; BRASIL, 2012).

Apesar dos achados da literatura mostrarem que a maioria das cepas isoladas de pacientes usuários de prótese sejam *C. albicans* (LYON et al., 2008; KAMIKAWA et al., 2014; KABAWAT et al., 2014) o presente estudo encontrou nas amostras de mucosa palatina a *C. albicans* e a *C. tropicalis* em 17,39% cada.

Pode-se observar que as cepas isoladas apresentaram comportamento variado frente aos diferentes antifúngicos testados, mostrando considerável resistência ao fluconazol e itraconazol. O antifúngico que todas as cepas testadas apresentaram-se sensíveis foi a anfotericina B, seguida da nistatina, cetoconazol, miconazol e fluconazol com respectivamente 90%, 80%, 50%, 10% das cepas sensíveis. Todas as cepas foram resistentes ao itraconazol. De acordo com Khan et al. (2009), as espécies de *Candida* tem mostrado comportamento resistentes mediante a aplicação de alguns medicamentos antifúngicos sintéticos. Estes resultados evidenciam a necessidade da busca de terapias alternativas e/ou complementares para o tratamento de infecções que envolvem esses micro-organismos.

Historicamente, pode-se notar que o uso de extratos vegetais com fins terapêuticos é uma das mais antigas formas de prática medicinal da humanidade. E atualmente pode-se vivenciar um crescimento do interesse pelos produtos de origem natural devido a grande procura por terapias alternativas para tentar superar os entraves encontrados nos produtos sintéticos. Sendo geralmente a ineficácia de alguns produtos sintéticos diante de cepas específicas, o alto custo dos medicamentos convencionais e à busca da população por tratamentos que possam ser menos agressivos ao organismo humano (GONÇALVES; ALVES FILHO; MENEZES, 2005; SOARES et al., 2008).

Segundo Santos et al. (2009) levantamentos baseados no conhecimento popular de plantas usadas em afecções orais devem ser realizados, identificando espécies vegetais com potencial para uso comprovado e seguro na odontologia. É importante destacar que a região nordeste é o local onde tem sido realizada a maioria dos estudos do uso popular de plantas em odontologia no Brasil (VIEIRA et al., 2014).

S. terenbithifolius Raddi tem sido relatado na literatura como uma planta medicinal utilizada empiricamente para diversos problemas bucais (LIMA et al., 2009; PEREIRA et al., 2011; FREIRE et al., 2013; MELO et al., 2014) Em um estudo etnobotânico sobre a indicação de plantas medicinais para tratamentos de patologias bucais, bem como investigar sobre o uso de plantas medicinais entre usuários de serviços odontológicos na cidade de João Pessoa, Brasil foi observado que *S. terenbithifolius* Raddi (17,24% das vendas) estava entre as plantas de uso odontológico mais vendidas pelos raizeiros, sendo antecedida apenas pelo Babatenon (20,70% das vendas) e 27% dos usuários faziam uso desta planta, ocupando o quinto lugar na lista de plantas medicinais utilizadas por usuários de serviços odontológicos. Também foi indicada para inflamação (SANTOS et al., 2009). No levantamento da literatura de Vieira et al. (2014) nas fontes de pesquisa Biological Abstracts, Chemical Abstracts, Medline, Lilacs, Web of Science e Scielo no período de 1996 a 2011 foram referidas 111 espécies vegetais utilizadas popularmente em afecções orais, dentre elas estava *S. terenbitiflius* Raddi, uma planta de origem brasileira, empregada em inflamação.

Segundo Ferreira et al. (2015) ainda não existem evidências científicas que comprovem o uso de algum produto natural no tratamento de candidíase bucal, devido aos ensaios clínicos encontrados na literatura apresentarem

diferenças metodológicas, quer sejam no projeto do estudo ou na escolha do produto testado, nas concentrações e formas farmacêuticas que permitam a confirmação da eficácia destes produtos. Tendo em vista essa lacuna na literatura evidencia-se a necessidade de estudos que abordem produtos naturais com potencial para utilização no tratamento dessa patologia.

Nos testes para determinação da CIM, os valores do óleo essencial extraído das folhas de *S. terebinthifolius* Raddi variaram entre 128 e 256 µg/mL, apresentando o menor valor as cepas *C. albicans* LM-16B, *C. albicans* LM-19A*, C. albicans* ATCC-76485*, C. tropicalis* LM-2A*, C. tropicalis* LM-7A*, C. tropicalis* ATCC-13803 e responsáveis pelo maior valor as cepas *C. albicans LM-1A, C. albicans LM-3A, C. tropicalis* LM-11A e *C. tropicalis* LM-13A . Assim, a $CIM_{60\%}$ do óleo essencial de *S. terebinthifolius* Raddi frente as cepas testadas foi de 128µg/mL. Para a nistatina a CIM variou entre 8µg/mL e 16µg/mL, sendo $CIM_{60\%}$ 8µg/mL. A relação CFM/CIM do óleo essencial reflete uma atividade fungicida da substância para a maioria das cepas, exceto para *C. tropicalis* LM-7A e *C. tropicalis* LM-13A. As diferenças encontradas na CIM do óleo essencial desta planta frente as cepas testadas pode ser explicada por serem de origem clínica e possivelmente apresentarem diferentes perfis genéticos e mecanismos de resistência adquiridos. O presente estudo corrobora um estudo encontrado na literatura que objetivou avaliar a atividade antifúngica da tintura de *S. terebinthifolius* frente a uma cepa padrão de *Candida* que foi observada CIM de 312,5µg/mL (ALVES et al., 2013).

Diante de todos os achados da literatura e do presente estudo, *S. terebinthifolius* tem mostrado grande potencial para o desenvolvimento de novos produtos à base de plantas (CARVALHO et al., 2013).

Esta investigação representa um estudo da atividade antifúngica do óleo essencial de *Schinus terebinthifolius* Raddi frente as espécies de *Candida*, incluindo algumas resistentes a antifúngicos sintéticos, isoladas de usuários de prótese.

Este estudo apresenta limitações como o número reduzido de coletas de material biológico para traçar o perfil epidemiológico da presença de *Candida* em usuários de prótese da comunidade em questão, sendo a pouca procura pelo atendimento justificada pelas características culturais da comunidade. Ainda, trata-se de um estudo inicial, *in vitro*, porém com cepas clínicas.

Entretanto, os resultados obtidos refletem boas condições para prosseguir com os ensaios da cinética de inibição do crescimento fúngico, elucidação do modo de ação da substância, alterações na micromorfologia fúngica, atividade antibiofilme, avaliação da citotoxicidade, ação antimicrobiana dos compostos isolados e associação com antifúngico sintético.

6 CONCLUSÃO

As espécies mais prevalentes nos usuários de prótese foi *C. albicans* e *C. tropicalis, com* perfil de sensibilidade variado em relação aos antifúngicos sintéticos, com destaque para um padrão de resistência de algumas cepas, especialmente ao fluconazol e itraconazol. O óleo essencial de *Schinus terebinthifolius* Raddi possui atividade fungicida sobre a maioria das cepas testadas.

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ANEXO

UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE CIÊNCIAS DA SAÚDE
COMITÊ DE ÉTICA EM PESQUISA

CERTIDÃO

Certifico que o Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da Universidade Federal da Paraíba – CEP/CCS aprovou por unanimidade na 8ª Reunião realizada no dia 21/08/2014, o Projeto de pesquisa intitulado: **"IDENTIFICAÇÃO DE ESPÉCIE DE CANDIDA DA CAVIDADE ORAL DE INDIVÍDUOS COM ESTOMATITE PROTÉTICA E SENSIBILIDADE IN VITRO A PRODUTOS NATURAIS E/OU SINTÉTICOS"** da Pesquisadora Ana Luiza Alves de Lima Pérez. Protocolo 0462/14. CAAE: 34309614.3.0000.5188.

