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Dissertação

Caracterização química, técnicas de extração, ensaios biológicos e potencial uso odontológico de óleos e extratos vegetais de *Arctium lappa, Butia sp. e Copaifera reticulada* Ducke

Lara Rodrigues Schneider

Pelotas, 2017

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Orientador: Prof. Dr. Rafael Guerra Lund

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(Marthin Luther King)

Resumo

SCHNEIDER, Lara Rodrigues. **Caracterização química, técnicas de extração, ensaios biológicos e potencial uso odontológico de óleos e extratos vegetais de *Arctium lappa, Butia sp. e Copaifera reticulada* Ducke *sp.*** 2017. 148f. Dissertação Mestrado em Ciências – Programa de Pós-Graduação em Bioquímica e Bioprospecção , Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2017.

O objetivo deste trabalho foi avaliar realizar uma revisão sistemática (RS) e um levantamento tecnológico dos potenciais usos e perfis químicos de extratos e óleos do gênero *Butia*, bem como comparar diferentes técnicas de obtenção de extratos e óleos de *Arctium lappa* ("bardana"), *Butia* sp.("butiá"), e *Copaifera reticulada* Ducke ("copaíba"), determinar seus principais constituintes fitoquímicos e determinar sua atividade antioxidante e antimicrobiana frente a cepas de *Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, Candida albicans, C. famata e C. parapsilosis.* Devido ao potencial antimicrobiano demonstrado pelo óleo de resina de *Copaifera reticulada* Ducke também neste estudo foi formulado e testado um material odontológico contendo copaíba quanto às suas características biológicas (teste antimicrobiano de contato direto modificado) e físico-químicas (tempo de escoamento, tempo de presa, grau de conversão, alteração dimensional e radiopacidade). A RS foi realizada dois revisores independentes (LS e DC) até dezembro de 2015, seguido as recomendações do PRISMA para revisão Sistemática. Os artigos incluidos nesta revisão foram extraidos das bases de dados MEDLINE via Pubmed, Scopus, Web of Science, Scielo e Scifinder. Sem restrições de data ou idioma. A literatura cinza foi extraida pelo portal Capes e Google Acadêmico. Foi feito levantamento tecnológico em bases de dados do INPI, Google Patents, USPTO, ESPACENET. O perfil fitoquímico foi avaliado, onde foi detectado qualitativamente a presença de saponinas, flavonóides, esteróides, taninos, glicosídeos cardiotônicos, compostos fenólicos, alcalóides e ácidos fixos fortes. Para avaliar a atividade antioxidante dos extratos, foi utilizado o método do DPPH. A atividade antimicrobiana dos extratos e óleos foi avaliada pela técnica de microdiluição em caldo e foi determinada a concentração inibitória mínima (CIM). O óleo de copaíba foi adicionado a um cimento experimental endodôntico nas concentrações de 0,5%, 1% e 2%. Os

tempos de escoamento e de presa, alteração dimensional e radiopacidade foram avaliados segundo a ISO 6876. O grau de conversão foi determinado por espectroscopia no infravermelho por transformada de Fourier (FTIR). Na RS foram inicialmente identificados 12 artigos e 14 patentes sobre o uso terapeutico do butiá, porém apenas 4 artigos e 1 patente se encaixaram nos critérios de elegibilidade. Os compostos fitoquímicos, encontrados tanto no butiá quanto na bardana, foram os fenóis e taninos. Nas folhas de butiá, foram encontrados alcalóides e esteróides e na bardana saponinas, alcalóides e ácidos fixos fortes. O extrato hidroalcólico das raízes de bardana apresentaram melhor atividade antioxidante quando comparados com o extrato das folhas, enquanto que os extratos cetônicos das folhas de butiá apresentaram melhor atividade antioxidante que os extratos etanólicos. Os extratos do butiá e da bardana apresentaram maior atividade antimicrobiana frente aos fungos testados, porém pouca atividade contra as bactérias. O óleo da copaíba revelou elevada atividade antimicrobiana tanto nos fungos como nas bactérias testadas, porém quando incorporado ao cimento endodôntico, não demonstrou a atividade antimicrobiana no tempo de 1 h, somente em 24 h. O escoamento e a alteração dimensional do cimento odontológico experimental foram semelhantes ao comercial, o tempo de presa e grau de conversão foram superiores ao comercial, enquanto que a radiopacidade foi inferior ao cimento comercial e apresentou valores menores que aqueles estipulados pela ISO 6876. Com base nas metodologias empregadas, conclui-se que: (1) Estudos *in vitro* e *in vivo* sugerem potencial farmacológico do *Butia sp*. como atividade antioxidante, atividade anti-inflamatória e antimicrobiana e antitumoral. (2) o óleo de copaíba é um promissor agente antimicrobiano, podendo ser empregado em cimentos odontológicos e em diversas formulações, assim como extratos cetônicos das folhas de butiá são promissores antioxidantes e antifúngicos. Os extratos das raízes da bardana apresentam atividade antifúngica e antioxidante, o uso associado com sonicador potencializa atividade antimicrobiana e a extração de compostos fitoquímicos Porém, outros estudos *in vitro,* como citotoxicidade, e *in vivo* são necessários para complementar os resultados desta pesquisa.

Palavras-Chave: *Butia sp; Arctium lappa*; óleo de copaíba; revisão sistemática; análise fitoquimica; atividade antioxidante; atividade antimicrobiana; cimentos endodônticos

Abstract

SCHNEIDER, Lara Rodrigues. **Chemical characterization, extraction techniques, biological tests and potential dental use of vegetable oils and extracts of *Arctium lappa, Butia sp.* and *Copaifera reticulata* Ducke.** 2017.148f. Dissertation (Master Degree in Ciências) – Programa de Pós graduação em Bioquímica e Bioprospecção , Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2017.

This study evaluated a systematic review (RS) and a technological monitoring of the terapeutical uses and phytochemical compounds of extracts and oils of the *Butia sp.* Due to compare different techniques for extracts obtaining and oils from *Arctium lappa (burdock) , Butia sp. ("Butiá") and Copaifera reticulada* Ducke ("copaíba"), determine this phytochemical compounds and determine the antimicrobial and antioxidant activity against *Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, Candida albicans, C. famata and C. parapsilosis.* Due to the antimicrobial potential demonstrated by Copaifera sp. resin oil, in this study a dental material containing copaíba was also formulated and tested for its biological characteristics (antimicrobial modified direct contact test) and physico-chemical characteristics (flow, setting time, conversion of degree, dimensional stability and radiopacity). The systematic review was performed by two independent reviewers (LS and DC) until December 2015 followed by PRISMA recommendations for Systematic review. The articles included in this review were extracted from the MEDLINE data via Pubmed, Scopus, Web of Science, Scielo, and Scifinder. No date or language restrictions. The gray literature was extracted by Capes and Google Scholar. It was done a technological survey in databases of INPI, google patents, USPTO, ESPACENET. The phytochemical compounds were evaluated by the methodology of Ndam *et al.* (2014) with modifications, where the presence of saponins, flavonoids, steroids, tannins, cardiotonic glycosides, phenolic compounds, alkaloids and strong fixed acids were qualitatively evaluated. To evaluate the antioxidant activity of the extracts, the DPPH method was used. The antimicrobial activity of the extracts and oils was evaluated by broth microdilution technique and the minimum inhibitory concentration (MIC) w

determined. Copaiba oil was added to an experimental endodontic sealer at concentrations of 0.5%, 1% and 2%. The flow, setting time, dimensional stability and radiopacity were evaluated according to ISO 6876. The degree of conversion was determined by Fourier transform infrared (FTIR) spectroscopy. In RS we initially identified 12 articles and 14 patents on the therapeutic use of the buti, but only 4 articles and 1 patent were the eligibility criteria. The phytochemicals found in both butia and burdock were phenolic compounds and tannins. In the butia leaves alkaloids and steroids. in the burdock were saponins, alkaloids and strong fixed acids. The hydroalcohol extract of the burdock roots presented better antioxidant activity when compared to the leaves extract, where as the ketone extracts of the butia leaves presented better antioxidant activity than the ethanolic extracts. Butiá and burdock extracts presented higher antimicrobial activity against the tested fungi, but little activity against the bacteria. Copaiba oil showed high antimicrobial activity in both the fungi and bacteria tested, but when incorporated into the endodontic sealer, it did not demonstrate the antimicrobial activity in the time of 1 h, only in 24 h. The flow and the dimensional stability of the experimental sealer were similar to the commercial sealer, the time setting and conversion of degree were superior to the commercial sealer, whereas the radiopacity was inferior to the commercial sealer and have smaller values than stipulated by this 6876. With Based on the methodologies employed, it is concluded that: (1) In vitro and in vivo studies suggest the pharmacological potential of *Butia sp*. As antioxidant activity, anti-inflammatory, antimicrobial and antineoplasic activity. (2) copaiba oil is a promising antimicrobial agent and could be used in dental applications and in various formulations, as well as ketone extracts from butiá leaves are promising antioxidants and antifungals. Burdock roots extracts have antifungal and antioxidant activity, The use associated with sonicador potentiates antimicrobial activity and the extraction of the phytochemical compounds. However, other in vitro studies, such as cytotoxicity, and in vivo are necessary to complement the results of this research.

Key-words: *Butia sp.; Arctium lappa;* copaiba oil resin; systematic review; phytochemical compounds; antioxidant activity; antimicrobial activity; endodontic sealer

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Lista de Abreviaturas e Siglas

AA	**Antioxidante**
ATCC	***American Type Culture Collection***
CIM	**Concentração Inibitória Mínima**
INPI	**Instituto Nacional da Propriedade Industrial**
OMPI	**Organização Mundial da Propriedade Intelectual**
RL	**Radicais Livres**
ROS	***Reactive Oxygen Species*** **(Espécies reativas de oxigênio)**
RS	**Revisão Sistemática**

Sumário

1 Introdução

Há milhares de anos as plantas têm sido utilizadas na medicina popular para tratar diversas doenças (GROPPO *et al.,* 2008; BOUTERFAS *et al*., 2016) sendo utilizadas pela maioria da população e como uma alternativa para curar doenças e sintomas, independentemente da classe econômica, tanto em subúrbios como em grandes metrópoles. Atualmente, ainda existem comunidades que as empregam como única fonte terapêutica (MONTES *et al*., 2009).

Produtos de origem vegetal são conhecidos por ser uma fonte de valor inestimável para a investigação e descoberta de novos fármacos. Nas ultimas décadas, suas propriedades medicinais têm sido investigadas devido à sua eficácia farmacológica, baixa toxidade e viabilidade econômica (GROPPO *et al*., 2008). Há uma abundância de plantas medicinais em todo o mundo e o Brasil é um dos países que apresenta a maior biodiversidade vegetal do planeta (MONTES *et al*., 2009).

As plantas medicinais são de grande aceitação pela população, entretanto, muitas destas ainda não possuem eficácia e comprovação científica (MONTES *et al*., 2009). Muitos fármacos usados atualmente como quimioterápicos, antimicrobianos e antiparasitários são isolados de fontes naturais provenientes de países de clima tropical e subtropical como o Brasil (MONTES *et al*., 2009; SANTOS *et al*., 2011;).

Revisões sistemáticas são importantes pois através delas pode-se reunir provas para poder nortear o desenvolvimento de projetos, indicando novos rumos para futuras pesquisas (SAMPAIO; MANCINI, 2006). O conhecimento tradicional associado ao uso de plantas medicinais brasileiras tem um papel importante no processo de geração de inovações, pode ser na localização de novas plantas, ou na sugestão de uma atividade farmacológica (REZENDE ; RIBEIRO, 2005)

Os fitoquímicos são compostos presentes no vegetais que apresentam como principal função, proteção das plantas contra agentes externos. Geralmente apresentam atividades biológicas interessantes, sendo fontes promissoras de diversas moléculas. Os principais compostos fitoquímicos são:

alcalóides, compostos fenólicos, óleos essenciais, esteróis, flavonoides, glicosídeos cardiotônicos, saponinas ligninas, taninos (PAL, 2007; SIMÕES *et al.,* 2007)

A atividade antioxidante tem sido pesquisada em muitos produtos à base de plantas para o tratamento de diversas doenças (CHAN *et al*., 2011). Antioxidantes são capazes de reduzir espécies reativas de oxigênio (ROS) que podem danificar o organismo. A formação de radicais livres é associada ao metabolismo celular, a ação dos radicais livres causa desequilíbrio eletrolítico resultando em danos celulares e teciduais, causando diversas doenças como envelhecimento, doença de Alzheimer, diabetes mellitus, aterosclerose e hipertensão (SANTOS *et al*., 2011; SALIMIKIA *et al*., 2016).

Um dos grandes problemas das ultimas décadas é o aumento de resistência bacteriana, devido ao aumento do uso indevido e indiscriminado de antibióticos. Muitas plantas têm sido investigadas pelo potencial antimicrobiano (GROPPO *et al*., 2008). Extratos de plantas aromáticas e medicinais são potencialmente úteis como agentes antimicrobianos e seu uso como medicamentos têm sido reconhecido (KIM *et al*., 1995; BURT, 2004; HOLLEY; PATEL, 2005). O uso de óleos e extratos como agentes antimicrobianos apresentam baixo risco de aumento da resistência microbiana a sua ação, porque estes são misturas de vários compostos que, aparentemente, apresentam diferentes ações antimicrobianas tornando mais difícil a adaptabilidade microbiana (MELO e SILVA *et al*., 2009; BUENO-SILVA *et al*., 2013).

Na área odontológica, há uma procura muito grande por novos produtos com potencial antimicrobiano e de baixa toxidade, já que a maioria das patologias orais é causada por microrganismos. Um dos desafios clínicos e de interesse odontológico é o desenvolvimento de materiais capazes de obturar o canal radicular conferindo vedamento adequado (HAMMAD *et al*., 2009). A importância da presença de características antimicrobianas nos materiais seladores do canal radicular se dá principalmente pela incidência de infecções endodônticas recorrentes e resistentes, os efeitos adversos de alguns agentes químicos antibacterianos atualmente utilizados em odontologia e considerações financeiras dos países em desenvolvimento, além da necessidade de

prevenção e opções de tratamento alternativas que sejam seguras, eficazes e econômicas (KESLER SHVERO *et al.*, 2012; HEYDER *et al.,* 2013). Embora haja diferentes agentes endodônticos com características antimicrobianas disponíveis comercialmente, há a preocupação em relação à biocompatibilidade destas substâncias e os seus efeitos tóxicos, alérgicos e mutagênicos (MOZAYENI *et al*., 2012). Assim, a busca pelo desenvolvimento de produtos alternativos, seguros e eficazes continua, sendo os fitoquímicos naturais isolados de plantas considerados como potenciais alternativas (PALOMBO, 2011). Visto isso, na tentativa de proporcionar um maior conjunto de características positivas e favoráveis para um tratamento endodôntico de sucesso, pesquisas vêm sendo realizadas visando o desenvolvimento de seladores e obturadores para aplicações endodônticas (PAMEIJER; ZMENER, 2010; CHADA *et al.,* 2012).

O Brasil é um país, que apresenta a maior reserva florestal do planeta, caracterizado por sua biodiversidade, o uso desta flora na medicina é destacado no meio cientifico, devido a descoberta de novas substâncias (MONTES *et al*., 2009).

Neste contexto, o presente trabalho esta dividido em 5 capitulos, sendo o primeiro capitulo revisão de literatura e os demais, cada qual com um artigo cientifico. No primeiro destes artigos e apresentado uma revisão sistemática e prospecção tecnológica sobre os usos terapêuticos do butiá. O segundo artigo é relatado a atividade antioxidante e antimicrobiana de extratos cetonicos e hidroalcoolicos das folhas e do óleo de *Butia* sp. O terceiro artigo relata a atividade antioxidante e antimicrobiana de extratos aquosos e hidroalcoolicos das folhas e raízes da *arctium lappa.* O quarto e ultimo artigo relata a atividade antimicrobiana e propriedades físicos-químicos de um cimento endodôntico experimental contendo óleo de *Copaífera reticulada* Ducke.

2 Objetivos do Trabalho

Objetivo Geral

Devido aos enormes benefícios dos fitoterápicos, o objetivo foi avaliar a atividade antioxidante, antimicrobiana e desenvolvimento de um material endodôntico a partir de extratos e óleos vegetais.

Objetivos Específicos

1) Revisar sistematicamente a literatura existente e bases de dados de patentes de uma espécie vegetal nativa da região com potencial terapêutico.

2) Obter e padronizar extratos e óleos *Butia*, *Arctium lappa* e *Copaifera reticulata* Ducke.

3) Avaliar qualitativamente os principais compostos fotoquímicos dos diferentes extratos vegetais.

4) Determinar e comparar as atividade antioxidante e antimicrobiana dos diferentes extratos e óleo vegetais.

5)Caracterizar físico-quimicamente e biologicamente cimentos endodônticos experimentais contendo diferentes concentrações (0,5%, 1% e 2%) de óleo de copaíba.

3 Revisão de Literatura

Revisão Sistemática

A revisão sistemática (RS) é um artigo planejado para responder uma pergunta específica, onde utiliza métodos explícitos e sistemáticos para identificar, selecionar, avaliar, coletar e analisar os dados incluídos no estudo, este pode ou não estar associado à meta-análise. A meta-análise é um método estatístico usado para integrar os resultados incluídos no estudo. Cada vez mais as revisões sistemáticas e meta-análises tornam-se importantes na área da saúde. São frequentemente utilizados pelos clínicos para se manter atualizados em seu campo de trabalho. Seu objetivo é agregar evidências de pesquisa para guiar a clinica. (SAMPAIO; MANCINI, 2007; COCHRANE, 2009; GOMES; CAMINHA, 2014;).