Outrossim, informo que a autorização para posterior publicação fica condicionada à apresentação do resumo do estudo proposto à apreciação do Comitê.

Andrea M. C. Lima
Andrea Márcia da C. Lima
Mat. SIAPE 1117510
Secretária do CEP-CCS-UFPB

ANEXO

Normas para submissão do artigo ao periódico “Planta Medica”

Guidelines for Authors

1. Editorial Policy

PLANTA MEDICA – Journal of Medicinal Plant and Natural Product Research is published in 18 issues a year. The following areas of medicinal plant and natural product research are covered:
1. Biological and pharmacological activity
2. Pharmacokinetic investigations and clinical studies (Pharmacokinetic investigations examining the kinetics of drug disposition and bioavailability including the use of *in vitro*, *in vivo* and human studies)
3. Natural product chemistry
4. Analytical studies

Only papers of highest scientific quality, concisely written and complying with these Guidelines for Authors can be considered for publication. All contributions are peer-reviewed by independent referees.

Submission of a manuscript to *Planta Medica* implies that it represents **original research not previously published and that it is not being considered for publication elsewhere.**
The corresponding author must declare that the manuscript is submitted on behalf of all authors. Copyright belongs to the publisher upon acceptance of the manuscript. There are no page charges.

The language of publication is **English**. Manuscripts written by authors whose mother tongue is not English should be checked by a native speaker or a professional language editing service before submission. **Manuscripts which do not meet acceptable standards will be returned to the authors.**

Authors investigating the chemistry of a single species should aim to publish their results in a single manuscript rather than in a series of papers. Manuscripts should not report fragmentary parts of a larger study. **Pharmacological investigations of extracts require detailed extract characterisation** (see below).

Submission of a manuscript signifies acceptance of the journal's Guidelines for Authors. Submissions which are not in line with these principles may be returned directly to the authors by the Editorial Office.

A statement clarifying the **conflicts of interests** of **all** authors must be included at the end of the manuscript (before the references); this will be published. Conflicts of interest also need to be declared durring the submission process. Declaration of conflicts of interest is mandatory; if none, this also needs to be stated.

2. Submission of Manuscripts

Manuscripts can be submitted exclusively online at http://mc.manuscriptcentral.com/plamed or using the link at http://www.thieme.de/plantamedica. Submissions of hardcopy manuscripts or by e-mail will not be accepted.

A **sample manuscript** (for Original Papers) is available at http://mc.manuscriptcentral.com/plamed → Instructions and Forms, and at www.thieme.de/plantamedica. In addition to the Guidelines, authors are urged to follow these formats when preparing a manuscript.

10 Basic Rules for a Publication in *Planta Medica*

Manuscripts will not be considered for publication in *Planta Medica* unless the following conditions, if applicable, are fulfilled:

1. Ethical considerations: Submission of a manuscript to *Planta Medica* implies that it represents **original research** not previously published and that it is **not being considered for publication elsewhere**. Authors investigating the chemistry of a single species should aim to publish their results in a single manuscript rather than in a series of papers. **Manuscripts should not report fragmentary parts of a larger study**.
2. Language of publication is **English**. Manuscripts written by authors whose mother tongue is not English should be checked by a native speaker or a professional language editing service before submission.
3. Plant material (as well as other organisms) must be properly identified. The scientific name (in *italics*), the author of this name and the family must be given. It should be mentioned who identified the material. The manuscript must include references to **voucher specimens** of the plants (deposited in a major regional herbarium) or the material examined.
4. Isolation of compounds: Extraction and isolation should be described in detail. The kind and amount of material, solvents and extraction methods must be indicated. The description of chromatographic systems should contain the quantitative information that allows the reader to repeat the work. Column dimensions, elution volumes, fraction sizes, etc. should be reported.
5. Analytical studies: Key data on method validation must be provided and should typically include information on specificity, linearity, limit of detection, limit of quantification, accuracy, precision, intermediate precision, and some robustness studies. Information on the purity of reference compounds, and on the methods used for the determination of purity must be given. Recoveries of extraction and sample pre-purification steps have to be indicated. Adequate statistical treatment of data is required. Analytical studies of a routine nature will not be considered for publication.
6. Pharmacological investigations of extracts require detailed extract characterisation. Chromatographic profiling (e.g. HPLC profile with at least the major peaks identified) should be carried out, or qualitative and quantitative information on active or typical constituents should be provided.
7. Pharmacological investigations: *Planta Medica* will only consider manuscripts in which conclusions are based on adequate statistics. In each case **positive controls** (reference compounds) should be used and the dose/activity dependence should be shown.
8. Pharmacological investigations: When working with **experimental animals**, reference must be made to principles of laboratory animal care or similar regulations, and to approval by the local ethical committee. The approval number and the corresponding date must be provided.
9. Clinical studies must be designed, implemented and analyzed in a manner to meet current standards of randomised controlled trials. Reference must be made to approval of the study by the local ethical committee. The approval number and the corresponding date must be provided.
10. Biological screening: Papers dealing with the biological screening of a meaningful number of extracts of plants or other organisms can be considered for publication in *Planta Medica*. Identification of the material must be properly documented, and preparation of the extracts must be clearly described. Biological activities should be reported by listing IC_{50} values, or at least a dose-response relationship should be shown by using at least two test concentrations. Positive controls (reference compounds) must be included. Results should be presented in a concise format, and the discussion should be kept to a minimum.

Planta Med 2013; 79: 93–96

Commonly used text processors should be used for preparation of the manuscripts. No pdf files must be submitted. The manuscript has to be accompanied by a **cover letter**, in which the authors briefly explain the significance of their findings and the interest to the readership of *Planta Medica*.

The **manuscript** (main text, tables, structural formulas and figures) should be submitted as **one file**. Figures will be automatically rendered in colour online and black and white in print. Colour reproduction in print will be subject to fees of EUR 440 for the first colour figure and EUR 80 for any further figure (incl. 19% VAT).
Authors are strongly encouraged to provide non-essential but useful data, figures and tables as **Supporting Information** (see below).

3. Format of Manuscripts

3.1. Original Papers. Original papers are research articles describing original experimental results. The material should be arranged in the order: Title Page / Abstract / Keywords / Abbreviations / Introduction / Results and Discussion / Materials and Methods / Acknowledgements / Conflicts of Interest / References / Figure Legends / Tables / Structural Formulas / Figures. Results and Discussion sections may appear as two separate parts or as a combined "Results and Discussion" section. No subheadings are allowed within this section. The normal length of the **main text** of an Original Paper, **excluding** references, tables, figures and figure legends, is about **3,000 words**. In exceptional and well justified cases longer manuscripts may be accepted. When submitting such manuscripts, authors should provide a justification statement, giving compelling reasons for the length of the paper.

3.2. RapidCommunications are intended for the publication of exceptionally significant new and original results, such as unusual structures, bioactivities and innovative analytical techniques that deserve rapid publication, in the format of an Original Paper.
If authors want their submission to be considered as a Rapid Communication, they should provide a justification statement for this with their manuscript. However, also regular submissions can be selected by the Editors for rapid communication after the review process.

3.3. Reviews will generally be **invited** by the Editor-in-Chief or the Review Editor. They should be as concise as possible and do not need to include experimental details. The main purpose of reviews is to provide a concise, accurate introduction to the subject matter and inform the reader critically of the latest developments in this area. Reviews should contain an abstract, and 4–6 keywords should be listed.

3.4. Minireviews and **Perspectives** will generally be **invited** by the Editor-in-Chief or the Review Editor. Minireviews provide concise and critical updates on a subject of high interest. Perspectives are written by leading experts in an emerging field and provide a concise assessment of the current state-of-the-art and an outlook on future developments. The normal length of the **main text** of Minireviews and Perspectives, **excluding** references, tables, figures and figure legends, is about **1,500 words**.

3.5. Editorials addressing topical issues of general interest to the readership of *Planta Medica* will be published on an irregular basis. They are written by the Editor-in-Chief, other Editors, or by experts on a specific issue in the form of an Invited Editorial.

4. Preparation of Manuscripts

Please note that papers published in *Planta Medica* now follow the IRDMAR structure: Introduction, Results and Discussion, Materials and Methods, Acknowledgements, References.

In addition to the Guidelines, authors should consult the **sample manuscript** (for Original Papers) at http://mc.manuscriptcentral.com/plamed → Instructions and Forms, or at www.thieme.de/fz/plantamedica prior to preparing their contribution. Commonly used text processors should be used for preparation of the manuscripts.

For submission of all manuscripts, follow the instructions of the online submission system. Before submission, prepare the cover letter, and keep ready all information on the manuscript (title, full name and affiliation of all authors, abstract, name of all files to be submitted). **The author submitting the manuscript will be corresponding author.**

4.1. The **Title Page** must contain the title of the manuscript, the full names referenced by numerical superscripts with affiliation and addresses of all authors, and the full address of the corresponding author.

4.2. The **Abstract** should contain brief information on purpose, methods, results and conclusion (without subheadings).

4.3 The **Keywords** should include the scientific name and family of the plant(s) or other organism(s) investigated. 4–6 keywords should be listed.

4.4. Abbreviations should generally be used sparingly. Standard abbreviations such as m.p., b.p., K, s, min, h, µL, mL, µg, mg, g, kg, nm, mm, cm, ppm, mmol, HPLC, TLC, GC, UV, CD, IR, MS, NMR can be used throughout the manuscript. Non-standard abbreviations must be defined in the text following their first use. Provide a list of all non-standard abbreviations after the keywords. Define all symbols used in equations and formulas. If symbols are used extensively, provide a list of all symbols together with the list of abbreviations.

4.5. The **Introduction** should state the purpose of the investigation and relate to current knowledge in the specific topic addressed.

4.6. Results should be presented in a concise manner. Tables and figures should be presented in a manner which maximises clarity and comprehension. The **Discussion** should provide an interpretation of the data and relate them to existing knowledge. Subtitles are only admitted in exceptional cases.

4.7. Materials and Methods. Specific details about test materials and test compounds, instrumentation and experimental protocols should be given here. This section should contain sufficient details so that others are able to reproduce the experiment(s). Purity (%) of all reference and standard compounds should be mentioned, as well as the method how it was determined. Previously reported methods should be referenced only. Suppliers for major equipment, cell lines, chemicals, biochemical reagents and major disposables should be indicated.

4.7.1. Documentation of plants and other organisms or starting materials. Use the correct scientific nomenclature. For plants, the Index Kewensis (electronic Plant Information Centre ePIC, Royal Botanic Gardens, Kew, UK: http://www.kew.org/epic), and/or the Inter-

national Code of Botanical Nomenclature (www.bgbm.fu-berlin.de/iapt/nomenclature/code/tokyo-e/default.htm) should be followed. Give the scientific name (in *italics*), the author of this name and the family. Indicate who identified the material. The manuscript must include references to voucher specimens of the plants (deposited in a major regional herbarium) or the material examined including their registration number(s). It should be mentioned which plant parts have been used.

4.7.2. Description of the preparation of extracts and isolation of compounds. The kind and amount of starting material, solvents and extraction methods must be indicated. The description of chromatographic systems should contain the quantitative information that allows the reader to repeat the work. Column dimensions, stationary phase, particle size, mobile phase composition, flow rate, sample amount, and elution volumes (or retention times, k' values) of fractions should be given. E.g.: "MPLC on silica gel (40–63 µm; 2 × 50 cm), MeOH/EtOAc 8 : 2, 3 mL/min; t_R of **1**: 60–70 mL, **2**: 120–140 mL, **3**: 145–175 mL; detection of eluates by TLC (SiO_2, MeOH/H_2O 9 : 1; Dragendorff reagent), Rf **1**: 0.35, **2**: 0.55, **3**: 0.73)." When using gradients the volumes of solvents should be presented; fractions should be defined by their elution volume. Similar information is necessary for HPLC, GLC, DCCC, MLCC and all other methods of purification. Figures of chromatograms will only be accepted if they are essential for understanding the methods or the results described. GC identifications of constituents of essential oils must be supported by retention indices on a polar and an apolar column. Identification by GC-MS is preferred.

4.7.3. Physico-chemical characterisation of compounds. Data provided for new compounds should enable an unambiguous identification of the substance and have to appear in the following order, if available: visual appearance, chromatographic mobility in TLC, GC, or HPLC, mp, UV-vis, specific optical rotation, CD, IR, ^{1}H-NMR, ^{13}C-NMR, low resolution MS, high resolution MS, elemental analysis. Note that for specific optical rotation $[\alpha]_D^{temp}$, the symbol *c* is defined as mass of substance (in g) in 100 mL of solution. For specific optical rotation no unit should be specified; the "degree" symbol "°" should not be used. In case of spectroscopic work on known substances refer, if possible, to published data; the manuscript should then contain the following indication: *Copies of the original spectra are obtainable from the corresponding author*. Such original spectra and/or spectral assignments can be provided as Supporting Information (see below), as well as structural formula outlining NMR spectral correlations, MS fragmentations, etc. IR, NMR, mass, and UV spectra should normally not be given in the manuscript as figures, but only if the listing of characteristic signals is not sufficient.

4.7.4. Chemical nomenclature used should be based on the systematic rules adopted by Chemical Abstracts and IUPAC. Trivial names should be avoided unless they are definitely advantageous over the corresponding systematic names. Trivial names are not accepted for close analogues and derivatives of known compounds. For reference drug substances the INN names should be used.

4.7.5. X-Ray crystallographic data must include a line drawing of the structure, a perspective drawing, and a discussion of bond lengths and angles. A supplement describing full details of the structure and methods and means of its determination in a form suitable for deposition must be submitted to the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44 (0) 1223 33 60 33 or e-mail: deposit@ccdc.cam.ac.uk). Deposition of the data has to be prior to submission of the manuscript, and appropriate reference has to be made in the Materials and Methods section, including the deposition number.

4.7.6. Analytical studies. Key data on method validation must be provided and should typically include information on specificity, linearity, limit of detection, limit of quantification, accuracy, precision, intermediate precision, and some robustness studies. Information on the purity of reference compounds, and on the methods used for the determination of purity must be given. Recoveries of extraction and sample pre-purification steps have to be indicated. Adequate statistical treatment of data is required. For more information regarding validation issues, prospective authors should also refer to ICH guidelines. Analytical studies of a routine nature will not be considered for publication.

4.7.7. Pharmacological investigations. *Planta Medica* will only consider manuscripts in which conclusions are based on adequate statistics that incorporate the appropriate tests of significance, account for the type of data distribution and are based on the number of experimental observations required for the application of the respective statistical method. In each case **positive controls** (reference compounds) should be used and the dose/activity dependence should be shown. When working with experimental animals, reference must be made to principles of laboratory animal care or similar regulations, and to approval by the local ethical committee. The approval number and the corresponding date must be provided.

Pharmacological investigations of extracts require **detailed extract characterisation.** This includes botanical characterisation of plant material, solvent(s), duration and temperature of extraction, plus other method(s) used for preparation(s). The drug to extract ratio (DER) must be given. Chromatographic profiling (e.g. HPLC profile with a reference compound recorded at different wavelengths) should be carried out, with at least the major peaks identified, or qualitative and quantitative information on active or typical constituents should be provided. Altogether the phytochemical standardisation of an extract and/or fraction(s) require state-of-the-art methods.