Conforme definido pelo Manual Cochrane, os elementos necessários de uma RS são: objetivos definidos, critérios de elegibilidade, metodologia explícita e facilmente reprodutível, pesquisa sistemática, avaliação da validade dos resultados, apresentação sistemática das características e resultados dos estudos incluídos (COCHRANE, 2009; GOMES; CAMINHA, 2014).

O PRISMA é um conjunto mínimo de itens baseados em evidências, relatados em revisões sistemáticas e meta-análises. A recomendação PRISMA, baseia-se em um checklist com 27 itens e um fluxograma com 4 etapas. Seu objetivo é ajudar no relato das RS. Seu foco é em estudos clínicos randomizados, porém pode ser usado como uma base para relatos de revisões sistemáticas com outros tipos de pesquisa. No entanto, o checklist PRISMA não é um instrumento de avaliação de qualidade da RS, ele é uma ferramenta de auxilio para os autores melhorarem o relato de revisões sistemáticas e meta-análises (GALVÃO *et al*., 2015)

Agências de fomento podem solicitar uma RS para se assegurarem de que há necessidade de pesquisas adicionais. Alguns periódicos da área clínica também estão seguindo nessa mesma direção. Apesar de RS ser considerada o melhor nível de evidência em pesquisas científicas, geralmente levam muitos meses, ou mesmo anos, para serem produzidas, então elas podem não ser

concluídas a tempo de informar decisões urgentes (GOMES; CAMINHA, 2014; GALVÃO *et al.,* 2015)

Prospecção Tecnológica

A prospecção tecnológica é o meio sistemático de mapear desenvolvimentos científicos e tecnológicos futuros capazes de influenciar de forma significativa uma indústria, a economia ou a sociedade como um todo. As prospecções tecnológicas são utilizadas há várias décadas em diversos países como uma ferramenta para orientar os esforços empreendidos para a pesquisa, desenvolvimento e inovação (AMPARO *et al*., 2012). É extremamente útil para apresentar o estado de determinada área tecnológica, com o objetivo de gerar informações sobre as tendências de mercado e percepção de sinais fracos. Estudos de prospecção tecnológica são de fundamental importância porque constituem uma ferramenta básica para orientar os esforços no desenvolvimento de uma tecnologia. A prospecção tecnológica utiliza informações oriundas de documentos de patentes, e consiste em uma ferramenta eficaz no apoio à tomada de decisão, tendo em vista o seu conteúdo. Porém, no Brasil, são poucas as empresas e instituições que desenvolvem tecnologia na área de pesquisa (AMPARO *et al*., 2012).

A documentação de patentes é a mais completa entre as fontes de pesquisa. Segundo o Instituto Nacional de Propriedade Industrial (INPI), 70% das informações tecnológicas contidas nestes documentos não são disponíveis em qualquer outro tipo de base de dados. Segundo a Organização Mundial da Propriedade Intelectual (OMPI), o número de pedidos de patente é aproximadamente 2,5 milhões a cada ano, sendo concedidas 1,2 milhões de patentes por ano. Empresas de diversos países como Estados Unidos, na Ásia e na Europa utilizam cada vez mais este instrumento como estratégia no desenvolvimento de novas tecnologias, monitoramento de concorrentes, identificação de tendências tecnológicas e investimentos. O documento de patente inclui, as patentes já concedidas, como também os pedidos de patente ainda não examinados. Estes apresentam informações tecnológicas que podem ser úteis para inventores, empresários e instituições de pesquisa. Tanto os documentos nacionais como estrangeiros, são disponibilizados em bases de patentes que podem ser acessadas via internet. Sua busca é muito importante

na fase inicial de desenvolvimento com a finalidade de evitar gastos desnecessários de tempo e recursos. Seus resultados podem revelar que uma invenção não é nova, como também a existência de outras soluções técnicas (AMPARO *et al*., 2012; INPI, 2016).

Produtos naturais

A Fitoterapia é um tratamento caracterizado pela utilização de matérias-primas vegetais, sem o isolamento de substâncias ativas, e que apresente qualidade constante e eficácia comprovadas (SOUZA *et al*., 2010, TAN *et al*., 2010; SANTOS *et al*., 2011). As plantas medicinais são uma das mais antigas "armas" utilizadas pelo homem no tratamento de enfermidades de todos os tipos. A utilização de plantas na prevenção e/ou na cura de doenças é um hábito que sempre existiu na história da humanidade. Neste contexto, a fitoterapia é encarada como opção na busca de soluções terapêuticas, utilizada principalmente pela população de baixa renda, pois se trata de uma alternativa eficiente, barata e culturalmente difundida (OLIVEIRA; ARAUJO, 2007).

Células vegetais produzem dois tipos de metabolitos. Os metabólitos primários estão envolvidos diretamente no crescimento e desenvolvimento vegetal, são carboidratos, lipídios, proteínas e nucleotídeos. Os metabólitos secundários são considerados produtos de metabolismo e geralmente não estão envolvidos em atividades essenciais ou vitais (alcalóides, compostos fenólicos, óleos essenciais, terpenos, esteróis, flavonóides, ligninas, taninos, etc. (PAL, 2007; SIMÕES *et al*., 2007). Metabólitos secundários, também conhecidos como biosubstâncias, fitoquímicos ou fitonutrientes, apresentam importantes propriedades biológicas e estão relacionados ao sistema de defesa das plantas como radiação e agressões de agentes internos e externos.

A eficácia ótima de uma planta medicinal pode não ser devido ao principal componente ativo, mas pode ser devido à ação combinada de diferentes compostos originalmente na planta. Vários fatores podem interferir nas propriedades biológicas das plantas como alterações climáticas, geográficas, estágio de maturação (SIMÕES *et al*., 2007; BOUTERFAS *et al*., 2016; SALEH-E-IN *et al.,* 2016). A produção de metabólitos secundários ocorre como uma função da interação da planta com o meio ambiente, em resposta a fatores biológicos e abióticos. Este fato pode explicar os resultados divergentes

observados a partir de extratos de uma mesma espécie, mas coletados em diferentes épocas do ano (PEREIRA *et al*., 2005). A figura abaixo (Figura 1) mostra a biossíntese dos metabólitos secundários produzidos pelas plantas.

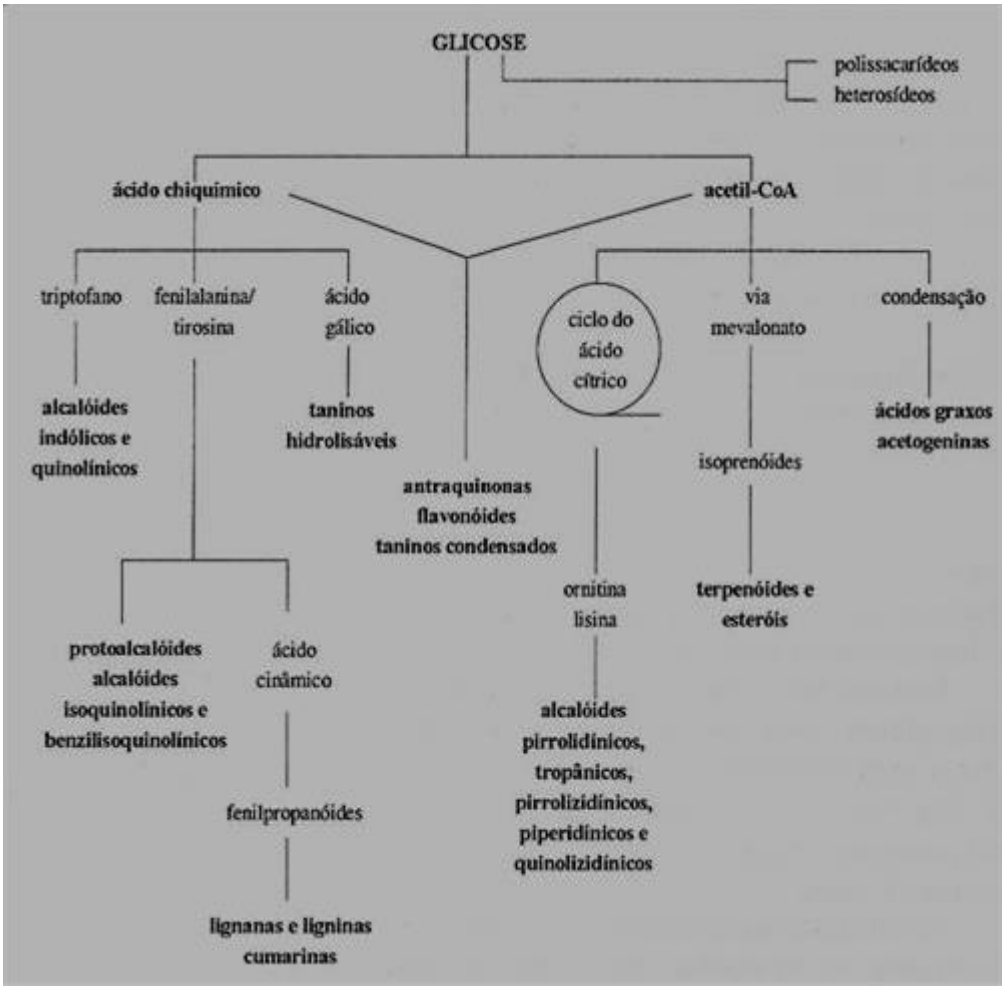

Figura 1- Biossintese de metabólitos secundários (SIMÕES *et al*., 2007)

A pesquisa fitoquímica tem por objetivos conhecer os constituintes químicos de espécies vegetais ou avaliar a sua presença. Quando não se dispõe de estudos químicos sobre a espécie de interesse, a triagem fitoquímica preliminar pode indicar os grupos de metabólitos secundários relevantes na mesma. Caso o interesse esteja restrito a uma classe específica de constituintes ou às substâncias responsáveis por uma certa atividade biológica,

a investigação deverá ser direcionada para o isolamento e a elucidação estrutural das mesmas. (SIMÕES *et al*., 2007).

As análises fitoquímicas fornecem informações relevantes da presença de metabólitos secundários nas plantas, para que assim possa chegar ao isolamento de princípios ativos importantes na produção de novos fitoterápicos. É importante ressaltar que essas variações de composição química determinada por fatores extrínsecos e intrínsecos podem ocasionar diferenças nas ações farmacológicas das espécies vegetais, o que reforça a importância dos estudos de prospecção química de espécies obtidas em diferentes localidades (Rodrigues *et al* ., 2010)

Os compostos fenólicos são substâncias que possuem pelo menos um anél aromático ligados a um ou mais grupo hidroxila em sua estrutura. Apresentam estrutura variável e são responsáveis por diversas funções. Existem mais de 8000 substâncias fenólicas descritas, sendo subdivididas em diversos grupos (DREOSTI, 2000; SIMÕES *et al*., 2007). Este grupo de sustâncias são metabólitos secundários que apresentam diversas propriedades biológicas sendo uma delas atividade antioxidante (PREDES *et al*., 2011), sua atividade antioxidante é principalmente devido às suas propriedades redox, que lhes permitem agir como agentes redutores, doadores de hidrogênio, varredores de radicais livres, supressores de oxigênio singlete e quelantes de metais. Os intermediários formados pela ação dos radicais livres com os antioxidantes são estáveis devido a ressonância do anel aromático, presente na estrutura. (PREDES *et al.,* 2011; SALEH-E-IN *et al.,* 2016)

Flavonóides e taninos são compostos fenólicos de plantas, Sabe-se também que os flavonóides são substâncias antimicrobianas eficazes *in vitro* contra uma grande variedade de microrganismos por inibição das enzimas ligadas à membrana. Foi relatado que possuem atividades substancialmente anti-carcinogênicas, antivirais, antialérgicas, anti-agregante plaquetária e anti-mutagênicas devido às suas propriedades antioxidantes e inflamatórias. Eles também são ativos na redução da pressão arterial elevada. Por outro lado, os taninos desempenham um papel importante como agente anti-hemorrágico e têm demonstrado ter imenso significado como propriedades anti-hipercolesterolemia, hipotensora e depressora cardíaca (BOUTERFAS et al., 2016; SALEH-E-IN *et al.,* 2016)

Glicosídeos cardiotônicos servem como mecanismos de defesa contra predação por muitos microrganismos, insetos e herbívoros. Além disso, os glicosídeos, flavonoides, taninos e alcalóides têm atividades hipoglicêmicas . Os alcalóides são um composto natural que contém azoto, comumente encontrado para ter propriedades antimicrobianas devido à sua capacidade de intercalar com o DNA dos microrganismos (SALEH-E-IN *et al.,* 2016)

As saponinas são outro grupo de constituintes químicos bioativos que estão envolvidos na resistência às doenças das plantas devido à sua atividade antimicrobiana. Por outro lado, as saponinas demonstram possuir propriedades benéficas (redução do colesterol) e deletérias. Embora algumas saponinas tenham se mostrado altamente tóxicas sob condições experimentais, o envenenamento agudo é relativamente raro tanto em animais quanto no homem. Apresentam propriedades hipocolesterolêmicas e antidiabéticas. As plantas medicinais são as melhores fontes para ingredientes químicos.(SALEH-E-IN *et al.,* 2016).

Butia sp.

O gênero *Butia sp*. é uma palmeira nativa da América do Sul, compreende aproximadamente 18 espécies diferentes predominantes nas áreas do sul do Brasil, leste do Paraguai, nordeste da Argentina, e no noroeste e sudeste do Uruguai (BÜTTON *et al*., 2009; HOFFMANN *et al.*, 2014). Esta palmeira ocorre em áreas de cerrado ou solos arenosos, como dunas e pântanos salinos. Produzem frutos de epicarpo liso e fibroso, mesocarpo carnoso, fibroso, endocarpo lenhoso, marrom escuro com 1-2 lóbulos e 1-2 sementes (FARIA *et al.*, 2008). Seus frutos são conhecidos como “butiá” ou “coquinho-azedo”, e estes são comestíveis e popularmente utilizados no preparo de geléias, sucos, sorvetes, licores, assim como sua folha é utilizada em artesanato (BÜTTON *et al*., 2009; PERALTA *et al*.,2013, PEREIRA *et al*.,2013; AGUIAR *et al*., 2014; AMMAR *et al*., 2014).

Atualmente, o butiá esta em risco de extinção devido ao aumento da área urbana e atividades agricolas sendo o *Butia eriospatha* incluído na Lista Brasileira de Espécies Ameaçadas de Extinção, e *Butia purpuracens Glassman* e *B. eriospatha* (Martius ex Drude) Beccari aparecem na Lista Vermelha Internacional de Conservação da Natureza. As outras espécies também

apresentam informações limitadas, pois existe um número limitado de artigos científicos sobre *Butia sp*. (HOFMANN *et al.*, 2014).

Os frutos de butiá são ricos em carotenóides, compostos fenólicos, ácidos graxos, ácido ascórbico e minerais como Ca, K, P, Mg, S, Fe, Mn, Na, Al, Cu e Zn e são também uma fonte de provitamina A e vitamina C (; FARIA *et al.,* 2008; AGUIAR *et al*., 2014). O consumo desses frutos é especialmente adequado em condições frescas, sem drástico tratamento térmico (LOPES *et al*., 2012). O óleo obtido da semente do butiá apresenta alto teor de ácido láurico, seguido por ácido oleico (FARIA *et al*., 2008; PERALTA *et al*., 2013).

Arctium lappa

Arctium lappa é uma planta da família Asteraceae, facilmente cultivável, de origem japonesa, que foi introduzida no Brasil onde é amplamente utilizada na medicina popular (CHAN *et al.,* 2011; PRESTES *et al.,* 2011;). “Bardana”, como é popularmente conhecida no Brasil, também é uma planta popular na China, Japão e outros países asiáticos, onde é utilizada há mais de 3.000 anos. Na cultura chinesa, é chamada de “Niubang” e é utilizada como alimento, por ser considerada saudável e nutritiva (CHAN *et al*., 2011)

Apresenta ampla ação terapêutica, sendo tradicionalmente utilizada para tratar hipertensão, gota, hepatite, doenças inflamatórias, a dor de garganta, infecções como erupções cutâneas, furúnculos e outras condições dermatológicas, além de ser diurética, antioxidante, ansiolítica, antiagregante plaquetário, anticancro, antidiabética, antibacteriana, antiviral e antifúngica (PEREIRA *et al.,* 2005; CHAN *et al*., 2011; PRESTES *et al.,* 2011)

A bardana age em doenças inflamatórias como a artrite reumatóide, doenças autoimunes, inflamação crônica e aterosclerose. Sua ação é atribuída à sua elevada capacidade de eliminação de radicais livres e atividade antioxidante. Suas raízes apresentam efeito hepatoprotetor, anti-inflamatório, antiproliferativo, além de atuar na eliminação de radicais livres (PREDES *et al.,* 2011). Além das raízes, sementes e folhas também têm sido investigadas pelo seu potencial terapêutico, sendo as raízes a parte mais utilizada desta planta e popularmente conhecidas como desintoxicantes, pois seu extrato protege as células de substâncias tóxicas e reduz as mutações de células (CHAN *et al.,* 2011)

Arctium lappa é uma planta que representa uma fonte natural de metabólitos secundários importantes que possuem efeito terapêutico, como: tanino, arctigenina, arctiina, beta-eudesmol, ácido cafeico, acido clorogênico, inulina, rachelogenina 4, sitosterol-beta-D-glucopiranósido, lappaol e diarctigenina. Suas atividades anticancerígenas são devido à arctigenina, presente nas sementes de bardana, que é responsável de privar as células cancerosas de nutrientes (CHAN *et al.,* 2011; PRESTES *et al.,* 2011)

Também foi relatada atividade antimicrobiana das folhas de bardana contra microorganismos orais relacionados inclusive com infecções endodônticas, tais como: *Bacillus subtilis, Candida albicans, Lactobacillus acidophilus* e *Pseudomonas aeruginosa* (PEREIRA *et al*., 2005; LUBIAN *et al*., 2010).