4.7.8. Clinical studies. Studies reporting on plant preparations tested in humans will be accepted for review and publication. Clinical studies must be designed, implemented and analyzed in a manner to meet current standards of randomised controlled trials. For guidelines see the following reviews: Begg C et al. JAMA 1996; 276: 637–639 and Altmann DG. BMJ 1996; 313: 570–571. Reference must be made to approval of the study by the local ethical committee. The approval number and the corresponding date must be provided. All methods and variables used in a trial should be described; the data must be based on adequate statistics. Herbal medicinal products used must be characterised as described above for pharmacological investigations.

4.7.9. Biological screening. Papers dealing with the biological screening of a meaningful number of extracts of plants or other organisms can be considered for publication in *Planta Medica*. Identification of the material should properly be documented, and preparation of the extracts should clearly be described (see above, sections 4.6.1 and 4.6.2). Biological activities should be reported by listing IC_{50} values, or a dose-response relationship should be shown by using at least two test concentrations. Positive controls (reference compounds) should be included. Results should be presented in a concise format, and the discussion should be kept to a minimum.

4.8. Acknowledgements should list persons who made minor contributions to the investigation and organisations providing support.

4.9. References should be numbered in the order in which they are cited in the text, using arabic numbers between square brackets, e.g. [1]; for multiple references, e.g. [1–3] or [1,2,5].The list of references should be arranged consecutively according to the numbers in the text. Use Index Medicus abbreviations for journal titles. Authors bear complete responsibility for the accuracy of the references. The following examples illustrate the format for references:

a) Journals

Trute A, Nahrstedt A. Separation of rosmarinic acid enantiomers by three different chromatographic methods and the determination of rosmarinic acid in *Hedera helix*. Phytochem Anal 1996; 7: 204–208

Article in press without doi:
Lim EK, Ashford DA, Hou B, Jackson RG, Bowles DJ. Arabidopsis glycosyltransferases as biocatalysts in fermentation for regioselective synthesis of diverse quercetin glucosides. Biotech Bioeng, in press

Article in press with doi:
Lim EK, Bowles DJ. A class of plant glycosyltransferases involved in cellular homeostasis. EMBO J, advance online publication 8 July 2004; doi: 10.1038/sj.emboj.7600295

b) Books

Citation to complete book:
Mabberley DJ. The plant book, 2nd edition. Cambridge: Cambridge University Press; 1997: 520–521

Citation to article within a book:
Lechtenberg M, Nahrstedt A. Cyanogenic glycosides. In: Ikan R, editor. Naturally occurring glycosides. Chichester: Wiley & Sons; 1999: 147–191

Lorberg A, Hall MN. TOR: the first ten years. In: Thomas G, Sabatini DM, Hall MN, editors. TOR – target of rapamycin. Heidelberg: Springer Verlag; 2004: 1–18

Multi-volume books and encyclopedias:
Warren SA. Mental retardation and environment. In: International encyclopedia of psychiatry, psychology, psychoanalysis and neurology, Vol. 7. New York: Aesculapius Publishers; 1977: 202–207

Pharmacopoeia of China, Part 1. Beijing: People's Health Press; 1977: 531–534

c) PhD and Diploma Theses
Dettmers JM. Assessing the trophic cascade in reservoirs: the role of an introduced predator [dissertation]. Columbus: Ohio State University; 1995

d) Patents
Cookson AH. Particle trap for compressed gas insulated transmission system. US Patent 4554399; 1985

e) Conference Paper
Okada K, Kamiya Y, Saito T, Nakagawa T, Kaawamukai M. Localization and expression of geranylgeranyldiphosphate synthases in *Arabidopsis thaliana*. Annual Meeting of the American Society of Plant Physiologists, Baltimore, MD; 1999

f) Electronic Sources
Agatep R, Kirkpatrick RD, Parchaliuk DL, Woods RA, Gietz RD. Transformation of *S. cerevisiae* by the lithium acetate/single-stranded carrier DNA/polyethylene glycol protocol. Technical tips online. Available at http://research.bmn.com/tto. Accessed September 22, 2005.

If no author is given, the title is used as the first element of the citation.

If reference is made to papers submitted or in press, authors are requested to add a file of the manuscript or galley proof to the online submission. Avoid references to unpublished personal communications.

4.10. Structural formulas should be prepared with ChemDraw® or a similar program using the following settings: bond lengths 0.508 cm, bond width 0.021 cm, bold bond width 0.071 cm, bond spacing 18% of length, hash spacing 0.088 cm, atom labels Helvetica 10, compound numbers Helvetica 10 bold. These settings correspond to American Chemical Society document settings preset in ChemDraw®. The configuration of all stereocenters present should be indicated; use of bold and dashed lines rather than solid and dashed wedges is recommended. The formulas should be integrated into the manuscript file (see above: 2. Submission of Manuscripts). They will be reproduced without reduction and the charts should be prepared with maximum widths of up to 8.0 cm for single column print and up to 17 cm for double column print.

4.11. Supporting Information: To keep articles as concise and at the same time as informative as possible, authors are strongly encouraged to submit part of their tables and figures as Supporting Information. The following type of data will be preferentially published as Supporting Information rather than in the print article: High-resolution halftone and colour illustrations, spectra, chromatograms, structural drawings outlining NMR correlations, experimental procedures of secondary importance, tables summarising data that are non-quintessential but useful to the understanding of an article. Tables, figures and text provided as Supporting Information must be referred to in the manuscript as follows: (Table 1S, Supporting Information, etc.).
The cover page for Supporting Information must contain the title of the manuscript, names and affiliations of all authors, and the full address of the corresponding author. Legends for Figures and Tables must appear directly on the respective figure pages. Pages have to be numbered consecutively. **Supporting Information has to be submitted as a separate file.**
Supporting Information is published on the journals homepage at http://www.thieme-connect.de/ejournals/toc/plantamedica.

5. Proofs and Reprints

Galley proofs will be sent to the corresponding author as a PDF file. An electronic author reprint will be supplied free of charge after online publication.

January, 2013

For further information, please contact plantamedica@thieme.de

APÊNDICE

Termo de Consentimento Livre e Esclarecido

Título do Projeto: **"Identificação de espécies de *Candida* da cavidade oral de indivíduos usuários de prótese e sensibilidade *in vitro* a produtos naturais e/ou sintéticos"**
Pesquisador Responsável: **Ana Luíza Alves de Lima Pérez.**
Orientador: **Edeltrudes de Oliveira Lima.**

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Prezado (a) Senhor (a)

Estamos realizando um estudo com o objetivo de identificar os micro-organismos do gênero *Candida* presente nos indivíduos usuários de prótese e avaliar a sensibilidade dos mesmos frente a produtos naturais utilizados em uma comunidade da cidade de João Pessoa-PB. Esta pesquisa se justifica pela necessidade de terapia alternativa no tratamento de infecções por *Candida* na cavidade oral devido a inconvenientes encontrados nos produtos alopáticos como alto custo, ineficiência de alguns produtos sintéticos e agressividade ao organismo humano. Espera-se que, a partir dos resultados deste estudo, haja o aumento do interesse pelo desenvolvimento de produtos naturais com potencial aplicabilidade clínica, visando criar novas estratégias de controle químico das infecções da cavidade oral por *Candida*, bem como suprir os inconvenientes e fragilidades dos produtos do mercado. Participarão da pesquisa indivíduos que fazem uso de prótese dentária, têm idade superior a 18 anos e aceitaram participar da pesquisa. As informações serão coletadas por meio de formulários de pesquisa e coleta de material biológico da prótese e da boca do participante. Informamos que esta pesquisa oferece riscos mínimos a seus participantes, como constrangimento. Não possui nenhum meio de discriminação aos autores dos documentos envolvidos no estudo. Sua participação é voluntária, sendo garantido o direito de desistir da pesquisa, em qualquer tempo, sem que essa decisão o prejudique quanto aos aspectos éticos, morais, financeiros, sociais e de acesso à saúde. Todas as informações obtidas em relação a esse estudo permanecerão em sigilo, assegurando proteção de sua imagem e dos autores dos documentos envolvidos no estudo. Serão respeitados valores morais, culturais, religiosos, sociais e éticos. Os resultados dessa pesquisa poderão ser apresentados em congressos ou publicações científicas, porém sua identidade não será divulgada nestas apresentações, nem serão utilizadas quaisquer imagens ou informações que permitam a sua identificação.

Esperando contar com o seu apoio, desde já agradecemos a sua colaboração.

Contato com o pesquisador responsável:
Caso necessite de maiores informações sobre o presente estudo, favor ligar para a pesquisadora Ana Luíza Alves de Lima Pérez. Telefone: (83) 8839-4162, Endereço: Rua General Renato Ribeiro de Morais, Bairro dos Estados. João Pessoa – PB. CEP: 58030229. E-mail: analuiza_perez@yahoo.com.br
Contato com o Comitê de Ética em Pesquisa do CCS/UFPB
Endereço: Centro de Ciências da Saúde da Universidade Federal da Paraíba - 1º andar / Campus I / Cidade Universitária / CEP: 58.051-900**.** Telefone: (83) 3216 7791. E-mail: eticaccsufpb@hotmail.com

AUTORIZAÇÃO
Após ter sido informado sobre a finalidade da pesquisa **"Identificação de espécies de *Candida* da cavidade oral de indivíduos usuários de prótese e sensibilidade *in vitro* a produtos naturais e/ou sintéticos"**, **AUTORIZO** a utilização dos dados por mim fornecidos.

João Pessoa, _____ de _____ de 2014.

_____
Assinatura do voluntário da pesquisa

Impressão Datiloscópica

_____
Assinatura da Pesquisadora Responsável
(Ana Luíza Alves de Lima Pérez)

_____
Assinatura da Orientadora da Pesquisa
(Edeltrudes de Oliveira Lima)

APÊNDICE

Formulário aplicado aos participantes do estudo

Título do Projeto: **"Identificação de espécies de *Candida* da cavidade oral de indivíduos usuários de prótese e sensibilidade *in vitro* a produtos naturais e/ou sintéticos".**
Pesquisador Responsável: **Ana Luíza Alves de Lima Pérez.**
Orientadora: **Edeltrudes de Oliveira Lima.**

Comunidade:_____

Data de Nascimento:_____ Idade:_____ Gênero:_____

Raça:_____

Naturalidade:_____

Escolaridade:_____

Profissão:_____

Estado civil:_____

Doenças Sistêmicas

Doença	**SIM**	**NÃO**
Hipertensão Arterial		
Diabetes		
Outra:		

Faz uso de algum medicamento?

Sim	
Não	

Qual (is)?_____

Fez uso de algum medicamento nas últimas duas semanas?

Sim	
Não	

Qual (is)?_____

Já foi a uma consulta com o dentista?

Sim	
Não	

Quando foi a última consulta?

Foi ao dentista por qual motivo?

Motivo especial	
Consulta de rotina	

Qual motivo especial? _____

Existe alguma Unidade de Saúde que abrange a área da sua residência?

Sim	
Não	

Tem alguma queixa (dor ou incômodo) na boca?

Sim	
Não	

Quando você ou algum parente sente algum incômodo na boca, vocês costumam procurar ajuda de alguém?

Sim	
Não	

Quem?_____

Faz ou já fez uso, alguma vez, de plantas para curar doenças da boca?

Sim	
Não	

Qual (is)?_____ Para que fim (ns)?_____
Qual a forma de utilização? _____
Qual a parte da planta utilizada? _____
Onde conseguiu a planta? _____

Faz uso de próteses dentárias?

Sim	
Não	

Quem fez a prótese?

Protético	
Dentista	

Realiza a higienização da prótese todo dia?

Sim	
Não	

Como realiza a higienização da prótese?

Faz uso de algum produto para higienizar a prótese?

Sim	
Não	

Qual (is)?_____

sFaz uso de plantas para higienizar a prótese?

Sim	
Não	

Qual (is)?_____

Tira a prótese da boca para higienizá-la?

Sim	
Não	

Dorme com a prótese?

Sim	
Não	

Como armazena a prótese?_____

Quanto tempo faz que usa essa prótese que está na sua boca?

APÊNDICE - Artigo publicado

DOES SCIENTIFIC EVIDENCE FOR THE USE OF NATURAL PRODUCTS IN THE TREATMENT OF ORAL CANDIDIASIS EXIST? A SYSTEMATIC REVIEW

Gabriela Lacet Silva Ferreira, Ana Luíza Alves de Lima Pérez, Ítalo Martins Rocha, Mayara Abreu Pinheiro, Ricardo Dias de Castro, Hugo Lemes Carlo, Edeltrudes de Oliveira Lima, Lúcio Roberto Castellano

Artigo elaborado durante as atividades de elaboração da Dissertação de Mestrado. Programa de Pós-Graduação em Odontologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, Campus I, João Pessoa, Paraíba, Brasil, 2015.

Periódico: Evidence-Based Complementary and Alternative Medicine (eCAM)
Qualis-Capes: B1 (Ano-Base 2014)
Área: Odontologia
ISSN: 1741-4288
Fator de impacto: 1,880 (JCR-2014)

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2015, Article ID 147804, 8 pages
http://dx.doi.org/10.1155/2015/147804

Review Article

Does Scientific Evidence for the Use of Natural Products in the Treatment of Oral Candidiasis Exist? A Systematic Review

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Received 27 November 2014; Accepted 12 March 2015

Academic Editor: Arndt Büssing

In view of the limitations of antifungal agents used in the treatment of oral candidiasis and the wide variety of natural products that have been studied as treatment of this disease, this systematic literature review proposed to evaluate whether scientific evidence attesting to the efficacy of natural products in the treatment of this disease exists. A systematic search in PubMed, MEDLINE, SciELO, Lilacs, and Cochrane Library databases was accomplished using the associations among the keywords Candida albicans, phytotherapy, biological products, denture stomatitis, and oral candidiasis in both English and Portuguese. Four independent observers evaluated the methodological quality of the resulting articles. Three studies were included for detailed analysis and evaluated according to the analysis protocol based on the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement. The tested products were different in all studies. Two studies mentioned random samples, but no study described the sample allocation. No study mentioned sample calculations, a prior pilot study, or examiner calibration, and only one trial reported sample losses. Differences between the tested products and the methodological designs among these studies did not allow the existence of scientific evidence related to the effectiveness of these products for the proposed subjects to be confirmed.

1. Introduction

Oral candidiasis, which is produced by yeast of the genus *Candida*, is the mucocutaneous mycosis present in the oral cavity [1]. Generally, oral candidiasis affects users of complete upper dentures and is called denture stomatitis. Denture stomatitis is characterized by the presence of edematous and erythematous mucosa beneath an area covered by the prosthesis [2, 3].

Candida albicans is the most important microorganism in the pathogenesis of candidiasis and is present in the normal flora of the oral cavity. However, the transition from normal mucosal conditions to a parasitism situation may occur when an imbalance between host and fungus arises, which can lead to the onset of the oral candidiasis. The predisposing factors for oral candidiasis and denture stomatitis include systemic diseases, immune deficiencies, reduced salivary flow, broad-spectrum antibiotic usage, continuous prosthesis usage nightly, smoking, and poor oral and denture hygiene [4].