Copaifera sp.

As copaibeiras são árvores da família Leguminosae – Caesalpinoideae, que apresentam crescimento lento, podendo alcançar de 25 a 40 metros de altura e viver até 400 anos. Seu tronco é áspero, de coloração escura, medindo de 0,4 a 4 metros de diâmetro. O gênero *Copaifera* apresenta 72 espécies, sendo 16 destas encontradas no Brasil, Amazônia e região centro-oeste do país. Dentre as espécies mais abundantes, destacam-se: *C. officinalis* L.*, C. guianensis* Desf.*, C. reticulata* Ducke*, C. multijuga* Hayne*, C. confertiflora* Bth.*, C.langsdorffii* Desf., *C. coriacea* Mart., *C. cearensis* Huber ex Ducke (VEIGA; PINTO, 2002; MONTES *et al*., 2009).

O óleo de copaíba é um produto de excreção ou desintoxicação do organismo vegetal e funciona como defesa da planta contra animais, fungos e bactérias (MONTES *et al*., 2009). É um óleo resina, constituído por ácidos resinosos e compostos voláteis, transparente, cuja coloração pode *variar* de amarelo a marrom, com exceção do óleo da *C. langsdorfii,* que apresenta-se vermelho, recebendo a denominação de “copaíba vermelha” (VEIGA JUNIOR; PINTO, 2002).

Existem muitos métodos relatados para a retirada do óleo de copaíba. Antigamente se retirava o óleo com cortes de machado no tronco, com cortes em “V” semelhantes à extração da borracha, o que acabava retirando o óleo em seu total esgotamento e, consequentemente, ocorria a morte da planta,

porém é um método que entrou em desuso. Atualmente, é utilizado uma pratica não agressiva, onde é realizada a coleta através de uma incisão com trado a cerca de 1 metro de altura do tronco. Terminada a coleta, o orifício é vedado com argila para impedir a infecção da árvore por fungos ou cupins. A argila pode ser facilmente retirada, permitindo que se façam outras coletas no mesmo tronco, obtendo-se quantidade de óleo igual ou mesmo superior a da primeira retirada. Nesta primeira extração a quantidade de óleo obtido varia bastante, uma única árvore pode gerar até 40 ou 50 litros de óleo por ano (VEIGA JUNOR; PINTO, 2002).

O óleo resina de copaíba é composto principalmente por sesquiterpenos e diterpenos. Os principais compostos encontrados normalmente são caurano, labdano e cleorodano, o ácido copálico, ácido hardwickiico e o ácido carenóico, alfa-copaeno, beta-cariofileno, beta-bisaboleno, alfa e beta-selineno, alfa-humuleno e ômega e gama-cadineno, sendo o ácido carenóico responsável pela citotoxicidade do óleo (VEIGA JUNIOR; PINTO, 2002; MONTES *et al*., 2009; SIMÕES *et al*., 2016).

No Brasil, o óleo revela as seguintes comprovações científicas: diurético, laxante, antitetânico, anti-séptico do aparelho urinário, cicatrizante, antiinflamatório e inibidor tumoral, muito embora ainda não sejam totalmente esclarecidos os princípios ativos, mecanismo de ação e características de citotoxicidade. Além dos usos já descritos anteriormente, a óleo resina de copaíba é utilizado na indústria de fragrâncias como fixador para perfumes, cosméticos e sabões. (MONTES *et al*., 2009).

Antioxidantes

O termo radical livre (RL) é designado para qualquer átomo ou molécula que apresente elétrons não pareados nos orbitais externos, os radicais livres são produzidos no corpo humano a partir do metabolismo normal ou induzida por fatores físicos e / ou químicos no ambiente, como por exposição ao álcool, fumo, drogas, raios ultravioleta e estilo de vida (SOARES 2002). As principais fontes de R.L são organelas citoplasmáticas que metabolizam o oxigênio, nitrogênio, cloro (HALLIWELL; GUITTERGGE, 2004; MENDEZ FILHO; RODRIGUEZ, 2004; BOUTERFAS *et al.,* 2016;). Os radicais livres podem

danificar proteínas, lipídeos e DNA, a sua eliminação contribui para doenças como envelhecimento, doença de Alzheimer, diabetes mellitus, aterosclerose e artrite hipertensão.

Os antioxidantes são importantes, pois protegem o organismo, reduzindo espécies reativas de oxigênio (ROS) que podem danificar o mesmo, atuam inibindo ou retardando a formação de radicais livres. Os antioxidantes podem ser exógenos, obtidos através da dieta ou endógenos, presentes no nosso organismo (BOUTERFAS *et al.,* 2016; SALIMIKIA *et al*., 2016; RODRIGUES *et al*., 2011). Os antioxidantes enzimáticos presentes no nosso organismo são superóxido dismutase (SOD), glutationa peroxidade (GPx), glutationa redutase (GR) e catalase, os antioxidantes não enzimáticos são glutationa (GSH) e ceruloplasmina. Os antioxidantes dietéticos principais são vitamina E e C, minerais como Mg, Cu, Zn, Se, Fe, carotenóides e compostos fenólicos. Juntos com os antioxidantes endógenos eles formam uma rede integrada (VALKO *et al*., 2007). Dietas ricas em frutas e vegetais com elevada atividade antioxidante podem causam diminuição destas doenças relacionadas aos R.L devido a presença de antioxidantes (RODRIGUES *et al*., 2011)

Os AA são importantes, pois não atuam somente na eliminação de R.L, eles são utilizados em cosméticos, alimentos e na indústria farmacêutica em medicamentos. A busca por antioxidantes naturais para usos dietéticos, cosméticos e farmacêuticos tornou-se um grande desafio de pesquisa industrial e científica ao longo dos últimos anos. Antioxidantes sintéticos são comumente usados em alimentos processados, porém apresentam efeitos colaterais e carcinogênicos. Atualmente, tem havido um interesse crescente de muitos grupos de pesquisas para identificar antioxidantes naturais que são farmacologicamente potentes e têm valor nutricional e terapêutico (BOUTERFAS *et al*., 2016). Os metabólitos secundários das plantas, principalmente na forma de compostos fenólicos e nitrogenados, bem como carotenóides e ácido ascórbico, apresentam estas características (SALEH-E-IN *et al.,* 2016; KAMALI *et al*., 2016; ASOWATA-AYODELE et al., 2016)

Os compostos fenólicos destacam-se por serem compostos que apresentam atividade antioxidante, são metabólitos secundários e estão presentes em frutas, legumes, folhas, nozes, sementes e flores. Muito utilizados como alimentos funcionais com a finalidade de melhorar a saúde

(RODRIGUES *et al*., 2011) existem mais de 8000 estruturas de fenóis conhecidas atualmente, variando desde estruturas simples a substâncias altamente polimerizadas como taninos (BOUTERFAS *et al.,* 2016). Compostos fenólicos como o ácido cafeico, ácido clorogênico, fenilpropanóides e flavonóides são provavelmente responsáveis por apresentar atividade antioxidante (KAMALi *et al*., 2016; SANTOS *et al*., 2011)

Em particular, muitos compostos fenólicos estão a atrair a atenção de pesquisadores, são importantes constituintes da planta, porque eles apresentam atividade antioxidante por inativação de radicais livres de lipídeos ou impedir a decomposição de H_2O_2 em radicais livres. Além disso, os flavonóides e compostos fenólicos são eficazes na prevenção da formação de ROS e proteger lipoproteínas de baixa densidade de produção de radicais livres e ferro-mediadas por cobre, diminuem o risco de tromboses e doenças cardiacas. Os flavonóides são compostos fenólicos hidroxilados e são antioxidantes solúveis em água que ajudam na eliminação de radicais e à prevenção de danos às células oxidativas (ASOWATA-AYODELE, OTUNOLA and AFOLAYAN 2016; SALIMIKIA *et al*., 2016)

A utilização de substâncias antioxidantes com o intuito de captar radicais livres, surge como uma alternativa na terapia de varias doenças, essa procura por novos compostos capazes de inibir processos oxidativos *in vivo* sem a presença de riscos para a saúde é de extrema importância.

Metabisulfito

Muitos antioxidante sintéticos são utilizados em alimentos sendo o metabisulfito de sódio um deles, este é um sal de sulfito, muito utilizados, pois são agentes multifuncionais, atuam como controlador do desenvolvimento microbiológico, previnem o escurecimento enzimático e não enzimático , atua como agente branqueador, antioxidante ou redutor. Usados em vários produtos como frutas desidratadas, vinhos, sucos, bebidas carbonatadas, que contenham suco de frutas, biscoitos, geleias, mostarda, hortaliças desidratadas, licores, salsichas, peixes, linguiças, com a finalidade de aumentar a vida útil dos alimentos. Este produto funciona como agente inibidor do oxigênio molecular (O_2), impedindo que o alimento seja oxidado por bactérias aeróbias. Seu resíduo é o dióxido de enxofre (SO_2), que não se

constitui como fator prejudicial à saúde dos consumidores, quando sua concentração encontra-se numa faixa de 40 a 100 ppm (partes por milhão). (FAVERO *et al*., 2011)

Porém a utilização de sulfitos apresentam efeitos colaterais como redução da biodisponibilidade de algumas vitaminas como a tiamina (B1), ácido fólico (B9), piridoxina, nicotinamina. Em algumas pessoas, podem causar reações denominadas como intolerância, esta é uma reação semelhante a alergia, porém não envolve o sistema imunológico. Os sulfitos podem causar anafilaxia, urticaria, angioedema, hipotensão, naúsea, irritação gástrica local, dores de cabeça, distúrbio do comportamento, erupções cutâneas, diarreia e crise asmática em indivíduos asmáticos sensíveis a sulfitos. A maioria das reações de intolerância adota a forma de ataques asmáticos e urticária. (FAVERO *et al*., 2011)

Antimicrobianos

Antibacterianos

Os antibióticos foram uma das grandes conquistas da humanidade, pois além de aumentar a expectativa de vida das pessoas salvaram milhares de vida. Porém as bactérias ajustam-se as mudanças ambientais e criam fatores de resistência, que inativam ou limitam a atividade do antibiótico. As bactérias são microorganismos que podem ser divididas em sete grandes grupos: bactérias Gram positivas, Gram-negativas, micobactérias, riquétsias, micoplasmas, espiroquetas e clamídias (TAVARES, 2009). Apresentam uma parede celular bacteriana constituída de peptidoglicano, este é um polímero que confere rigidez e sobrevivência as variações osmóticas. A camada de peptidoglicano é presente em bactérias gram-positivas e gram-negativas. Nas bactérias Gram-positivas esta parede é mais espessa que nas gram-negativas e é a camada mais externa da célula. As bactérias gram- negativas apresentam uma estrutura mais complexa, onde apresenta uma membrana externa que atua como barreira para alguns antibióticos (MENDES, 2014).

Os antibióticos mais relevantes na prática médica afetam essencialmente cinco processos metabólicos importantes da bactéria: a síntese da parede celular, a síntese de proteínas, a replicação do DNA e a transcrição

para o RNA, a síntese de ácido fólico e a membrana citoplasmática (WALSH; TIMOTHY, 2014; TAVARES, 2009). Os antibióticos podem atuar como bactericidas ou bacteriostáticos, estes efeitos são determinados pelo mecanismo de ação e concentração da droga utilizada (TAVARES, 2009). A morte bacteriana depende das condições de crescimento e multiplicação das bactérias , e fatores físico-químicos, tais como: a temperatura, o pH, pressão osmótica, a concentração de oxigénio, dióxido de carbono e o substrato . (SIMÕES *et al*., 2016)

A resistência bacteriana é um fenômeno genético, esta resistência pode ser intrínseca ou adquirida. A resistência intrínseca ou natural é característico de espécies bacterianas em particular. Por exemplo, pode ser devido a falta do alvo susceptível ao antibiótico. A resistência adquirida ocorre quando os genes que conferem resistência as bactérias não estão presentes, os genes são obtidos por elas por mutação ou transferência genética (MENDES, 2014).

Segundo a OMS “o mundo caminha para uma era pós-antibiótica, onde infecções comuns e ferimentos leves, que por décadas foram tratáveis, podem voltar a matar” (ORGANIZAÇÃO MUNDIAL DE SAÚDE [OMS], 2014).
Atualmente já existem bactérias patogênicas que não respondem mais a nenhum dos agentes antibacterianos disponíveis. Inclusive algumas espécies apresentam mecanismos de resistência a qualquer tipo de antibióticos que possam ser desenvolvidos (MENDES, 2014). Nos últimos anos, a resistência bacteriana a múltiplos fármacos foi desenvolvida devido ao uso incorreto e indiscriminado de medicamentos antimicrobianos. A resistência aos antibióticos levou a busca por novas moléculas. As biomoléculas de plantas (fitoquímicos) têm sido relatadas como alternativas à resistência aos antibióticos de patógenos humanos devido à sua comprovada eficácia e disponibilidade. Tem sido relatado que as plantas devem suas propriedades antimicrobianas, principalmente, à presença de alcalóides, fenóis, glicosídeos, esteróides, óleos essenciais, cumarinas e taninos (ASOWATA-AYODELE, OTUNOLA and AFOLAYAN, 2016)

Antifúngicos

Infecções causadas por fungos são um grande problema, devido ao número limitado de fármacos antifúngicos disponíveis e a exposição repetida a

estes agentes, levaram ao rápido desenvolvimento da resistência aos fármacos (KUSUMA *et al*., 2014). A frequência de micoses invasivas causadas por patógenos fúngicos oportunistas aumentou significativamente na última década. Estima-se que 46% dos indivíduos saudáveis são portadores de *Candida albicans* como parte de sua microbiota oral normal (WECKWERTH *et al.,* 2015).

Candida albicans é um importante patógeno, é um fungo versátil, apresenta grande capacidade de sobrevivência, é um fungo comensal e vive em diversos órgãos do corpo humano, se adapta a qualquer ambiente com diferentes pH. É responsável por aproximadamente 50% dos casos de candidemia associada à colonização de dispositivos de internamento, como cateteres, tubos endotraqueais. Além disso, as infecções causadas por *C. albicans* continuam sendo as infecções fúngicas nosocomiais predominantes, devido à crescente população de pacientes cujo sistema imunológico está comprometido pela AIDS ou terapia imunossupressora ou anticancerígena (KUSUMA *et al*., 2014). *C. Albicans*, e sua participação efetiva na etiopatogenia da infecção endodôntica e/ou periodontal ainda é controversa. No entanto, a presença desta espécie já foi detectada em estudos microscópicos eletrônicos em lesões periapicais assintomáticas (WECKWERTH et al., 2015)

A procura por novas substâncias antimicrobianas a partir das plantas medicinais é importante, pois, pode ser feita o isolamento e a caracterização dos seus constituintes (SALEH-E-IN *et al.,* 2016; KUSUMA *et al*., 2014; MENDES, 2013)

Cimentos endodônticos

Na área odontológica, há uma procura muito grande por novos produtos com potencial antimicrobiano e de baixa toxidade, pois a maioria das patologias orais é causada por microrganismos. A cavidade oral apresenta mais de 500 espécies de microorganismos, já isolados e identificados. Estes são responsáveis pela formação de biofilme nos tecidos, sendo os principais causadores das doenças periodontal, periapical e cárie (SBORDONE; BORTOLAIA, 2003; ZAURA et al., 2009). Uma das bactérias de maior relevância na odontologia é *E. faecalis,* esta é a mais prevalente e resistente

em casos de infecções, sendo a principal razão para o fracasso do tratamento endodôntico (SUNDQVIST *et al.,* 1998; PINHEIRO *et al.,* 2003; SJOGREN *et al*., 1997)

Um dos desafios clínicos de interesse odontológico e de desenvolvimento são materiais capazes de obturar o canal radicular conferindo ao mesmo vedamento adequado (HAMMAD *et al*., 2009). A importância da presença de características antimicrobianas nos materiais seladores do canal radicular se dá principalmente pela incidência de infecções endodônticas recorrentes e resistentes, os efeitos adversos de alguns agentes químicos antibacterianos atualmente utilizados em odontologia, além da necessidade de prevenção e opções de tratamento alternativas que sejam seguras, eficazes e econômicas (KESLER SHVERO *et al.,* 2012; HEYDER *et al*., 2013). Embora haja diferentes agentes endodônticos com características antimicrobianas disponíveis comercialmente, há a preocupação em relação à biocompatibilidade destas substâncias e os seus efeitos tóxicos, alérgicos e mutagênicos (MOZAYENi *et al*., 2012). Assim, a busca pelo desenvolvimento de um produto alternativos seguro e eficaz continua, sendo os fitoquímicos naturais isolados de plantas considerados como potenciais alternativas (PALOMBO, 2011). Visto isso, na tentativa de proporcionar um maior conjunto de características positivas e favoráveis para um tratamento endodôntico de sucesso pesquisas vêm sendo realizadas visando o desenvolvimento de seladores e obturadores para aplicações endodônticas (PAMEIJER; ZMENER, 2010; CHADA *et al*., 2012).