Although these diseases can be asymptomatic, some patients may experience discomfort such as swelling, pain, and burning sensations in the mouth [5], impairing the ingestion of liquids and food and, consequently, the quality of life of these patients [6]. Several commercially available antifungal agents are used to treat oral *Candida* infection, including nystatin, amphotericin B, clotrimazole, miconazole, itraconazole, fluconazole, and ketoconazole. However, despite their effectiveness, these drugs may produce adverse effects such as bitter taste, allergic reactions, and drug interactions [2, 3].

The development of natural products capable of clinical application is needed to create new strategies to control oral

candidiasis because of the drawbacks and weaknesses of commercially available products. Natural products are promising therapeutic alternatives because they tend to display much smaller and lower intensity adverse reactions compared to allopathic drugs. Notably, the study of natural products can provide health professionals with alternative, feasible, and low-cost therapies for treating oral diseases [7].

Therefore, the use of medicinal plants and natural products for the treatment of these diseases has been extensively investigated; however, the scientific evidence from these studies has not yet been consolidated. Randomized clinical trials are the most suitable study design for providing evidence regarding the effects of an intervention study. However, the results of only one of these studies are not sufficient to clarify certain issues. In this sense, systematic reviews and meta-analyses are the most appropriate and current methods to summarize and synthesize evidence regarding the effectiveness and effects of interventions [3, 8].

Thus, the aim of this study was to use a systematic literature review to evaluate whether scientific evidence attesting to the efficacy of natural products in the treatment of oral candidiasis exists.

2. Materials and Methods

A systematic literature review was performed using the methodology proposed by Higgins and Green [9]. The screening and selection of articles adopted the following criteria.

Inclusion Criteria. We included studies in English, Spanish, and Portuguese that were randomized controlled trials and systematic reviews of all ages and both genders that examined products for use in dentistry based on natural substances with or without reduced clinical and/or microbiological signs and symptoms of oral candidiasis.

Exclusion Criteria. We excluded all studies that did not meet the inclusion criteria of this research that evaluated the associations of synthetic and natural products.

Search Strategies. The identification of articles was accomplished using a systematic search in the PubMed (National Library of Medicine), MEDLINE (International Literature on Health Sciences), SciELO (Scientific Electronic Library Online), Lilacs (Latin American and Caribbean Literature on Health Sciences), and Cochrane Library databases.

The search strategy in PubMed was performed based on the association of the following words using the search option "all fields": (*Candida albicans* AND phytotherapy) OR (*Candida albicans* AND biological products) OR (stomatitis, denture AND phytotherapy) OR (stomatitis, denture AND biological products) OR (candidiasis, oral AND phytotherapy) OR (candidiasis, oral AND biological products). To refine the search, filters such as controlled trial, systematic review, and humans were used.

The search for articles was performed such that the greatest number of studies was found. The strategy used in the MEDLINE (search option "subject descriptor"), SciELO (search option "subject"), Lilacs (search option "all indexes"), and Cochrane (search option "title, abstract, and keywords") databases was as follows: (*Candida albicans* AND phytotherapy) OR (*Candida albicans* AND biological products) OR (denture stomatitis AND phytotherapy) OR (denture stomatitis AND biological products) OR (oral candidiasis AND phytotherapy) OR (oral candidiasis AND biological products) OR (*Candida albicans* AND fitoterapia) OR (*Candida albicans* AND produtos biológicos) OR (estomatite sob prótese AND fitoterapia) OR (estomatite sob prótese AND produtos biológicos) OR (candidíase bucal AND fitoterapia) OR (candidíase bucal AND produtos biológicos).

All articles related to these word associations and published by May 2014 were selected for analysis. Four independent observers evaluated the methodological quality of the selected articles (the title and abstract) to verify whether these articles met the inclusion criteria. In cases where the data contained in the abstract were insufficient for determining the inclusion of the study, the full text was reviewed. After individual assessments, the examiners came to a consensus regarding the inclusion of studies for the evaluation of the full text.

Finally, the selected studies were screened using the Jadad scale [10], and those studies with scores greater than or equal to 3 were evaluated according to the analysis protocol based on the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement [11].

Protocol followed by the examiners for the analysis of articles included in this systematic review is as follows:

(1) preliminary analysis: title, primary author, country, language, journal, impact factor, and year of publication;

(2) methodological review:

- (2.1) primary outcome of interest: with or without reduced clinical and/or microbiological signs and symptoms of oral candidiasis;
- (2.2) assessment of the quality of clinical trials: Jadad scale [10], with studies that obtained scores less than 3 being excluded from this review;
- (2.3) methodological design;
- (2.4) type of blinding and type of sample allocation;
- (2.5) profile, sample size, and sample size calculation;
- (2.6) loss of sample and reasons;
- (2.7) masking of product color, smell, and taste;
- (2.8) presence and characterization of placebo or control group;
- (2.9) comparison between control and experimental groups at the beginning of the study: description of groups to assess the equivalence between them at the initial phase;
- (2.10) quote of a pilot study;
- (2.11) quality of result measurement: inter- and intraexaminer calibration;
- (2.12) criteria used for clinical and/or microbiological evaluation for the disease diagnosis;
- (2.13) statistical analysis and significance level;

(2.14) type of clinical trial: phase I, II, III, or IV according to Chalmers et al. [12];

(3) analysis of intervention:

(3.1) pharmaceutical form of the test product: gel, paste, or mouthwash;

(3.2) product concentration;

(3.3) dose range: amount and frequency per day and the time when the product is being used;

(3.4) time of use (days or weeks);

(3.5) clinical condition assessment intervals;

(3.6) adherence to treatment, daily monitoring, and adverse effects (reports of discomfort caused by the product).

(4) analysis of results: verification of accuracy according to the confidence interval and the sample size;

(5) analysis of conclusions: determining whether conclusion meets the goals.

3. Results

According to the strategic search, 378 studies were found. After excluding repetitions, 301 different articles were identified (Figure 1). Of this total, fifteen articles met the inclusion criteria and were selected for further analysis. After careful analysis, three studies were considered of high importance and were included in this systematic review. The following three were the controlled clinical trials included in this analysis:

(i) treatment of oral thrush in HIV/AIDS patients using lemon juice, lemon grass (*Cymbopogon citratus*), and gentian violet [13];

(ii) comparison of the therapeutic effects of an aqueous garlic extract and a nystatin mouthwash on denture stomatitis [3];

(iii) miconazole gel compared with *Zataria multiflora* Boiss. gel in the treatment of denture stomatitis [2].

3.1. Data Description. The three trials were conducted in English ($n = 3$) in two countries, Iran ($n = 2$) and South Africa ($n = 1$). All three trials scored 3 on the Jadad scale ($n = 3$).

Table 1 presents data regarding the study design and characterization. All three trials were randomized ($n = 3$), and two were performed as a double-blind ($n = 1$) or triple-blind ($n = 1$) trial. The three studies described the sample profile; however, none of them mentioned the type of sample allocation.

One study reported follow-up losses; however, none of the studies mentioned conducting sample calculations or performing a pilot study or inter- and intraexaminer calibration. The three studies characterized the control group regarding the concentration of the product and the form of use. All articles provided the concentration of the test product, the quantity and time of use, and the intervals of clinical evaluation (Table 1).

Table 2 presents the data regarding the initial comparison between groups, the criteria used for the initial evaluation, and descriptions of the statistical analyses. All studies presented complaints of adverse effects and conclusions that corresponded to the study objectives (Table 2).

4. Discussion

Given the large amount of publications testing new products for clinical use, researchers, clinicians, and managers do not likely have access and time to evaluate all of these publications. Discerning and condensing all the information contained in these manuscripts to apply this knowledge to different clinical situations are even more difficult [14]. In this context, in dentistry, systematic reviews have been proposed for evaluating existing scientific evidence to respond to specific questions and to present the evidence in an accessible format.

The primary features of a systematic review are as follows: the creation of preestablished goals with inclusion and exclusion criteria for selecting studies, clear and reproducible methodology, systematic searches that provide access to the largest number of studies that meet the selection requirements, careful evaluation of the methodology and conclusions of the included studies, and organization and synthesis of results and conclusions [9] to minimize bias and to provide reliable results that support decision making [14].

Considering the limitations of commercially available antifungal agents for treating oral candidiasis, which involve increased fungal resistance [15], high cost, and adverse effects [2, 3] related to treatment, natural products have been investigated as important alternatives for the treatment of this pathology. The diversity of clinical and laboratory studies in the literature that have tested the different natural products raises this important question: is there clinical evidence for the use of natural products in the treatment of oral candidiasis?

To answer a clinical question, clinical trials are the studies of choice. The evaluation of a clinical trial includes careful methodological analyses of sample size, randomization, blinding, control usage, and sample losses [10].

The determination of sample size is an important part of the design of a clinical study because it attempts to eliminate both bias and predictable errors. A smaller sample than necessary can compromise the quality of the study, making understanding and inferring the results difficult; however, an extremely large sample may induce the existence of differences between groups when compared [16]. None of the studies included in this systematic review mentioned sample size calculations for sample determination.

The use of a control group is recommended to enable comparisons of test products preferably with the gold standard for treating the studied pathology. Thus, this review study included only controlled clinical trials.

Randomization and blinding are requirements cited in the literature [10] to assess the quality of clinical trials because randomization is a process in which each individual has the

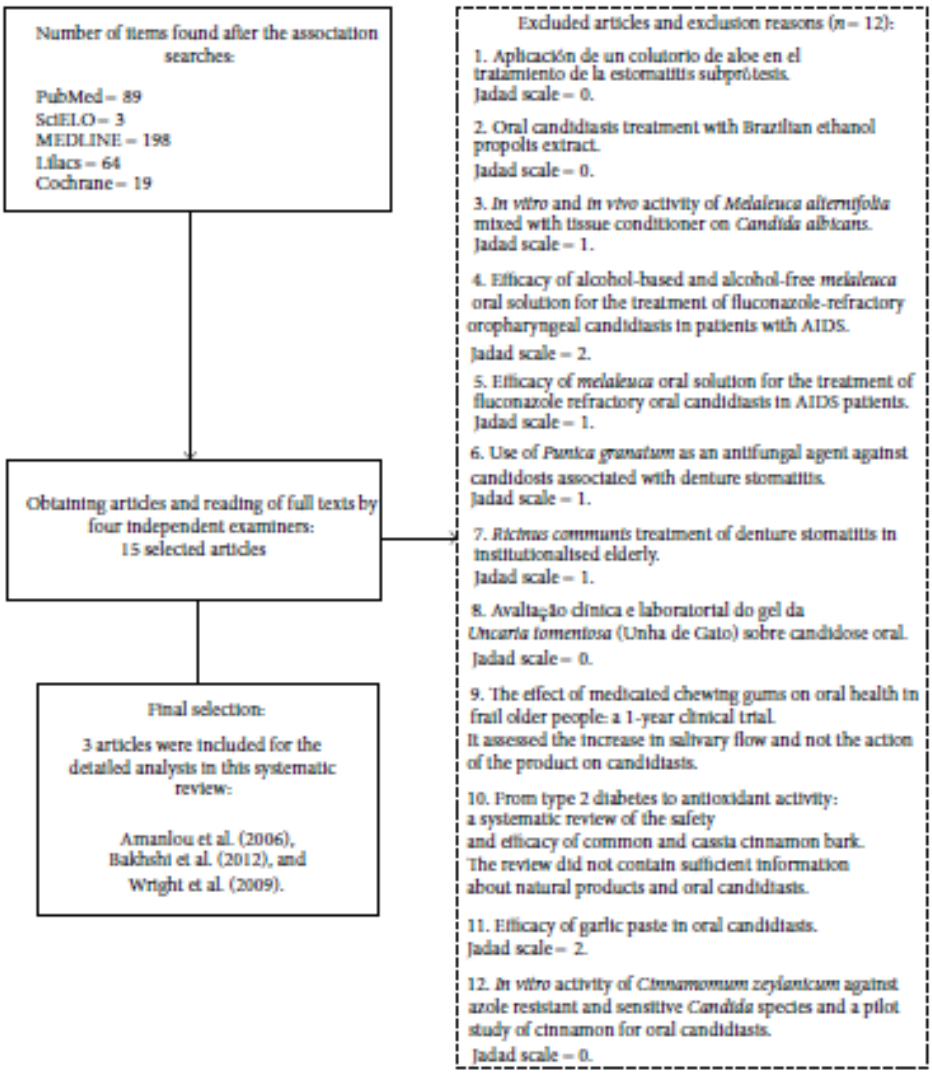

FIGURE 1: Flowchart of the search strategy.

same chance to participate in one of the groups. Blinding prevents the researcher, the individual, or the statistician to influence the results [11]. Both requirements prevent errors and biases during the study and were considered in the screening of manuscripts by applying the Jadad scale. Many studies were excluded because they did not mention randomization and blinding in the methodological procedures. Although most studies have indicated the use of these resources, Wright et al. [13] described the randomization process but did not perform blinding. Bakhshi et al. [3] described the process of randomization and blinding of researchers and statisticians involved; however, these authors did not detail the process of randomization and the masking of product color, taste, and smell, which would confirm the blinding of study participants. Amanlou et al. [2] characterized the blinding process, including the masking of product, but only mentioned the sample randomization process without describing it.

Because clinical studies involving monitoring participant follow-up for a certain period are subject to withdrawal due to several factors, losses are expected and should be mentioned [17]. Only Wright et al. [13] mention this fact. Because the greater the follow-up loss, the larger the questions regarding the study validity due to the higher occurrence of systematic errors [18], these data may be frequently omitted.

Another important aspect that should be considered in studies involving the follow-up of participants is maintaining a daily personal contact or media that try to encourage adherence to treatment and the correct use of products. None of the three included studies reported this form of contact with participants; however, in the study by Wright et al. [13],

Table 1: Methodological aspects, design and quality of studies, and characterization of groups.

Primary author	Study design and Blinding type	Primary outcome	Sample allocation and profile	Sample loss and calculation	Test product masking	Pilot study and examiner calibration	Control group characterization	Test product pharmaceutical form, concentration, and usage time
Wright [13]	Randomized controlled clinical trial. Study was not blind.	Treatment of oral candidiasis: clinical regression of lesions.	90 patients with HIV/AIDS in a sanatorium. These patients were diagnosed with oral candidiasis and were not being treated for this purpose. Not mentioned: sample allocation.	The study began with 90 patients and finished with 52. Not mentioned: calculation.	There was no masking.	Not mentioned: pilot study and examiner calibration.	Gentian violet aqueous solution (0.5%). Painted on the inside of the mouth three times daily for 10 days.	Lemon juice (experimental treatment 1) for 10 days or until clinical cure; lemon grass (experimental treatment 2) for 10 days. Not mentioned: concentrations.
Bakhshi [3]	Controlled randomized double-blind clinical trial. Not mentioned: the blinding of study subjects.	Treatment of denture stomatitis under dentures: clinical reduction of lesions after mouthwash.	40 institutionalized elderly subjects diagnosed with denture stomatitis. Not mentioned: sample allocation.	Not mentioned: calculation and loss.	Garlic aqueous solution and nystatin mouthwash were in similar bottles with the same shape, size, and color. Not mentioned: masking of smell, color, and taste.	Not mentioned: pilot study and examiner calibration.	Nystatin mouthwash (100,000 UI/mL); 5 with 20 drops for 60 seconds, three times a day.	Garlic aqueous solution (40 mg/mL) for 4 weeks.
Arunabh [2]	Randomized controlled triple-blind clinical trial.	Treatment of stomatitis under denture associated with Candida: reduction of clinical signs and symptoms and microbiological findings.	24 users of dentures diagnosed with denture stomatitis (clinical and microbiological), aged between 45 and 85 years. Not mentioned: sample allocation.	Not mentioned: calculation and loss.	Masking of smell, color, and taste as much as possible.	Not mentioned: pilot study and examiner calibration.	Miconazole 2% gel. Apply 2.5 mL on the base of the denture and place it in, four times a day for a period of 2 weeks.	Z. multiflora essential oil 0.1% gel for 2 weeks.