A terapia endodôntica se divide em três fases: instrumentação, desinfecção e obturação, tem por objetivo restabelecer a integridade dos tecidos perirradiculares e preservar o elemento dental. Após a desinfecção e limpeza dos canais infectados através do preparo químico- mecânico é necessário promover vedamento do espaço afim de evitar a recontaminação (HAMMAD *et al*., 2008). Seu sucesso depende da atividade antimicrobiana dos materiais utilizados, desde o preparo físico-químico, medicação intracanal e também nos cimentos endodônticos. Além disto, a obtenção do selamento ideal é extremamente difícil, o cimento ideal deve fluir ao longo de toda a superfície da parede do canal, preencher todos os espaços e as lacunas entre o material de obturação e da dentina, aderir na dentina e na guta-percha, ter efeito

antimicrobiano e ser biocompativel (WU et al., 2000; GEURTSEN et al., 2000). Além disso, o mercado nacional de materiais odontológicos ainda é predominantemente dominado por produtos importados. Fabricantes como Dentsply(EUA), 3M ESPE (EUA), Ivoclar-Vivadent (Liechenstein), Kerr (EUA), Bisco (EUA), Heraeus-Kulzer (Alemanha), Kuraray (Japão), Voco (Alemanha) e SDI (Austrália) são exemplos de empresas que exportam materiais odontológicos e vendem amplamente no Brasil.

Há um desafio clinico permanente na Odontologia em diminuir a incidência de infecções endodônticas recorrentes e resistentes e um questionamento tecnológico a respeito dos efeitos adversos (toxidade, alergias e mutagênese) de alguns agentes químicos antibacterianos atualmente utilizados em odontologia. Materiais endodônticos, com potencial antimicrobiano e utilizando alta tecnologia à base de resina e extratos naturais com potencial terapêutico são escassos no mercado odontológico voltado para a Endodontia, tanto estrangeiro quanto nacional.

Capitulo II

Therapeutic uses and phytochemistry of *Butia sp*: a systematic review and a technological monitoring process overview.

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Situação do artigo: a ser submetido

Abstract

A systematic review of literature and a technological overview were carried out to summarize the available evidence on the therapeutic uses and phytochemical compounds of *Butia sp*. The following electronic databases were searched: MedLine (PubMed), Web of Science, Scopus, Scielo, and gray literature. Furthermore, the online system USPTO, ESPACENET, INPI, Google Patents was accessed to obtain patent data. The inclusion criteria were: articles that describe either the therapeutic uses of *Butia sp.* (antimicrobial activity, antioxidant activity, anti-inflammatory activity, antineoplastic activity) or studies describing phytochemical compounds of *Butia sp*. A limited amount of handsearching was undertaken. Reference lists were scanned to identify other relevant studies, and requests for unpublished data were made to people working in the field. There wasn`t language or data restriction. Of 12 papers and 14 patents, 9 full texts of scientific articles and 1 patent were scrutinised by two reviewers. Analyzing the results of articles on therapeutic activity of *Butia sp*. we concluded that *Butia* has shown antioxidant activity, anti-inflammatory and antimicrobial activity. *In vitro* studies have proved the pharmacological potential of *Butia sp*. However, evidence of its therapeutic uses has not been extensively studied, and the available evidence need further confirmation.

Keywords: *Butia sp.* herbal medicines, phytochemicals, patents, systematic review

INTRODUCTION

Arecaceae, known as Palmae family, lies between the largest families of plants in the world in terms of number of species and in plenty. It has between 2,500-3,500 species. Its fruits are attractive due to their organoleptic characteristics, nutritional and functional use. These plants have economic uses and biological activities such as a tonic, diuretic, leprosy treatment, asthma, bronchitis, fatigue, tuberculosis, abdominal pain, fever and vomiting (Ammar, *et al.*, 2014; Button *et al.*, 2009; Hoffmann *et al.*, 2014).

The genus *Butia* is native from South America, has about 18 different species prevalent in areas of southern Brazil, eastern Paraguay, northeastern Argentina, and in the northwest and southeast of Uruguay (Button *et al.*, 2009; Hoffmann *et al.*, 2014). This palm occurs in areas of cerrado or sandy soils, such as dunes and salt marshes. It offers fruits of smooth and fibrous epicarp, mesocarp fleshy, fibrous, woody endocarp,

dark brown with 1-2 locules and 1-2 seeds (Faria *et al.*, 2008). Their fruits are known as Butia or coquinho-azedo, these they are edible and popularly used in the preparation of jelly, juices, ice cream, licor, and this leaves is used in handicrafts (Button *et al.*, 2009; Peralta *et al.*, 2013; Pereira *et al*., 2013; Aguiar *et al*., 2014;. Ammar *et al.*, 2014).

The fruits of *Butia* are rich in carotenoids, phenolic compounds, fatty acids, ascorbic acid and minerals (Aguiar *et al*., 2014). They are considered a source of unsaturated fatty acids, especially suitable for fresh consume without drastic heat treatment drastic (Lopes *et al*., 2012). The volatile compounds can vary depending on the stage of maturity, as well as the conditions and the storage period and postharvest can also influence the chemical composition of the compounds. Chemical characterization of fruit and the quantitation of their volatile components are important to understanding nutritional value, during the maturation process some organic acids can be a precursor for the synthesis of esters (Aguiar *et al*., 2014). The importance of bioactive compounds derived from diet has been established, which can help overcome such deficiencies and also promote the protection, prevention or reduction of the effects caused by oxidative stress (Pereira *et al.*, 2013).

Hoffmann´s study was an overview that compiled general articles of *Butia* and focused mostly in agronomy aspects of this plant genus (Hoffmann *et al.*, 2014). Our article is the first to systematically review the literature of *Butia* based on a technological monitoring process and a systematic review.

MATERIALS AND METHODS

Protocol and Registration

The study was followed the recommendations of the PRISMA statement for the report of this systematic review.

Informations Sources and Search

The literature search was carried out by two independent reviewers (LS and DC) until december 2015. The following seven databases were screened: MedLine (PubMed), Web of Science, Scopus, Scielo, and Scfinder. No restrictions were placed on the publication date or languages. The grey literature was explored using the database Google Scholar and Portal Capes. Moreover, the online system United States

Patent and Trademark Office (USPTO), Google Patents, National Institute of Industrial Property (INPI) and Espacenet patent search (EPO) was accessed to recover patent documents related to *Butia*. A limited amount of handsearching was undertaken. Reference lists were scanned to identify other relevant studies, and requests for unpublished data were made to people working in the field.

The search strategies defined for the databases described above are listed in Table 1. The search strategy was appropriately modified for each database and performed by two reviewers (LS and DC) to identify eligible studies and then checked by a third one in the case of any divergences about some of the screened articles by the two reviewers.

Full-text versions of the papers that appeared to meet the inclusion criteria were retrieved for further assessment and data extraction.

Study selection and data collection process

Initially, the articles were selected by title and abstracts according to the previously described search strategy (Table 1). Articles appearing in more than one database were considered only once. Full reports were also obtained when there was insufficient information in the title and abstract to make a clear decision. Subsequently, full-text articles were acquired and two reviewers (LS and DC) classified those who met the inclusion criteria. **Search in patent:** Patent search was also made using International Patent Classification (IPC) with the following codes:#A61P 29/00 (anti-inflamatóry),#A61P 31/00 (Anti-infectives, antibiotics, antiseptics, chemotherapeutics), #A61P 31/10 (antimycotic), #A61P 31/02 (antibacterial agent). #A61P 31/02 (antiseptic), #A61P 35/00 (antineoplasic) and #A61K 8/97 (vegetable origin).

Characteristics of included articles

The characteristics of included article were all studies that describe the therapeutic uses of *Butia sp.* The antimicrobial activity, antioxidant activity, anti-inflammatory activity, antineoplastic activity and study were have phytochemical compounds.

RESULTS

Study selection

After research in databases Pubmed (3), Scielo(0), Scifinder (15), Web of science (7) e Scopus (7) and removal duplicates 12 Studies were identificates. After reading the titles and abstracts were 9 selected items, after complete reading of the studies were selections articles 4 the full text of these studies, were evaluated to see if they were eligible.

In the patent database, the search strategy initially retrieved 27 patents, with 12 being excluded after reading the title and abstract (Fig. 2) since they were not related to therapeutic use of butia. Of the remaining two patents, one patents were excluded: A total of one patents were included in the analysis.

From all the studies included, only one was *in vivo* (an animal experimentation). That article was an in vivo and in vitro study. 75% of studies included were from Brazil, tree were about antioxidant activity, one about antimicrobial activity and one about antiinflamatory activity. As regards the patents, only one patent about antineoplasic activity of this fruit was selected.

Characteristics of excluded articles

The characteristics of studies excluded were: risk of bias, multiple publications, literature reviews, theses, processed products and studies with deficiency of data.

DISCUSSION

Phytochemical compounds

The bioactive constituents in various parts of *Butia sp.* are presented in table 3. The compounds present in fruits of *Butia eriosphata* were gallic acid derivatives, protocatechuic acid derivatives, caffeic acid derivatives, chlorogenic acid derivatives, isoquercitrin, quercetin derivatives, hyperoside, and rutin. (Denardin *et al.*, 2015). Peralta *et al.,* (2013), evalued the oil of B*utia capitata,* it was indicated the composed 24% of the unsaturated fatty acids and 76% of saturated fatty acids. In fruits were describe phenolic compounds, carotenoids, fatty acids unsaturated, the linoleic acid presented were considered essential in food (Pereira *et al.,* 2013). The results in leaves of *Butia capitata* identificated volatile constituents, the major compounds identificated were decanol (28,69%). Was identificated oxygenated compounds (64,58%), non-

oxygenated compounds (35,41%), monoterpenes, sesquiterpenes and monoterpene esters (Ammar *et al*.,2014).

Therapeutic Uses

Antimicrobial activity- Peralta *et al*., (2013), investigated and compared the antimicrobial effect of an experimental self-adhesive resin containing oil derived from *B. capitata* seeds than commercial self-etching adhesives, of which has been claimed to have an antimicrobial component its in formulation. This study evalued the antimicrobial activity in a model biofilm microcosm, it was also reported physical and mechanical tests of the final product. The antimicrobial effect of the experimental adhesive of *B. capitata* oil was similar to bacterial effect of commercial adhesives. The antimicrobial effect of butia oil might be explained by the presence of fatty acids as lauric acid, oleic acid, linoleic acid and palmitoleic. Lauric acid is a most active of saturated fatty acids present in the oil *B. capitata* and palmitoleic acid is the major unsaturated fatty acid.

Anti-inflamatory activity- Ammar *et al*., (2014), evaluated the anti-inflammatory activity of butia leaves. The carrageenan type IV was used for induction of acute inflammation in rats. They use for anti-inflammatory activity polar and non-polar extracts of *B. capitata*. The inflammation model in rats, using the method of hind paw edema. The results showed that both polar and non-polar extracts, possess significant anti-inflammatory activity on all monitored different times of the test; the maximum effect was 51% and 41% respectively after four hours from the carrageenan injection.

The polar extract of anti-inflammatory activity can be attributed to the presence of flavonoids in the extract, this were detected from the phytochemical part. It was reported that most flavonoids which exhibit antioxidant activity also have anti-inflammatory activity. The nonpolar extract leaf also showed anti-inflammatory activity. Phytochemical analysis of this plant revealed the presence of β-sitosterol and β-amyrin that have been reported to anti-inflammatory activity, by blocking the inflammatory enzyme modifying the shape prostaglandin pathway. It was also shown that β-sitosterol of inhibits that myeloperoxidase and adenosine desaminase activity or IL-1β, and level of tumor necrosis factor alpha reduce inflammation. Triterpene such as β-amyrin was reported to inhibit inflammation by activating cannabinoid receptors and by inhibiting the production of cytokines and the expression of the nuclear factor and

cyclooxygenase 2. The alpha-tocopherol has been shown to activity anti- inflammatory lower than the tocotrienol rich fraction nevertheless still an anti-inflammatory effect. The anti-inflammatory activity of α-tocopherol has been reported. This, both sterol and α-tocopherol may effect synergism as anti-inflammatory in the non-polar extract leaves of *B. capitata* (Ammar *et al*., 2014).

Antioxidant- Many studies related antioxidants in plants, the most important are the tocopherols, ascorbate, thiols, β-carotene and phenolic compounds such as flavonoids, lignans and chromones (Ammar *et al*., 2014). Polyphenols are important, because protect the body from oxidative stress, scientific evidence indicates that oxidative stress is associated with neurodegenerative diseases related to age (Denardin *et al*., 2015).

Polyphenols have high antioxidant activity in vitro, this can removing reactive oxygen species, nitrogen, chlorine, superoxide anion, hydroxyl radical, radical peroxyl, hypochlorous acid and peroxynitrous. Also take away metal ion chelate that have pro-oxidant activity (Denardin *et al.,* 2015). Antioxidant activity of polyphenols occurs by different mechanisms. The most important is the scavenging free radicals, which depends on the structure of the compound involved, it is differents because the number and position of hydroxyl groups in the molecule. These facts may explain the higher antioxi dant activity observed in the fruits with a red and purple pulp, which had a higher content of phenolic compounds and a predominance of flavonoids and anthocyanins such as cyanidin and quercetin derivatives (Denardin *et al.,* 2015)

Sterols are compounds that can antioxidant activity the reason acting as hydrogen donor (Ammar *et al.,* 2014). Tocopherols are effective antioxidant, they have free phenolic hydroxyl groups. In their study Ammar *et al*., (2014) related the presence of α-tocopherol in nonpolar extracts made in this study is the responsible of antioxidant activity (Ammar *et al.,* 2014).

Denardin *et al.,* (2015) evaluated the antioxidant activity of various extracts from araçá, pitanga (red, purple and orange), *Butia eriosphata* and blackberry (xavante and Cherokee), where the butia had the lowest value of FRAP compared with araçá and pitanga (red, purple and orange) and blacberry (xavante and cherokee). The correlation between bioactive compounds and antioxidant activity of the extracts suggests that phenolic compounds are most responsible for antioxidant activity in DPPH and FRAP assays. In addition, it was observed that Butia one had the highest vitamin C compound,

although its activity antioxidant in DPPH and FRAP assays was lower, which indicates that the ascorbic acid is not related than antioxidant activity (Denardin *et al.*, 2015).

Ammar *et al*., (2014), evaluated the antioxidant activity of different extracts of butia capitata leafes. This used DL-α-tocopherol method. The extracts obtained were: petroleum ether, ether, chloroform, methanol and aqueous methanol extracts 50%. The results showed potent antioxidant activity both leaves extracts of methanol and petroleum ether (78.85% and 71.33%, respectively). The aqueous solution methanol 50% and ether leaf extracts also showed a moderate antioxidant activity (62.24%), while the methanol extract showed activity 48 25%. In the present study, the antioxidant activity polar extract of *B. capitata* sheets can be attributed to the presence of flavonoids, tricin the 7-rutinoside, isorhamnetin 3-O-rutinoside, kaempferol 3-O-rutinoside, rutin, 7-O-glucoside, luteolin and apigenin, which were isolated and identified by chromatography (Ammar *et al.*, 2014).

The antioxidant activity of petroleum ether should occurred to the presence of sterols, such as β-sitosterol and campesterol and oil-soluble vitamin, α-tocopherol, all of which were identified by chromatographic analysis (Ammar *et al.*, 2014).

Pereira et al. (2013) evalued the pulp of *Butia capitata* and analyzed the antioxidant activity, DPPH and ABTS were methodology used. They compared with two fruits, *Rollinia sylvitica* (Araticu-do-mato) and *Cereus hildmannianus* (Mandaracatu de três-quinas) by the ABTS method, Butia fruit showed higher values, representing 6.74 times the value obtained for araticu-do-mato and 1.32 times the value obtained for mandacaru de três-quinas. However, for DPPH assay values obtained for Butia and mandacaru- de três-quinas were considered statistically equal and average, four times the value found in araticu-do-mato (Pereira *et al*. 2013).

Ascorbic acid:

The ascorbic acid is biologically active biologic form of vitamin C, it is commonly found and distributed mainly in citrus fruits and leaf of vegetables (Denardin *et al*., 2015). According Denardin et al., (2015), the Butia showed the highest concentration of ascorbic acid compared with araçá and pitanga (red , purple and orange), blackberry (Xavante and Cherokee), this concentration was more higher, nearly 73 times (Denardin *et al.*, 2015).

Denardin *et al.,* (2015) found a negative correlation between total phenolic compound and ascorbic acid, it suggested that the lower ascorbic acid compound, the higher total phenolic compound of these fruits studied.

Total phenolics:

Phenolic compounds are products of the secondary metabolism in plants, it is a large and complex group, these molecules are essential for plant growth and reproduction. This compounds is produced in the plant whenever conditions of stress, injury, infection, radiation. The phenolic composition is determined by genetic and environmental factors, but can be modified by oxidative reactions during storage and / or processing. They are responsible for the color, adstringency and flavor. (Denardin *et al.,* 2015). According Denardin *et al.,* (2015) have been found in butia extracts, compounds derived from gallic acid, derivatives protocatechuic acid, derivatives of caffeic acid derived from chlorogenic acid, isoquercitrin, derivatives of quercetin, hyperoside, and rutin.

Compared with araçá, red pitanga, and Orange pitanga Butia eriosphata had lower concentration of phenolic compounds, but high concentration compared to the Xavante and Cherokee blackberry (Denartin *et al.,* 2015)

Pereira *et al*. (2013) evaluated the concentration of total phenolic compounds using the pulp of *Butia capitata*, this homogenized with methanol and comparing with other two fruits (araticu do-mato and madacaru-de-três-quinas). The total phenolic compounds, the mandacaru-de-três-quinas) showed a significantly higher amount corresponding to twice that found in araticu do mato and butia fruits, the latter two being considered statistically equal (Pereira *et al.,* 2013).