Table 2: Data collection, statistical analysis, results, and conclusions.

Primary author	Initial comparison between groups	Criteria for diagnosis	Follow-up treatment	Statistical analysis	Analysis of results and conclusions
Wright [13]	Age, gender, BMI, oral candidiasis scale on the day of admission, and number of days with symptoms were compared, and no difference between groups was observed.	Oral candidiasis was diagnosed and characterized from a 0 to 4 scale, where 0 represents no disease and 4 severe degree (oral thrush scale).	Once the patients were institutionalized, the treatment was controlled by nurses.	Data were gathered as ordinal data and analyzed by the following statistical tests: Fisher's exact test, chi-square test, and chi-square test with the continuity correction and the likelihood ratio. Level of significance: 95%.	Whether the sample was adequate is unknown because the author does not mention sample calculations. The quality of the analysis is doubtful due to the test selection. The choice of statistical test was inadequate for the ordinal data. The conclusion answers the aims.
Bakhshi [3]	The groups were compared regarding the methods used for cleansing dentures and the size of erythematous lesions present before treatment. No significant difference was observed between groups.	Diagnosis was established by measuring the length and width of erythematous lesions underneath the dentures by an oral medicine specialist using an oral caliper.	Not mentioned: daily monitoring to assess adherence to treatment and the correct use of the products.	ANOVA repeated measures + LSD post hoc test Chi-square test is mentioned in the methodology but is not referred to in the results or in the analysis itself. The level of significance is not mentioned specifically. In the results, we find references for 99,9% and 99,99%.	The analysis is accurate because the tests chosen are suitable for the type of obtained data. However, whether the sample was adequate is unknown because the author does not mention sample calculations. The conclusion answers the aims.
Amarilou [2]	The groups were compared in terms of age, gender, history of systemic disease, and detection of *C. albicans* on denture surfaces and palatal sample. No significant difference was observed between groups.	Erythematous denture, covered palatal mucosa graded as moderate or severe. Diagnosed candidiasis was confirmed by microbiologic cultures from the palatal mucosa and from the denture surface.	There is no information regarding adherence to treatment or follow-up to control the proper use of medication.	Chi-square analysis, Student's *t*-test, and Mann-Whitney *U* test for independent samples. Level of significance: 95%.	Whether the sample was adequate is unknown because the author does not mention sample calculations. We cannot affirm the accuracy of the statistical analysis because the text does not mention a test to assess paired ordinal data and because the mentioned tests do not cover the entire analysis. The conclusion answers the aims.

this form of contact was assumed to be controlled because subjects were institutionalized and because the products were administered by trained nurses.

The three evaluated studies showed different forms of intervention, including amounts, usage frequency, and treatment duration. This difference can be attributed to the different nature of the products tested and forms of presentation. During the intervention period and even after its completion, clinical evaluations to monitor treatment progress, as well as the presence of adverse effects observed by participants, are extremely important. All studies have reported follow-up intervals and recorded adverse effects. Amanlou et al. [2] and Bakhshi et al. [3] conducted weekly meetings with individuals for this purpose. Wright et al. [13] mentioned personal contact every two days. These follow-up intervals are relevant because the greater the proximity to subjects, the smaller the chances of follow-up losses and the greater the maintenance and effectiveness of the interventions.

Because clinical examination is of paramount importance in the diagnosis of oral candidiasis, microbial examination becomes an important auxiliary method for its confirmation. However, several diagnostic and classification tools for this disease are available, and, in an attempt to better understand and to compare data from different studies, these criteria should be standardized. All studies mentioned the stage of clinical examination but different parameters for the measurement and classification of lesions. Only Amanlou et al. [2] used the mycological exam of mucosa and dentures for confirmation.

Statistical analysis is an important step in analyzing the results because it reduces the probability of events occurring randomly. The judicious choice of the statistical test increases the reliability and accuracy of results [19]. One of the items evaluated in the selected studies was the use of statistical tests and their properties to provide answers to the guiding questions of the study according to the types of variables involved, the number of groups, and the sample size. Among the three studies evaluated, only Bakhshi et al. [3] clearly showed that the tests used were able to evaluate the data obtained and to answer the study objectives. However, all studies have limitations for not presenting a review of the clinical significance because the statistical significance presents the possibility of the obtained differences being true, regardless of the clinical importance, as determined by clinical judgment [9, 20].

A major difficulty in comparing the studies included in this systematic review was the variety of natural products tested and the forms of presenting these products. Confirming the existence of scientific evidence for the treatment of oral candidiasis with natural products is difficult when few studies meet the inclusion criteria and most have large methodological differences, whether in study design or in choosing the test product, concentrations, and pharmaceutical forms. All selected studies differ regarding these criteria. Thus, further clinical trials that address the study products by standardized methodologies and that evaluate different usage periods and various concentrations should be performed.

5. Conclusion

Currently, affirming the existence of scientific evidence for the use of natural products in the treatment of oral candidiasis is not possible.

Conflict of Interests

All authors declare that no conflict of interests regarding the publication of this paper exists.

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APÊNDICE - Artigo submetido para publicação

ANTIFUNGAL AND ANTI-BIOFILM ACTIVITY, MECHANISM OF ACTION AND CYTOTOXICITY OF CHLORAMINE T ON *Candida* spp.

Gabriela Lacet Silva Ferreira, Larissa Rangel Peixoto, Ana Luíza Alves de Lima Pérez, Brenna Louise Cavalcanti Gondim, Fabíola Galbiatti de Carvalho Carlo, Lúcio Roberto Cançado Castellano, Edeltrudes de Oliveira Lima, Ricardo Dias de Castro

Colaboração na pesquisa da Dissertação de Mestrado da aluna Gabriela Lacet Silva Ferreira. Programa de Pós-Graduação em Odontologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, Campus I, João Pessoa, Paraíba, Brasil, 2015.

Periódico: Clinical Oral Investigations
Qualis-Capes: A1 (Ano-Base 2014)
Área: Odontologia
ISSN: 1436-3771
Fator de impacto: 2,352 (JCR-2014)

APÊNDICE - Apresentação de trabalho em Congresso

ATIVIDADE ANTIFÚNGICA DE PRODUTOS NATURAIS SOBRE Candida spp. DE ORIGEM CLÍNICA

Ana Luíza Alves de Lima Pérez*, Daniele Figueredo Silva, Cássio Illan Soares Medeiros, Ricardo Dias de Castro, Edeltrudes de Oliveira Lima

Trabalho elaborado a partir da Dissertação de Mestrado: Estudo da atividade antifúngica *in vitro* do óleo essencial de *Schinus terebinthifolius* Raddi contra espécies de *Candida* isoladas da cavidade bucal de usuários de prótese. Programa de Pós-Graduação em Odontologia, Centro de Ciências da Saúde, Universidade Federal da Paraíba, Campus I, João Pessoa, Paraíba, Brasil, 2015.

Congresso: 32ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica (SBPqO)
Ano: 2015
Local: Campinas- SP, Brasil

32ª REUNIÃO ANUAL

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Divisão Brasileira da IADR

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ALAL*, Silva DF, Medeiros CIS, Castro RD, Lima EO foi apresentado

na 32ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica, no período de 4 a 7 de setembro de 2015, em Campinas - SP - Brasil.

Carlos Eduardo Francci
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