Carotenoids:

According to Pereira *et al*., (2013) Butia has higher carotenoid levels compared to other tested fruits, the total content of carotenoids found in fruits of pindo palm corresponded to 82.85 and 44.68 times the values found araticu- do-mato and mandacaru-de-três-quinas, respectively (Pereira *et al.,* 2013).

The carotenoids are important compounds because are related to several benefits to health, the major benefits is associated with lutein, This evidence of a reduced risk of

developing macular degeneration in old age, protective effects against atherosclerosis, cataracts, cancer, damage of UV and other diseases. The result of butia showed a much greater amount of lutein compared to the other two fruits, b-carotene is considered the carotenoid with the highest vitamin potential, with an activity of 100%, this had a higher value when compared with other two fruits in addition, b-carotene is the most efficient in scavenging free radicals after lycopene, acting in the prevention of chronic degenerative diseases, like heart disease and cancer (Pereira *et al.,* 2013).

Patents

By means of this review, it was possible to obtain a scientific and technological overview of the field of uses the *Butia sp*. With regard to the technological protection of *Butia sp*. only one patent using the *Butia sp*. in therapeutic uses, were found. This patent with therapeutic use of butia was a drink used for the treatment in hyperplasia and prostate cancer. The produced is adjunct in treatment and prevention of prostate inflammation, in the process benign than evil process. The product is formulated with alcohol, *Butia eriosphata* and Milome liana, which is used 600ml of alcohol, 120g of Butia seeds and 172 gr of Milome, mix the alcohol and butia seeds for 1 year, after addict the milome for period about 90 days. This drink is administered 30ml before meals. This drink reduced the side effects of commonly therapies.

CONCLUSION

Actually have few studies about therapeutic effect of butia, however it is believed that butia has effect in many diseases. *Butia sp.* is the plant exhibit high therapeutic uses, studies showed anti-inflammatory, antioxidant, antimicrobial and antineoplasic activities. According studies, all parts of plant have effect therapeutic both pulp, seeds and leaves, however more studies in this area are necessary. Is important too, identify this phytochemical compounds to future discover the compounds responsible of therapeutic effect.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist

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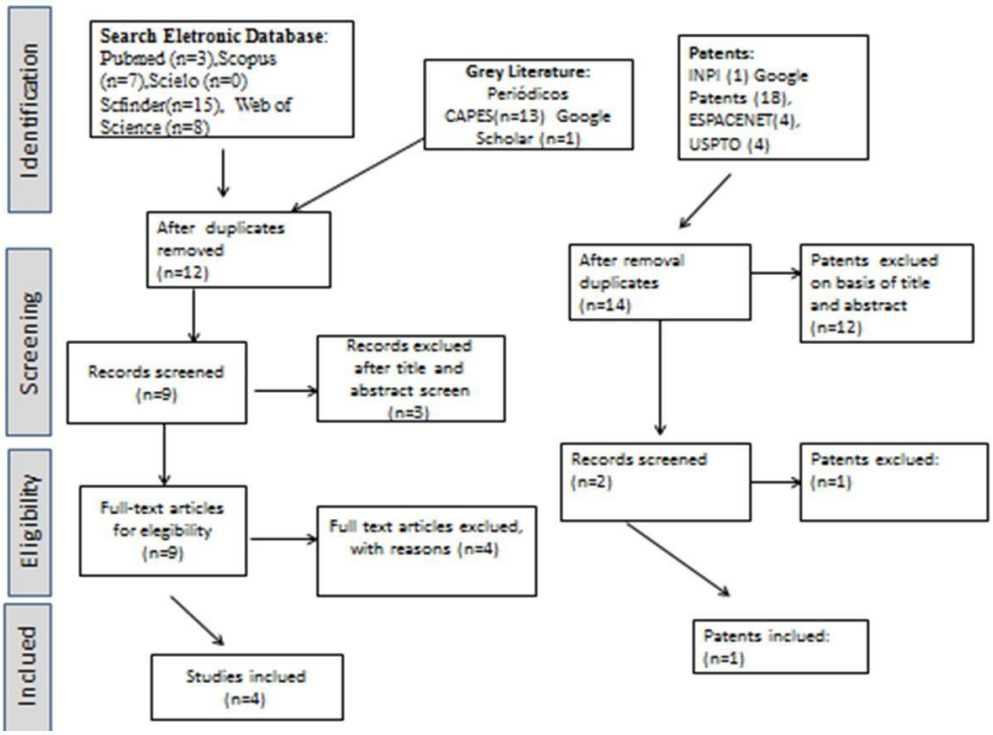

Fig. 1- Flow diagram of the study

Tables

Table 1- Electronic database and search strategy

Pubmed #1 "antimicrobial"[MESH] OR "antimicrobial" OR "anti-infective agents" OR "anti-infective agents" OR "Antibacterial"[MESH] OR "Antibacterial" OR "Agents, Anti-Bacterial" OR "Anti Bacterial Agents" OR "Antibacterial Agents" OR "Agents, Antibacterial" OR "Antibiotics" OR "Bacteriocidal Agents" OR "Agents, Bacteriocidal" OR "Bacteriocides" OR "Anti-Mycobacterial Agents" OR "Agents, Anti-Mycobacterial" OR "Anti Mycobacterial Agents" OR "Antimycobacterial Agents" OR "Agents, Antimycobacteria" OR "Biofilm"[MESH] OR "Biofilm" OR "Bacterial Adhesion" OR "Dental Deposits" OR "Prosthesis-Related Infections" OR "Adhesins,	#2 "Anti-inflammatory" [MESH] OR "Anti-inflammatory" OR "Anti Inflammatory Agents" OR "Agents, Antiinflammatory" OR "Antiinflammatories" OR "Antiinflammatory Agents" OR "Agents, Anti-Inflammatory" OR "Agents, Anti Inflammatory" OR "Anti-Inflammatories" OR "Anti Inflammatories"	#3 "antioxidant"[MESH] OR "antioxidant" OR ''Antioxidant Effect" OR "Effect, Antioxidant" OR "Anti-Oxidant Effects" OR "Anti Oxidant Effects" OR "Effects, Anti-Oxidant" OR "Antioxidant Effects" OR "Effects, Antioxidant" OR "Anti-Oxidant Effect" OR "Anti Oxidant Effect" OR "Effect, Anti-Oxidant"	#4 "Phytochemicals"[MESH] OR "Phytochemicals" OR "Phytonutrients" OR "Plant-Derived Compounds" OR "Compounds, Plant-Derived" OR "Plant Derived Compounds" OR "Dietary Phytochemicals" OR "Phytochemicals, Dietary" OR "Plant-Derived Chemicals" OR "Chemicals, Plant-Derived" OR "Plant Derived Chemicals"	#5 "antineoplastic"[MESH] OR "antineoplastic" "Agents, Antineoplastic" OR "Antineoplastic Drugs" OR "Drugs, Antineoplastic" OR "Antineoplastics" OR "Chemotherapeutic Anticancer Drug" OR "Drug, Chemotherapeutic Anticancer" OR "Antitumor Drugs" OR "Drugs, Antitumor" OR "Cancer Chemotherapy Agents" OR "Agents, Cancer Chemotherapy" OR "Chemotherapy Agents, Cancer" OR "Cancer Chemotherapy Drugs" OR "Chemotherapy Drugs, Cancer" OR "Drugs, Cancer Chemotherapy" OR "Chemotherapeutic Anticancer Agents" OR "Agents, Chemotherapeutic Anticancer" OR "Anticancer Agents" OR "Antitumor Agents" OR "Agents, Antitumor"	#6 "Butia"[MESH] OR "Butia"

Bacterial" OR "Biofouling" #1 AND #6 #2 AND #6 # 3 AND #6 # 4 AND #6 #5 AND 6					
Scopus #1 (TITLE-ABS-KEY) "(Phytochemical)" #1 OR #2 OR #3 OR #4 OR #5 AND #6	#2 (TITLE-ABS-KEY) "(Antioxidant)"	#3 (TITLE-ABS-KEY) "(Anti-inflammatory)"	4# (TITLE-ABS-KEY) "(Antimicrobial)" OR "(Biofilm)" OR "(Antifungical)" OR "(Antiparasitic)" OR "(Antibacterial)"	5# (TITLE-ABS-KEY) "(Antineoplasic)"	#6 (TITLE-ABS-KEY) "(Butia)"
Scielo "(Phytochemical)" #1 OR #2 OR #3 OR #4 OR #5 AND #6	"(Antioxidant)"	"(Anti-inflammatory)"	"(Antimicrobial)" OR "(Biofilm)" OR "(Antifungical)" OR "(Antiparasitic)" OR "(Antibacterial)"	"(Antineoplasic)"	"(Butia)"
Web of Science Topic: "(Phytochemical)" #1 OR #2 OR #3 OR #4 OR #5 AND #6	Topic: "(Antioxidant)"	Topic:"(Anti-inflammatory)"	Topic: "(Antimicrobial)" OR "(Biofilm)" OR "(Antifungal)" OR "(Antiparasitic)" OR "(Antibacterial)"	"(Antineoplasic)"	Topic: "(Butia)"
Scifinder	Butia have effect antimicrobial or antifungical or biofilm or antiparasitic	Phytochemical of butia	Effect antioxidant or anti-inflammatorty in butia		
Google Scholar:	phytochemical OR antioxidant OR anti-inflammatory OR antimicrobial OR biofilm OR antifungal OR antiparasitic OR antineoplasic OR antibacterial AND butia.				

Table 2 - Characteristics of studies included

Study	Part of Plant	Title	Study of type	Test of type	Methods	Results
Ammar *et al.*,2014	Leaves diferents extracts (petroleum ether, ether, methanol and 50% methanol aqueous)	Phytochemical and biological studies of Butia capitate Becc. Leaves cultivated in Egypt.	*in vitro/ in vivo*	Antioxidant activity, Antiinflammatory activity and Phytochemic compounds	β-carotene bleaching method , hind paw edema method, Gas Chromatograph, Thin layer cromatography (TLC), column cromatography and paper cromatography (PC)	Methanol extract possess the highest antioxidant activity and test showed that both polar and non polar extracts of the leaves of B. capitata possess a significant anti-inflammatory activity all over the tested times
Denardin *et al.*, 2015	Fruits extracts with 95% ethanol	Antioxidant capacity and bioactive compouns of four Brazilian native fruits.	*in vitro*	Antioxidante activity and Phytochemic compounds	Ferric-Reducing antioxidant power (FRAP), DPPH assay, Total reactive antioxidant potential (TRAP), Total antioxidante reactivity (TAR) and HPLC	Was observed that butia has antioxidant activity lowest when compared the other fruits tested.
Peralta *et al.*, 2013	Nuts oil	Self-eatching dental adhesive containing a natural essential oil: anti-biofouling performance and mechanical properties.	*in vitro*	Antimicrobial activity and Phytochemic compounds	Biofilm microcosmo and Gas Chromatography	The self-eatching adhesive oil was antimicrobial activity similar to that of an a commercial adesive.
Pereira *et al.*,	Fruits extracts with	Caracterization	*in vitro*	Antioxidant activity and	DPPH assay, ABTS method	Butia capita showed

2013	methanol and acetone	bioactive compounds and antioxidant potential of tree Brazilian fruits.		Phytochemic compounds	and high performance liquid chromatography (HPLC)	ntioxidant activity from ATBS method who compared to the other fruits tested, however han`t the better resulted of phenolic compounds

Table 3: Patents included

Ref. patent	Deposit year	Country	Year	Title	Inventor	Claimed
PI 0305287-7 A.	24/12/2003	Brasil	2005	Bebida composta utilizada na prevenção e tratamento de hiperplasia e câncer de próstata	Claudio Cadamuro, Joel Aparecido Cadamuro.	Drink use. Treatment of hyperplasia and prostate cancer. Adjunct treatment and prevention of prostate inflammation.

Table 4 – Phytochemical Compounds			
	Chemical Constituents	**Structure**	**References**
Nuts oil (Peralta *et al.*, 2013) and fruits (Lopes *et al.*, 2012)	Fatty acid	Caprylic	Peralta *et al.*, 2013
		Capric	Peralta *et al.*, 2013
		Lauric	Peralta *et al.*, 2013
		Mystiric	Peralta *et al.*, 2013
		Palmitic	Peralta *et al.*, 2013
		Stearic	Peralta *et al.*, 2013
		Oleic	Peralta *et al.*, 2013
		Linoleic	Peralta *et al.*, 2013
		Palmitoleic	Peralta *et al.*, 2013
		Linolenic	Peralta *et al.*, 2013
		Arachidic	Peralta *et al.*, 2013
		Beenic	Peralta *et al.*, 2013
Fruits	Carotenoids	Lutein	Pereira *et al.*, 2013
		Zeaxanthin	Pereira *et al.*, 2013
		5,6-Epoxy-β-carotene	Pereira *et al.*, 2013
		Cryptoxanthin	Pereira *et al.*, 2013
		13-ds-β-carotene	Pereira *et al.*, 2013
		β-carotene	Pereira *et al.*, 2013
		9-ds-β-carotene	Pereira *et al.*, 2013
Leaves	Aldehydes	Decanal	Ammar *et al.*, 2014
		Hexanal	Ammar *et al.*, 2014
		4-decanal	Ammar *et al.*, 2014
	Ketones	cyclohexanone 3-metyl	Ammar *et al.*, 2014
	Alcohols	Pyllocladanol	Ammar *et al.*, 2014
		Decanol	Ammar *et al.*, 2014
		Undecanol	Ammar *et al.*, 2014
	Monoterpenes	isomethone 2- ethyl	Ammar *et al.*, 2014
	Sesquiterpenes	Longipinanol	Ammar *et al.*, 2014
		Cidrol	Ammar *et al.*, 2014
		Liguloxine	Ammar *et al.*, 2014
	monoterpenes esters	α- terpinyl acetate	Ammar *et al.*, 2014
		geranyl tigalate	Ammar *et al.*, 2014
		allyl decanoate	Ammar *et al.*, 2014
		Linalool	Ammar *et al.*, 2014
		Butyrate	Ammar *et al.*, 2014

Capitulo III

Antimicrobial and antioxidant activities of ketone and ethanol extracts and oil from *Butia sp.*

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ABSTRACT

This study evaluated the phytochemical compounds, antioxidant and antimicrobial activity of the nut oil and five different extracts from the leaves of *Butia sp*. Furthermore, the antimicrobial activity of butia oil and antimicrobial activity of the extracts with preservative sodium metabisulfite were evalued too with the purpose of verifying synergism between them. Antioxidant activities of extracts were evaluated using the DPPH method, the antimicrobial activity was evaluated against strains of *Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, Candida albicans, C. parapsilosis* e *C. famata*. The minimum inhibitory concentration was determined using the broth microdilution technique using the 96-well microplate method. The main phytochemical compounds of *Butia* extracts were: tannins, steroids, flavonoids and phenolic compounds. The results of this investigation indicated that *Butia sp*. extract from leaves have high antioxidant activity and antifungal activity, specially the extract obtained using ketone solvent. The combination of sodium metabisulfite and butia leaves extracts didn´t showed better efficacy when compared with extracts with no-metabissulfite. The nuts oil of *Butia* didn't reveal antimicrobial activity. The extracts with ketone demonstrated good antimicrobial and antioxidant activities.

Key Words: antibacterial activity; antifungal activity; *Butia;* DPPH method; *in vitro* study

INTRODUCTION

For thousands of years, herbal products have been used in folk medicine for the purpose of treating various diseases.[1,2] In the modern medicine, plants have been replaced by synthetic drugs, but in the last years herbal products have been investigated due to their effectiveness, safety and quality [1]Many of these products come from tropical and subtropical countries such as Brazil.[3]

One of the great problems of the last decades is the increase of bacterial resistance, due to the increase of the incorrectly and indiscriminate use of antibiotics,

many plants have been investigated by the antimicrobial potential.[1] The antioxidant activity has also been investigated in many herbal products for the treatment of various diseases[4], The antioxidants are able to reduce reactive oxygen species (ROS) that can damage the body. The formation of free radicals is associated with cellular metabolism, the action of free radicals causes electrolyte imbalance resulting in cellular and tissue damage, causing diseases like diseases such as aging, Alzheimer's disease, diabetes mellitus, atherosclerosis, hypertension.[3,5]

Medicinal plants are potentially useful as antimicrobial agents.[6,7,8,9] The use of plants as antimicrobial agents present low risk of developing microbial resistance because plants are mixtures of several compounds that, apparently, presents different antimicrobial activity hindering the adaptation of the microorganism.[10]

Among several oils that may be useful as antimicrobial agents, the *Butia capitata*-seed's oil may have a considerable potential in industrial applications because it is rich in medium-chain fatty acids (capric, lauric and myristic acids) and also long-chain fatty acids (arachidonic, palmitoleic, γ-linoleic, oleic and linoleic acids) with significant antimicrobial activity against oral bacteria[11] and antioxidant activity .[12]

The *Butia sp*. is a native tree of South America, this have approximately 18 different species predominant in the areas of southern Brazil, eastern Paraguay, northeastern Argentina, and northwest and southeast Uruguay.[13,14] 'Butiá' or 'coquinho-azedo' is the name of the aromatic fruit of the *Butia* palm (family Arecaceae), native from Brazil and Uruguay. This fruit is consumed *in natura* and it is widely cultivated for use in beverages, juices and ice-creams. This fruits are known as butia or coconut, and these are popularly used in the preparation of jellies, juices, sorbets, liqueurs, as well as its leaf is used in handicrafts (Denartin et al., 2015).[15,16,17] Furthermore, the collection of the fruit plays important role in several low-income communities in Brazil. The seed is discarded in 'butiá' [14,18,19] and in this study the oil was recovered from the seed. The agricultural wastes are important feedstock because represent renewable sources and low cost materials .[20,21]

Moreover, the leaves of Butia present a wax on their surface. In this study, different techniques of extraction from Butia leaves was used to evaluate the biological activity of these leaves with the wax, without the wax or only the wax existent on these leaves. After systematic review, studies didn't found to evaluate the antimicrobial activity of the leaves of *Butia sp.*

Futhermore, metabisulfite is an antioxidant widely used in the food industry. The antimicrobial activity of butia extracts associated with sodium metabisulfite was also evaluated, in order to verify the presence of synergism between the extracts and the synthetic antioxidant.

Hence, the objective of this paper as to evaluate the antioxidant and antimicrobial activity by different extracts from the leaves and nuts of *Butia sp*. as well as to analyze the main phytochemical constituents from these extracts.

MATERIALS AND METHODS

Plant Materials

Leaves and fruits of *Butia sp*. weasqzdused for the extractions were obtained from cultivars of Embrapa Clima Temperado, Estação Experimental Cascata, Pelotas, Rio Grande do Sul, Brazil, location 31° 31'S 052°31'W, in May 2016.

Oil Extraction

The fruits used to obtain the oil were immediately demolished manually after collection, the nuts were oven dried for approximately 14 days, after the nuts were broken and separated manually and frozen immediately for later use.

The oil extraction was performed by the Bligh & Dryer[22] method with modifications associated with sonication, since it is a cold method with high yield. The nuts were removed from the freezer 24 hours before its extraction. These nuts were crushed in IKA mill model A11BS32. The nuts were triturated with a 1: 2 chloroform and methanol mixture respectively, and vortexed. After 6 mL of chloroform was added and stirred for 30 seconds, after stirring 6 mL of distilled water was added and vortexed. The mixture was filtered with Wathaman 1 filter paper and centrifuged for 5 minutes at 1000 rpm. After the centrifugation two higher phases were formed (methanol and debris) and the lower chloroform and oil the supernatant was removed. The oil was placed on SU-3HTE ultrasonic cleaner sonicator for 30 minutes and the oil was in a laminar flow hood until complete evaporation of chloroform.

Leaves Extraction

After harvesting the leaves were immediately submitted to extraction technique by five different methods using 75% ethanol and ketone as solvents. Method I consisted

in crushing 75% ethanol, the extract was allowed to stand for four days. Subsequently, the extract was filtered and concentrated in rotary evaporator. The method II constituted to crush the leaves of *Butia sp.* with ketone. The mixture was protected from light and left to stand for four days. After, it was and evaporated in a laminar flow hood until total evaporation of the solvent. Method III constituted leaving the leaves chopped with ketone at rest for four days. Subsequently it was placed in a laminar flow hood until the solvent was completely removed. Method IV and V constituted in removing the wax that is on the surface of the sheet with acetone and cotton and rubbing on the limb until its total elimination, where in method IV, the leaves were triturated with ketone, after the mixture was stored for four days and then left in a laminar flow hood for solvent evaporation for 4 days. Method V consisted in grinding the sheets without the ketone wax with ethanol 75% this misture was left to stand for four days and subsequently filtered and concentrated in a rotary evaporator. All extracts were frozen in ultrafreezer during seven days and after lyophilized for five days too.

Phytochemical analysis

The phytochemical screening of extracts were determined based on the methodology of NDAM *et al*.,(2014)[23] with modifications. Some chemical compounds were qualitatively determined: flavonoids, tannins, phenolic compounds, saponins, steroids, tannins, cardiotonic glycosides, alkaloids and strong fixed acids.

Antioxidant Assay

The antioxidant activities of the ethanol and ketone extracts were determined by measuring their effect against DPPH method. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is captured based on the methodology of Brand-Williams et al., (1995),[24] with modifications, where 0,01 g of lyophilized extract were added to this 1 mL of methanol PA and 700 µl of water, after being vortexed. The samples were diluted 10 times where 20 µl of this dilution was used for the reaction. Added 180 µl of methanol plus 3.8 mL of DPPH solution. The solution was rested for 24 hours. After this period, the samples were read in a spectrophotometer at 515 nm and the antioxidant activity was calculated. The tests were performed in triplicate. The results were expressed as mg trolox / g.

Antimicrobial Assay

The reference strains used in this study were chosen based on their pathological effects on dentistry. The strains were de *Staphylococcus aureus* (ATCC 19095), *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 4083), *Candida albicans* (ATCC 62342), *Candida parapsilosis e Candida famata.* The microorganism used in this study consisted from the American Type Culture Collection (ATCC) and of clinical isolates kept in the collection of the Research Laboratory of Microbiology of Odontology in Universidade Federal de Pelotas (UFPel).

*Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MB*C)

The minimum inhibitory concentration (MIC) were determined in triplicate by using the broth microdilution technique using a modified version of the reference documents M27-A3 (CLSI, 2008) and M7-A7 (CLSI, 2006). The susceptibility test was performed on 96-well microplates, 0.1 g of lyophilized extracts were diluted in 1 ml of ethanol PA and oil were dissolved in ethanol at 0, 5 g/mL. This diluted into the 96-well microtiter plates in culture medium (RPMI for fungi and Mueller-Hilton for bacteria) in Concentration of 2500µl - 4.88µl. Then, aerobic and microaerophilic bacteria were incubated for 24 h, and fungal for 48 h. *S. mutans* was incubated with microanaerobac for 24 h too.

Before addition of resazurin and in order to determine MBC, an aliquot of the inoculum was aseptically removed from each well and plated onto BHI agar supplemented for bacterial and Sabouroud for fungal. The plates were incubated as described previously. MBC was defined as the lowest concentration of the sample where no bacterial growth occurred.

RESULTS AND DISCUSSION

Yield of extracts

After lyophilization, the yields of the extracts were distinct (Table 1), especially the extracts extracted with ethanol. The III method, presented lower yield compared to the other extracts.

Phytochemical Compounds

In the present study, the phytochemical screening, antioxidant and antibacterial activities were performed with ethanol and ketone extracts of the leaf of *Butia sp.*. The study was made against tree bacteria and tree fungal bacteria using the MIC method. The leaves of *Butia sp.* were rich in flavonoids, steroids, phenols compound and tannins. These phytochemicals confer antioxidant and antimicrobial activity on the leaf extracts (Table 2). The various phytochemical classes detected are known to have high biological activity. For instance, flavonoids have been antibacterial, antiviral, anti-inflammatory and anti-allergic effects, reducing low-density lipoprotein (LDL), inhibiting platelet aggregation, eliminating free radicals, and preventing cell proliferation[2] flavonoids have been referred to as nature's biological response modifiers.[25]

Plant steroids are known inhibit inflammation, for cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. But the steroids were found only in extracts with ketone solvents (method II, III and IV).[25]

The tannins were found in all extracts but especially in extracts I and V. The ethanol is more efficient in recovery of tannins. According to literature, tannins have antioxidant, antimicrobial and antiviral activity anti-tumor activities, and some tannins were able to inhibit HIV replication and diuretic activity.[23,25] Alkaloids have been reported to exert analgesic, antispasmodic and antibacterial activity.[23]

Phenolic compounds were found in all extracts at high concentrations. The phenolic compounds were the compounds found in more concentrations, these are the most common compounds in aleopathic substances.[23]It is secondary metabolites that have several biological properties and are one of them antioxidant activity [26], its antioxidant activity is mainly due to its redox properties, which allow them to act as reducing agents, donors Hydrogen peroxide, free radical scavengers, singlet oxygen suppressors and metal chelators. In the literature were reports of correlation of phenolic compounds and antioxidant activity.[26]

The low table shows that the solvents used interfere with the results and concentrations of phytochemical compounds. As they indicate that the bioactive compounds studied have a wide biological activity.

Antioxidant activity

The results of the antioxidant activity were shown using the DPPH method, there were difference between extracts that used solvent ketone, these showed higher antioxidant activity when compared with extracts that used solvent ethanol. Method II was the extract that presented the highest antioxidant activity, approximately 3 times more antioxidant activity than the V method, which presented the lowest activity when compared all the extracts used. The wax interfered with antioxidant activity. Table 3 showed the values of the antioxidant activity found in the different leaf extracts of *Butia sp*.

Some authors have been found a correlation between the content of phenolic compounds and antioxidant activity, while others haven't relation.[27]

The use of antioxidant substances with the purpose of capturing free radicals appears as an alternative in the therapy of several diseases, searching for new compounds capable of inhibiting oxidative processes in vivo without the presence of risks to human health .[3]

Antimicrobial activity

According to the evaluation of the antimicrobial activity of the compounds tested the extracts which used ketone as solvent presented better antimicrobial activities. No extract tested had activity against *S. aureus* strain, only extracts II, III and IV obtained activity against *E. faecalis*, but in high concentrations. All extracts tested had good antifungal activity. The results of the minimum inhibitory concentration are shown in Table 4.

The activity antimicrobial of butia extract associates with sodium metabissulfite didn't demonstrated synergism between them, the results of this association were similar to extracts without metabisulfite. The sodium metabisulfite alone didn't antimicrobial activity. These results showed the butia extracts have better antimicrobial activity compared to preservative and have high activity antioxidant.

Butia oil didn't present antimicrobial activity, being lower Butia sp. leaves, which disagrees with the findings of some authors like Peralta *et al.*, 2013,[9] who identified antimicrobial activity against oral bacteria such as *S. mutans* and lactobacillos. According to Peralta *et al.*, 2013,[9] the antimicrobial effect of butia oil can

be explained by the presence of fatty acids such as lauric acid, oleic acid, linoleic acid and palmitoleic acid. Lauric acid is one of the most active saturated fatty acids present in B. capitata oil and palmitoleic acid is the main unsaturated fatty acid.

According to some authors, this difference in results can be explained by the amount and composition of bioactive compounds present in the plants, which are influenced by the genotype, extraction procedure, climatic and geographic conditions, and stage of maturation of the plants, which May be a justification for the low activity of *butia sp.* Compared with other studies.[2,23]

CONCLUSIONS

The extracts tested showed high antioxidant activity, being highlighted the extracts which used ketone as solvent. The extracts tested also presented high antifungal activity, but low antibacterial activity. However, further studies are needed in this area to confirm the results, especially antimicrobial activity against other microorganisms. Although extracts extracted in ethanol obtained higher yields they obtained worse results, when compared with extracts extracted in ketone.

From the present study that ketone extract of leaves of *Butia sp*. is a good natural source of antioxidants and a possible food supplement or food preservative as well as an antifungal agent in the pharmaceutical industry.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist

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Table 1 -Yield and prepare of different extracts of *butia sp.*

Extract	Parts of plant	solvent	Fraction	Wax	Time	Yeld
Method I	Leaves	Ethanol 75%	Crush	Yes	4 days	6.61%
Method II	Leaves	Ketone	Crush	Yes	4 days	2.87%
Method III	Leaves	Ketone	Chopp	Yes	4 days	1.51%
Method IV	Leaves	Ketone	Crush	No	4 days	2.72%
Method V	Leaves	Ethanol 75%	Crush	No	4 days	6.52 %
Butia oil	Nuts	Chloroform and methanol	Crush	-	1 day	25%

**Yield (%) = Weight of extract (g) / gr of powdered plant sample before extraction * 100*

Table 2-Phytochemical screening of leaves extracts of *Butia sp*

	Saponins	Flavonoids	Steroids	Tannins	Cardiotonic Glycosides	Phenolic Compounds	Alkaloids	Strong fixed acids
Method I	_	+	_	+++	_	+++	-	_
Method II	_	+	+	++	_	+++	-	_
Method III	_	+	+	+	_	+++	-	_
Method IV	_	+	+	++	_	+++	-	_
Method V	_	+	-	+++	_	+++	-	_

Legend: + Low concentration, ++ Moderate concentration, +++ High concentration, - Absent

Table 3- Antioxidant activity of different methods of extraction Butia *sp*. leaves

Leaves Extract of *Butia sp.*	**Mean (mg trolox equivalent/g tissue)**	**Standard deviation**
Method I	356.06	12.31
Method II	948.31	3.7
Method III	888.66	46.40
Method IV	671.36	23.67
Method V	269.42	5.68

Table 4- Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) compared to different microorganisms, using 0,1g of liofilized extracts and 0,01g of sodium metabisulfite.

Butia	***S.mutans***	***S.aureus***	***E.faecalis***	***Candida albicans***	***Candida parapsilosis***	***Candida famata***
			MIC/MBC			
Method I	1250/ 2500	-/-	-/-	39,06 /-	156,25 /156,25	4,88/4,88
Method II	1250/ 2500	-/-	625 /1250	625/ -	4,88 /4,88	4,88/4,88
Method III	312/-	-/-	625 /1250	312,5/-	4,88/2500	19,53 /-
Method IV	1250/ 2500	-/-	625/ 625	4,88/625	78,125/ 156,25	4,88/ 4,88
Method V	2500/-	-/-	-/-	19,53/-	625/-	4,88/ 4,88
Method I + Sodium Metabisulfite	2500/ 2500	-/-	2500/-	39,06 µ /-	4,88 /78,125	4,88/ 4,88
Method II + Sodium Metabisulfite	1250/ 2500	-/-	625/ 625	4,88 /-	4,88 /156,25	4,88/ 4,88
Method III+ Sodium Metabisulfite	2500/-	-/-	312,5/1250	78,125/-	4,88/ 625	19,53/-
Method IV + Sodium Metabisulfite	2500/-	-/-	312,5/ 312,5	4,88/312	4,88/312	4,88/4,88
Method V + Sodium Metabisulfite	2500/-	-/-	2500/-	155/-	4,88 /78,125	4,88/4,88
Sodium Metabisulfite	-/-	-/-	-/-	-/-	-/-	-/-
Butia oil	-/-	-/-	-/-	-/-	4,88/4,88	4,88 /4,88

Legend: concentration expressed in µg/mL, (-) didn't have effect in concentrations used

Capitulo IV

Antimicrobial, antioxidant activity and phytochemical screening of aqueous and ethanol extracts from *Arctium lappa*

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ABSTRACT

Context: *Arctium lappa* is a plant from the Asteraceae family with Japanese origin, and was introduced in Brazil, where it is widely used in folk medicine. Burdock as it is also called, is used for more than 3000 years ago, because it present therapeutically effects.

Objective: The objective of this paper was to evaluate the antioxidant and antimicrobial activities of aqueous and ethanol extracts obtained with and without the sonication of the leaves and roots of *Arctium lappa*. Moreover, it was analyzed the main phytochemical constituents from these extracts.

Materials and methods: Phytochemicals were profiled using Ndam et al. (2014) method. Antioxidant activities of extracts were evaluated using the DPPH method, and the antimicrobial activity was evaluated against strains of *Staphylococcus aureus* (ATCC 19095), *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 4083), *Candida albicans (*ATCC 62342)*, C. parapsilosis* e *C. famata.* The minimum inhibitory concentration was determined by the broth microdilution techique using the 96-well microplate method.

Results: A preliminary phytochemical screening of the different crude extracts showed the presence of secondary metabolites such as saponins, alkaloids, taninns. phenolic compounds and strong fixed acids were detected. Flavonoids, steroids, cardiotonic glycosides were not detected. The tested demonstrated high antioxidant activity, they extracts did not reveal antibacterial activity, but they has have antifungal activity.

Discussion and conclusion: These results showed that the *Arctium lappa* leaves and roots extracts are the important source of the antioxidant and antifungal agent. However, further research is necessary to isolate and characterize different phytoconstituents for pharmaceutical drug lead molecules and also to verify their traditional uses.

Keywords: *Arctium lappa;* burdock; antibacterial; antioxidant; antifungal; extracts; Phytochemical screening.

Introduction

Natural products have been known by their important role in the discovery of drugs, due to their medicinal properties, as they present efficacy, low toxicity and economic viability. There are plenty of medicinal plants around the world, however a small number are used and investigated for their biological and pharmacological properties (Groppo et al. 2008; Radulovi et al. 2013; Saleh-e-In et al. 2016).

Antimicrobial agents isolated from plants represent an unexplored and source of compounds with therapeutic potential. Some of these compounds are effective in treating infectious diseases and have as their advantage few side effects, often caused by the use of synthetic antimicrobials. Antioxidants derived from plant are being used for the purpose of treating various diseases, in addition to being used in foods, cosmetics and in the pharmaceutical industry (Bouterfas et al. 2016; Chan et al. 2011; Herrera et al. 2014).

Arctium lappa is a plant of the Asteraceae family, easily cultivable herb and with Japanese origin that was introduced in Brazil where it is widely used in folk medicine (Predes et al. 2011; Chan et al. 2011). Burdock as it is also called, is a popular plant in China, Japan and other Asian countries, where it's used for more than 3000 years ago. In Chinese culture is called "Niubang" where it is used in food, for being considered healthy and nutritious (Chan et al. 2011).

It has a wide therapeutic action and it is traditionally used to treatment of diseases such as hypertension, gout, hepatitis, inflammatory diseases, sore throat, infections such as rashes, boils and various skin problems. It is diuretic, antioxidant, anxiolytic, antiplatelet agent, anti-HIV, anti-diabetic, antimicrobial, antiviral activity, antifungal activity and anti-HIV activity. *A. lappa* were described for leukemic cells as well as antitumor effects of arctigen in on pancreatic cancer cell lines (Pereira et al. 2005; Chan et al. 2011; Predes et al. 2011).

Burdock acts on inflammatory diseases such as rheumatoid arthritis, autoimmune disease, chronic inflammation and atherosclerosis, its action is attributed to its high free radical scavenging capabilities and antioxidant activity (Predes et al. 2011). The roots, seeds and leaves have been investigated for their therapeutic potential (Chan et al. 2011). The roots are the most used part

of this plant, they are popularly known as detoxifying, and their extracts protects cells from toxic substances and reduce cell mutations , have hepato protective, anti-inflammatory, antiproliferative activity, besides acting in the elimination of free radicals (Chan et al. 2011; Predes et al. 2011).

Hence, the objective of this paper as to evaluate the antioxidant and antimicrobial activity by aqueous and ethanol extracts, with and without sonication, from the leaves and roots of *Arctium lappa* as well as to analyze the main phytochemical constituents from these extracts.

Materials and methods

Plant Material and Extraction

The leaves and roots of *Arcitum lappa* were obtained from Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil. The plants were grown on greenhouse at room temperature. Leaves and roots of this plant were collected and identified in March 04th, 2016 The plants were collected at 9:00 a.m. After the collection, the plants were submitted to the extraction process.

Seven difference extracts from the leaves and roots were obtained according to the method emploted: Extract 1- ethanol 50% extract from the leaves without sonicator; Extract 2- ethanol 50% extract from the leaves, with sonicator; Extract 3- aqueous extracts from the leaves, without sonicator; Extract 4- aqueous extracts from the leaves, with sonicator; Extract 5- Ethanol 50% extract of roots without sonicator; Extract 6- ethanol 50% extract of roots with sonicator; Extract 7- decoction of roots.

Hydroalcoholic extracts from leaves were obtained with maceration when 50 g of leaves ground in a mixer with 300 mL of ethanol 50%, this mixture was allowed to stand at room temperature protected from light for 3 days. After that, the extracts were filtered through whatman 1 filter paper and concentrate in a rotary evaporator at 25°C. The extracts were separate into two parts (Extract 1 and Extract 2), one of these parts (Extract 2) being placed on the sonicator ultrasonic cleaner SU-3HTE for 30 minutes.

Aqueous extract of leaves were obtained with maceration, 50 g of leaves were ground in a mixer with 300 mL of water, this mixture was allowed to stand at room temperature and protected from light for 3 days.After this period it was

filtered, and separate in two parts (Extract 3 and Extract 4), the extract 4 was pass one on the ultrasonic cleaner SU-3HTE sonicator for 30 minutes at room temperature.

Hydroalcoholic roots extract were obtained with maceration, 50 g of roots previously washed for the purpose of removing soil cracks from it. Roots were ground in 300 mL of ethanol 50%, this mixture was allowed to stand at room temperature protected from light for 3 days. After this period, it was filtred and concentrate in a rotary evaporator at 25°C. After was separate into two parts (Extract 5 and Extract 6) one of these parts (extract 6) being placed on the sonicator ultrasonic cleaner SU-3HTE for 30 minutes.

Watery Roots Extract was performed by a decoction method, according to the Brazilian pharmacopoeia, where 25 grams of the roots were used and placed in a flask with 150 mL of water and heated contiguously for 30 minutes at constant temperature (Farmacopeia Brasileira, 2011). All the extracts were take to ultrafreezer for a week and after they were lyophilized.

Phytochemical screening

The phytochemical screening of extracts were determined based on the methodology of Ndam et al. (2014) with modifications. Some chemical compounds were qualitatively determined: flavonoids, tannins, phenolic compounds, saponins, steroids, tannins, cardiotonic glycosides, alkaloids and strong fixed acids.

Antioxidant Assay

The antioxidant activities of the ethanol and hydroalcoholic extracts were determined by measuring their effect against DPPH method. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is captured based on the methodology of Brand-Williams et al. (1995), with modifications, where 0,01 g of lyophilized extract were added to this 1 mL of methanol PA and 700 µl of water, after being vortexed. The samples were diluted 10 times where 20 µl of this dilution was used for the reaction. Added 180 µl of methanol plus 3.8 mL of DPPH solution. The solution was rested for 24 hours. After this period, the samples were read in a UV-1600 PC spectrophotometer Shimatdzu at 515 nm and the antioxidant

activity was calculated. The tests were performed in triplicate. The results were expressed as mg trolox / g.

Antimicrobial Assay

The strains used in this study were: *Staphylococcus aureus* (ATCC 19095), *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 4083), *Candida albicans* (ATCC 62342), *C. parapsilosis e C. famata.* The microorganism used in this study consisted from the American Type Culture Collection and of clinical isolates kept in the collection of the Research Laboratory of Microbiology of Odontology in University Federal of Pelotas (UFPel).

*Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MB*C)

The minimum inhibitory concentration (MIC) were determined in triplicate by the broth microdilution technique (MIC) using a modified version of the reference documents M27-A3 (CLSI, 2008) and M7-A7 (CLSI, 2006). The susceptibility test was performed on 96-well microplates.0.1 g of lyophilized extracts were diluted in 1 mL of sterile water in culture medium (RPMI for fungi and Mueller-Hilton for bacteria) into the 96-well microtiter plates. Concentrations ranging from 4.88µL to 2.500µL - of the extracts were tested for fungus. The positive control were 100 µL of inoculum and 100 µL of culture medium, the negative control were 100 µL of extracts and 100 µL of culture medium. The 96-well microtiter plates with the mixture reactions and the suspensions of aerobic bacteria were incubated for 24 h, *S. mutans*, a microaerophilic bacteria, was incubated for 24 h too and with fungus for 48 h.

The colorimetric assessment for the determination of MIC was performed with resazurin. The resazurin method is simple, sensitive, rapid and reliable, and could be used successfully to assess antibacterial properties of natural products (Sarker et al. 2007). Resazurin solution with 0.1% 10µL was added and after 15 min incubated the reading was done.In order to determine MBC, an aliquot before added resarzurin of each suspension of the reaction mixture was aseptically removed from each well and plated onto BHI agar supplemented for bacterial and Sabouraud agar for fungus. The plates were incubated as

described previously for the determinationof MIC. The MIC and MBC was defined as the lowest concentration of the sample where no bacterial growth occurred.

Results

After lyophilization, the yields of the extracts were distinct, especially the extracts extracted with decoction of roots and the sonication extract has had more yield than non-sonicated extracts, the method 3, presented lower yield compared to the other extracts. The yield of extractables obtained from differences extracts of *Arctium lappa* was analyzed in Table 1.

The extracts of leaves and roots of burdock were rich in tannins, strong fixed acids and phenols compound. The saponins were found just in aqueous extract of leaves and the decoction extract of roots (Table 2). The various phytochemical compounds detected are known to have high biological activity. Table 2 shows the different parts of plants used, the solvents and use of sonication interfere with the results and concentrations of phytochemical screening. This method is qualitative and categorical and identifying classes of secondary metabolites. The tannins were found in all extract but especially in decotion extract from roots.

The results of the antioxidant activity were shown in Table 3. There were difference between leaves and roots, where roots revealed higher antioxidant activity when compared to other extracts. The major antioxidant activity was detected in the root ethanol extract, followed for that obtained by the dectotion of roots and the ethanol extracts from leaves. The smaller antioxidant activity was found in aqueous extract from leaves. Sonicated extracts did not influenced antioxidant activity when compared to non-sonicated extracts. Extracts that used ethanol as solvent showed higher antioxidant activity.

According to the evaluation of the antimicrobial activity (Table 4) of the compounds tested the extracts haven't effect in S. aureus, E. faecalis and S. mutans have effect however in high concentration 2500 µg /mL. The antifungal effect were better in ethanolic extract low effect. The antifungal activity was highlighted in the ethanolic extracts of the root and leaves with sonication, with

fungistatic and fungicidal effect, proving that sonification of the extract increases its antimicrobial activity.

Discussion

The correct choice of solvent can considerably improve the extraction yield of plants. Differences in the extraction efficiency of various solvents can be attributed to their polarities. The ultrasonic method lead to cell wall disruption, decrease in particle size, and enhancement of mass transfer across cell membranes. This is a simplicity, high efficiency, and inexpensiveness are among the main factors that have made the ultrasonic method to be one of the most industrially used processes to leach out the cellular extracts. The extraction yield of the polar protic solvents with ethanol, methanol, and water could be affected by the ultrasonic assisted process.

According to literature, tannins have antioxidant, antimicrobial, antiviral activity anti-tumor activities, and some tannins were able to inhibit HIV replication and diuretic activity (Ndam et al. 2014; Gowri & Vasantha 2010), tannins play a major role as antihemorrhagic agent and have been shown to have immense significance as antihypercholesterol, hypotensive and cardiac depressant properties (Saleh-e-In et al. 2016). Tannins are the most compounds found in burdock roots, however this are potentially toxic in nature. This can cause stomach pain and in high concentrations has some dangerous side effects such as nephrotoxicity and hepatic necrosis (Chan et al. 2011).

Alkaloids have been reported to exert analgesic, antispasmodic and antibacterial activity. The antimicrobial activity is because the alkaloids have been nitrogen-containing naturally occurring compound, this have ability to intercalate with the DNA of the microorganisms (Saleh-e-In et al. 2016; Ndam et al. 2014).

Phenolic compounds are substances having at least one aromatic ring attached to one or more hydroxyl groups in their structure. They have variable structure and are responsible for several functions. There are more than 8000 described phenolic substances, being subdivided into several groups, these are the most common compounds in aleopathic substances. The phenolic compounds act as primary antioxidants of free radical scavengers (Dreosti 2000; Saleh-e-In et al. 2016; Ndam et al. 2014).The saponins were related to

have hypocholesterolemic and antidiabetic properties. According the literature saponins may be highly toxic under experimental conditions and acute poisoning is relatively rare both in animals and in the man (Saleh-e-In et al. 2016).

Some authors have found a correlation between the content of phenolic compounds and antioxidant activity, while others did not showed any relationship (Asowata-Ayodele, et al. 2016). The use of antioxidant substances with the purpose of capturing free radicals appears as an alternative in the therapy of several diseases, searching for new compounds capable of inhibiting oxidative processes *in vivo* without the presence of risks to human health (Santos et al. 2011). Natural antioxidant sources has gained attention and researchs have been put into identifying compounds as suitable antioxidants to replace synthetic ones (Kusuma et al. 2014).

According to Chan et al. (2011) the leaves of *Arctium lappa*, have antibacterial activity in microorganisms of the oral cavity, which disagrees with this study where didn't antimicrobial activity was found against strains *of S. aureus, S. mutans* and *E. faecalis*. Chan et al. (2011) report that chlorogenic acid isolated from burdock leaves has effects against *E. coli*, *Staphylococcus aureus* and *Micrococcus luteus* the polyacetylene ingredients extracted from burdock root also have potent antibacterial and antifungal activities (Chan et al. 2011).

Pereira et al. (2005) evaluated the antimicrobial activity by different fractions of the leaves of *A. lappa* by the agar diffusion method, were used of microorganisms found in endodontic infections. *Enterococcus faecalis* (ATCC 29210), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633) and *Candida albicans* (ATCC 10231). The paper showed that the hexanic phase showed a better activity against the microorganisms tested.

Arctium minnus is other burdock species, in which it is widely used in folk medicine because of its vast therapeutic effect and has shown significant effects against microorganisms. Lubian et al. (2010) evaluate the antifungal activity of the aqueous extract from leaves of *Arctium minus* of differents species and strains of Candida sp. The species were *Candida albicans, C. tropicalis, C. glabrata, C. stellatoidea, C. dubliniensis*, and *C. Kruse, C. albicans*

e de *C.tropicalis*. The concentration of 12.5 mg/mL1 had fungistatic effect on most tested strains and species. However, fungicidal effect was only observed on the species *C. krusei* with concentration at 12.5 mg/mL (Lubian et al. 2015).

Ionescu et al. (2013) evaluated the antimicrobial activity of *Arctium lappa* roots. The antimicrobial activity of these products was tested by serial dilution method against bacterial strains (*Staphylococcus aureus, Escherichia coli* and *Salmonella abony*). The hydroalcoholic extracts obtained from the studied vegetable species, have shown an antimicrobial activity against the bacterial strains of *Escherichia coli* and *Salmonella abony*, but they have not shown any antimicrobial activity against *Staphylococcus aureus*.

In the literature were reported cases of glycolic extract of burdock at 500 mg/mL have been antimicrobial action for *Staphylococcus aureus, S. epidermidis, Streptococcus mutans, Candida albicans, C. tropicalis and C. glabrata*by the minimum inhibitory concentration method and didn't present cytotoxicity in macrophages (Oliveira et al. 2006).

Phenolic compounds have been reported in leaves and roots of burdock as chlorogenic acid, caffeic acid, p-coumaric acid, rutin, cinnarine, luteolin, arctin, quercetin (Predes et al. 2011; Lou et al. 2016). Studies have shown that caffeic acid and chlorogenic acid have antioxidant activity and bacterial biofilms activity. Routine, quercitrin, luteolin, p-coumaric acid, caffeic acid, and quercetin have been reported to have antibacterial and antioxidant activity (Zaixiang et al. 2016).

Researches has been carried with the aim of discovering new antifungal agents due to the increase in fungal resistance and due to the limited number of antifungal agents on the market. The potent activity of the extracts against *C. albicans* suggests the possibility for the treatment of this disease (Kusuma et al. 2014).

Conclusion

In the conditions that this study was carrieded, it was not possible verify that the extracts of leaves and roots possessed antibacterial activity. The extracts presented antioxidant properties. Among the antifungal activities the hydroalcoholic extracts of the leaves and roots sonicates were more activity.

The results serve as a scientific basis to further develop some studies of *Arctium lappa*.

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Author disclosure statement

There is no conflicts of interests.

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Table 1. Yeld of differents extracts of leaves and roots of *Arctium lappa.*

Extract	Parts of Plant	Solvent	Sonicator	Yield
Extract I	Leaves	Ethanol 50%	No	5.59%
Extract II	Leaves	Ethanol 50%	Yes	5.84%
Extract III	Leaves	Aqueous	No	1.98%
Extract IV	Leaves	Aqueous	Yes	2.09%
Extract V	Roots	Ethanol 50%	No	3.84%
Extract VI	Roots	Ethanol 50%	Yes	8.19%
Extract VII	Roots	Aqueous	No	10.08%

*Yield (%) = Weight of extract (g) / gr of powdered plant sample before extraction * 100

Table 2. Phytochemical constituents of *Arctium lappa.*

Extracts	Saponins	Flavonoids	steroids	Tannins	Cardiotonic Glycosides	Phenolic Compounds	Alkaloids	Strong fixed acids
Extract I (ethanol extract from the leaves)	-	-	-	+	-	+	-	+
Extract II (ethanol extract from the leaves with sonicator)	-	-	-	+	-	+	-	+++
Extract III (aqueous extracts from the leaves)	+	-	-	+	-	+	+	+
Extract IV (aqueous extracts from the leaves with sonicator)	++	-	-	+	-	+	+	++
Extract V (Ethanol extract of roots)	-	-	-	++	-	+	-	++
Extract VI (Ethanol extract of roots with sonicator)	-	-	-	++	-	+	-	++
Extract VII (decoct of roots)	+	-	-	+++	-	+	-	+

Legend: += Low concentration, ++ Moderate concentration, +++= High concentration, -=Absent

Table 3. Antioxidant activity of leaves and roots by *Arctium lappa.*

Leaves and roots of *Arctium lappa*	**Mean (mg trolox equivalent/g tissue)**
Extract I	93.30 ± 16.1
Extract II	93.30 ± 3.79
Extract III	47.86 ± 8.52
Extract IV	39,33 ± 11.36
Extract V	260.90 ± 12.31
Extract VI	268.00 ± 10.41
Extract VII	174.26 ± 20.83

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) compared to different microorganisms.

Arctium lappa	***S.mutans***	***S.aureus***	***E.faecalis***	***Candida albicans***	***Candida parapsilosis***	***Candida famata***
				MIC/MBC		
Extract I	2500/ 2500	-/-	-/-	1250/-	78.125/1250	78.125/ -
Extract II	2500/ 2500	-/-	-/-	1250/ 1250	78.125 /1250	78.125/ 1250
Extract III	-/-	-/-	-/-	-/-	625/ 625	-/-
Extract IV	-/-	-/-	-/-	1250/ 1250	4.88/ 312.5	-/-
Extract V	2500/-	-/-	-/-	1250/ -	312.5/ 625	1250/ 625
Extract VI	2500/-	-/-	-/-	39,06/-	312.5/ 625	78.125/ 625
Extract VII	2500/ 2500	-/-	-/-	1250/-	2500/ 312	2500/ -

(-) No antimicrobial activity at the concentrations tested. The results were expressed in μg/mL.

Capitulo V

Antibacterial activity and physical-chemical properties of a endodontic sealer containing copaiba oil-based compounds[δ]

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Situação do artigo: a ser submetido

Abstract

Aim: The aim of this study was to evaluate the antimicrobial activity and physical-chemical properties of an endodontic sealer containing copaiba oil. **Methods:** The sealers were formulated with three oil resin concentrations (0.5%, 1% and 2%). The negative control group was a sealer without oil and a commercial Real Seal® (Sybronendo, Orange ,USA). The positive control was the bacterial inoculum. To evaluate the antimicrobial activity, the determination of the minimum inhibitory concentration (MIC) and direct contact tests were used. To evaluate the physical-chemical properties of these sealers, the dimensional stability, flow, setting time, and radiopacity tests were made according ISO 6876. The degree of conversion was measured by Fourier Transform Infrared Spectroscopy (FTIR).

Results: To the MIC, the results against *S. aureus* was 19.53 µg/ml and the other strains were 4.88 µg/ml. In the direct contact test the sealer containg 0.5% copaiba oil showed the highest antimicrobial effect in 24h against *E. faecalis,* in the test against *S. aureus* the sealers with copaiba oil in tree differents concentrations is superior than the Real Seal in 24h. The dimensional stability and the flow, were similar to commercial sealer, the setting time and conversion of degree and is higher than commercial cement but the radiopacity in the three concentration is lower than commercial sealer.

Conclusion: The sealers containing copaiba oil revealed antimicrobial activity in 24 h in all concentrations tested. In special, mainly that containing 0.5% copaiba. However, it is necessary adjustment of the radiopacity, because it was not in accordance with ISO 6876.

Keywords: copaiba oil, endodontic sealer, antimicrobial activity, antimicrobial sealer, phototherapy.

Introduction

The purpose of phytotherapy treatment is based on the use of medicinal plants, whose quality and efficacy are proved by scientific studies (Santos *et al*. 2011).

Medicinal plants are the oldest "weapons" used by humans in treatment of several diseases and prevention of them. Moreover, this costume always has been present in the human history. In this context, phytotherapy is an option in the research about therapeutic solutions, mainly in low-income populations, since it is an efficient, cheap and culturally widespread alternative medicine (Oliveira e Araújo, 2007).

Copaiba oil is obtained through the secretion of a second metabolic product to excretion and detoxification that works as a defense system against animals, fungi and bacteria action (Montes *et al.*, 2009), making no injury to the *Copaifera sp*. tree (Veiga e Pinto, 2002). This product oil is a herbal medicine very useful in northern Brazil, more specific in Amazonian population, because of its antibacterial, anti-inflammatory, anesthetic, healing and antineoplastic effects (Simões *et al.*, 2016).

It is widely used in folk medicine, mainly in northern Brazil as antimicrobial, antifungal, diuretic, laxative, anti-tetanic, healing, anti-inflammatory and tumor inhibitor but the main activity, action mechanism and cytotoxicity characteristics are not very clear. In addition to being consumed as a component of products, such as ointments and syrups, it is also very consumed in nature, by oral administration or topical application. It is also important do not forget the commercial importance activity about copaiba oil export for the cosmetics industry (Veiga Junior e Pinto, 2002; Montes *et al.*, 2009).

The genus *Copaifera sp*. presents 72 species that 16 are found in Brazil, more specific in Amazon and the central-western region of the country. The species more common are *C. officinalis L.* (northern Amazonas, Roraima, Colombia, Venezuela and San Salvador), *C. guianensis* Desf. (Guianas), *C. reticulata* Ducke, *C. multijuga* Hayne (Amazon), *C. confertiflora* Bth. (Piauí), *C. langsdorffii* Desf. (Brazil, Argentina and Paraguay), *C. coriacea* Mart. (Bahia), *C. cearensis* Huber ex Ducke (Ceará) (Veiga Junior e Pinto, 2002).

Due to high antimicrobial activity, researches has been done in dentistry area, since was prove that it has activity against microorganisms strains that are responsible for caries and periodontal disease (Bardaji *et al.*, 2016). Economic, effective and safe products are required in dentistry mainly because of increase a microbial resistance (Palombo, 2011). Although, there are many dental products available in the market and the majority of them presents side effects and cytotoxicity in periapical area (Camargo *et al.*, 2016).

Regarding incidence of resistant and recurrent infections with different severity degrees, are essential dental materials capable to fill all system root with antimicrobial properties (Hammad, 2009, Kesler Shvero *et al.*, 2013, Heyder *et al.*, 2013).

Root canal sealers are generally irritating to the periapical tissues. Therefore, they may inhibit the healing processes and consequently influence the success of the endodontic treatments. Several root canal sealers are currently commercially available and they are classified into five large groups according to their chemical composition: Zinc oxide-eugenol-based sealers, sealers containing calcium hydroxide, resin-based sealers, glass ionomers-based sealers and based on silicone (Yoshimine *et al.*, 2003; Eldeniz *et al.*, 2007; Gandolfi *et al.*, 2008; Baraba *et* al., 2011). Despite the great variety of root canal sealers, there is still no material that fulfills the ideal requirements of the American National Standards Institute/American Dental Association (ANSI/ADA) (Eldeniz *et al.*, 2007). Thus, the development of new root canal sealers with adequate physical-chemical and biological properties is crucial (Gandolfi *et al.*, 2008).

Although there are endodontic sealers with antimicrobial characteristics on the market and many of them are cytotoxic or fail in biocompatibility (Mozayeni *et al.*, 2012). All these materials do not presents yet ideal features, such as flow through surface of canal wall, fill all spaces between material and dentin and adhering to dentin and gutta-percha (Wu *et al.*, 2000). The aim of this study was development a polymerizable endodontic sealer with copaiba resin oil of *Copaifera reticulada* Dunke and shows antimicrobial activity as well as desirable physicochemical characteristics for this material.

Methods

Material and Reagents

The oil resin of copaiba was obtained from the market and certificated by microbiological analysis and physical-chemical analysis (Copaiba da Amazonia, Guarujá - São Paulo). This oil were extracted from the sustainable development reserve of Tupé, Amazonas, Brazil. The certificate of analysis was issued by laboratory Hexalab consulting in clinical analyzes LTDA, under the supervision of UNISANTOS (Universidade Católica de Santos, São Paulo, Brazil).

In the formulation of experimental endodontic sealers were used these components: Exothane 8, ethoxylated bisphenol A diglycidil ether dimethacrylate with

30 ethylene oxide units (Bis-EMA 30), triethylene glycol dimethacrylate (TEGDMA), Polyethylene glycol dimethacrylate (PEG 400), silica particles as filler, photosensitizer camphorquinone (CQ), benzoate (EDAB), radiopacifier Ytterbium trifluoride (YT3), benzoyl peroxide, sulfinic acid derivatives and antioxidant BHT.

The copaiba oil was incorporated in the end of the formulation process at the concentrations: 0% (experimental material, negative control group), 0.5% (C0.5), 1% (C1) and 2% (C2). Real Seal® (SybronEndo, Orange ,USA) was the commercial sealer used in this study. Bipartite metal matrix (5 mm internal diameter and 1 mm high) were used for sample preparation. The photoactivation was performed on 1 side for 40 seconds. The photoactivation procedures were carried out using a light emitting diode unit with 800mW/cm².

Antimicrobial Assay

The reference strains used in this study were chosen based on their pathological effects on dentistry, and included: *Staphylococcus aureus* (ATCC 19095), *Streptococcus mutans* (ATCC 25175), *Enterococcus faecalis* (ATCC 4083), *Candida albicans* (ATCC 62342), *Candida parapsilosis e Candida famata* (clinical isolates). These microorganisms were obtained in the collection of the Research Laboratory of Oral Microbiology from the Universidade Federal de Pelotas (UFPel, RS, Brazil).

*Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MB*C)

This test evaluated antimicrobial activity of the crude copaiba oil. The minimum inhibitory concentration (MIC) was determined in triplicate by the broth microdilution technique (MIC) using a modified version of the reference documents M27-A3 (CLSI, 2008) and M7-A7 (CLSI, 2006). The susceptibility test was performed on 96-well microplates, the copaiba oil were dissolved in ethanol at 0.5 g/mL. This diluted into the 96-well microtiter plates in culture medium (RPMI for fungi and Mueller-Hilton for bacteria) in 2500µL - 4.88µL concentration. Then, aerobic and microaerophilic bacteria were incubated for 24h, and fungal for 48h. *S. mutans* was incubated with microanaerobac for 24h too. The positive control was bacterial inoculum and culture medium and the negative control group was oil with culture medium.

Before addition of the stain resazurin and in order to determine MBC, an aliquot of the inoculum was aseptically removed from each well and plated into BHI agar

supplemented for bacterial and Sabouraud for fungal. The plates were incubated as described previously. MBC was defined as the lowest concentration of the sample, which no bacterial growth occurred. The experiments were performed in triplicate.

Characterization of experimental resin sealers

For the characterization of experimental composites, the International Organization for Standardization (ISO) 6876 (2001) was used for root canal sealer materials. For each test, tree samples (n = 3) were prepared. The degree of conversion was evalued from Fourier transform infrared spectroscopy (FTIR).

Flow

The volume of 0.05 ± 0.005mL of the sealer were prepared and dispensed in glass plate of dimensions 40 x 40mm with 5mm thickness approximately using a syringe measuring 1mL. After 180 ± 5 seconds from the start of the manipulation, another glass plate weighing about 20g were carefully positioned on the sealer and, on this second plate, a weight of 100g, in a total 120 ± 2g. Ten minutes after the start of the mixture, the weight were removed and photoactivated for 40 seconds. The disc sealer formed was measured in its largest and smallest diameter by means of a digital caliper with 0.01mm accuracy. The experiments were performed in triplicate.

Dimensional stability

Bipartite silicone matrices were used in order to obtain cylindrical specimens measuring 3mm in height by 5mm in diameter. After manipulation, the sealer was inserted inside the matrix, forming a set with cellophane-type microscope slides at its top and at its base, this set was photopolymerizable and later the samples were removed from the matrix and its length measured with a digital caliper. Next, the specimens were stored for 30 days in eppendorfs with 1.5mL of distilled water, at a temperature of 37°C. The excess water of the samples were removed, after this period, with the aid of absorbent paper and a new evaluation of the length was performed. The experiments were performed in triplicate.

The following formula shall was use for calculation of rate dimensional change, used:

$$AD = \frac{C30 - C}{C} \times 100,$$

Where:

AD is the dimensional change;
C30 corresponds to sample length after 30 days of storage;
C is the initial length of the sample.

In order for the developed sealer to conform to the ISO specification, the samples should not exceed 1% contraction or 0.1% expansion.

Degree of conversion by Fourier transform infrared spectroscopy (FTIR)

The degree of conversion sealer was evaluated by Fourier Transform Infrared Spectrophotometer (Prestige 21, Shimadzu, Japan), contained an attenuated total reflectance (ATR) device, composed of a zinc selenide (ZnSe) crystal, with 45° angulation mirrors. A support was attached to fix the photoactivating unit to the spectrophotometer, allowing the standardization of a distance of 5mm between the end of the fiber optic tip and the sample. A small amount of the material was deposited on the crystal and two types of evaluation were performed: for chemical polymerization (evaluation up to 50min) and for chemical / photopolymerization after 30 or 40s of photoactivation.

The degree of conversion, per second, was calculated considering the intensity of the stretching type vibration of the carbon-carbon double bond at the 1635 cm^{-1} frequency. The symmetrical drawing of the aromatic ring at 1610 cm-1 *of the* polymerized and unpolymerized samples were used as an internal standard. The experiments were performed in triplicate.

Setting time

The experimental endodontic sealers were obtained using the metallic matrix. The determination of setting time was performed using an indenter with a head weight

of 200g. Each hour, the indenter was carefully lowered vertically on to the horizontal surface of the sealer. The setting time of each sealer was established by calculating the mean time elapsed from mixing until the indenter failed to leave an indentation on the surface of the samples

Radiopacity

From this test were using five (5) samples obtained with a metallic matrix with 5mm of internal diameter and 1mm high. The sealers' samples were positioned on an occlusal phosphor plates of the VistaScan Plus® digital system (Dürr Dental AG, Bietigheim-Bissingen, Germany) and radiographed with a x-ray unit (Ion 70x®, Procion, Ribeirão Preto, São Paulo, Brazil) with 70 kVp, 8mA, exposure time of 0.2s and a focal length of 40cm. On this phosphor plates were also be placed a aluminum stepwedge, with purity greater than 98%, with 50 x 20mm and thickness varying in step form every 1 mm.

The 5 samples of each group experimental (the sealer withot oil and the sealers with 0.5%, 1% and 2% of copaiba oil concentration) were placed on the occlusal phosphor plates together and with the aluminum stepwedge, and were made 5 radiographs, processed with the software of the VistaScan Plus (DBSWIN Imaging Software®, Dürr Dental AG, Bietigheim-Bissingen, Germany) and saved in Joint Photographic Experts Group (JPEG) format. These images were exported to the software Photoshop CC® (Adobe Systems Incorporated, São Jose, California, USA) and the radiopacity of the sealers and the steps of the aluminum stepwedge were measured using the histogram tool. With a standardized circle of 20x20 pixels, were made three measure in each sample of the experimental sealers and the steps of the aluminum stepwedge, in all five radiographs, and after were obtained the average and the standard deviation. The measures were acquire in pixel intensity and to sealers were transformed in millimeters of aluminum (mmAl), too. In order for the developed cement to comply with the ISO specification, the radiopacity of the material must be equal to or greater than the 3-mmAl radiopacity.

Modified Direct Contact Test (mDCT)

In this test, strains of *Enterococcus faecalis (*ATCC 4083) *and Staphylococcus aureus* (ATCC19095) were used.

Samples of 7mm diameter of the sealers were sterilized by gamma radiation using a dose of 4080Gy, the samples were placed 3cm from the source and sterilized with cobalt 60 irradiation with particles is 1.25Mev and subjected to 232.08Gy/Min with total dose of 4.08kGy. The total time was 18 minutes.

The methodology was performed according to the protocol used by Damlar *et al*. (2014). It was performed sowing of the microorganisms used 24h before in BHI broth, these sowings were placed in the oven at 37ºC. After this period the inoculum was diluted in BHI broth obeying the 0.5 McFarland scale.

For the test 96-well plates were used and the test was performed in triplicate. The positive control was the bacterial inoculum and the negative control group was a sealer without oil and commercial sealer. Each sample was covered by 10µl of inoculum and allowed to stand in the oven for 1 and 24h, then 240µl of broth was added and stirred for 5 minutes. Subsequently 100µl were collected and serially diluted for subsequent counting of the strains. Four dilutions were carried out 25µl agar of each dilution was plated in BHI, which will be returned to the oven at 37ºC for 24h. After this period the bacterial strains were counted.

Statistical Analyses

The testes were submitted to ANOVA and the Tukey's test.. All statistical tests were performed with significance level of 5% and were *perfomed* using SPSS® software, version 22 (IBM Corporation, Armonk, New York, USA).

Results

According the evaluation of the MIC and MBC, the strains used in this test were sensible to copaiba oil resin. *E. faecalis, S.mutans, C. albicans, C. parapsilosis and C. famata* were sensible to minimal concentrains used (4.88µg/mL) in this test (Table 1).

Antimicrobial effect

Os resultados sobre o teste de contato direto modificado contra E. faecalis estão expressos na Figura 1. Eles mostram que o selante comercial revelou a melhor atividade antimicrobiana em 1h, mas em 24h todos os cimentos contendo óleo de copaíba igualam a atividade do que o selo real. Os resultados do teste direto de contato contra S. aureus são mostrados na Figura 2. Em 1h, os selantes com óleo de copaíba não mostraram

atividade antibacteriana, mas em 24h os selantes em concentrações de 0,5% e 1% obtiveram melhores resultados, excedendo o Real Seal.

Physical- chemistry properties

There was no significant difference between the tested groups in the dimension stability and regarding flow. Setting time in copaiba oil with was significantly higher than the commercial one.

About conversion of degree, there was statistical difference between all sealers. The sealer containing copaiba oil 2% presented higher value than others. Degree of conversion in commercial sealer showed to be lower (Table 2).

The Table 3 showed that there was a statistically significant difference ($p<0.0001$) in the radiopacity of the different experimental materials evaluated, when compared to the aluminum stepwedge on the 3-mmAl step. Experimental cement without oil and the material with 1% copaíba concentration had higher values of radiopacity compared to the material with 2% copaíba concentration. The sealer with 0.5% of copaiba concentration had an intermediate radiopacity. However, even the materials with the highest values of density showed lower radiopacity when compared with 3-mmAl step of the aluminum stepwedge, which is the recommended minimum for sealers according to ISO 6876.

Discussion

The real objective of root canal treatment is remove bacteria through cleaning, shaping and filling the root system but unfortunately, residual bacteria can be present in endodontic failures. Based on that, root canal sealer with good sealing, ability and antimicrobial activity is very relevant to kill the surviving microorganisms (Saha *et al.*, 2010; Kangarlou *et al.*, 2016).

Microorganisms and their products are considered to be the primary etiological agents in endodontic diseases. Failure during and after endodontic treatment is linked to the presence of bacteria in the root canal. *Entrococcus faecalis* is a gram-positive facultative anaerobic microorganism and part of the normal flora of the mouth, this is microorganism as a resistant pathogen in endodontic treatments. This can result in periapical inflammation, and it has the ability to survive even after the application of conventional antimicrobial agents such as alkaline pH of calcium hydroxide (Kangarlou *et al*., 2016).

Current sealers and materials for root-end filling are of highly varying chemical composition and antimicrobial properties (Prestegaard *et al.*, 2014) general idea is in most instances, the antibacterial activity of canal sealers is greater at the freshly-mixed-state, but reduces with time (Prestegaard *et al.*, 2014).

The diterpenes present in the plants act on the components of the bacterial cell, increasing the permeability to wall cell of the bacteria, the terpenoids act through the membrane, swell the bacterial cell interfering in the gradient, PH, resulting in cell damage. This may be a possible mechanism of antibacterial action of copaiba oil (Bardajì *et al.*, 2016).

Copaiba oil consisted in mixtures of sesquiterpenes, and diterpenes. Main of the diterpenes are cauran, labdane, and cleorodan. By spectrophotometry was possible identify most commonly structures in these diterpenes wich are copalic acid, hardwickiic acid and carenoic acid. The copalic acid was found in all copaiba oils analyzed (Montes *et al.*, 2009; Veiga e Pinto, 2002). According to Bardaji *et al*. (2016) analyzes of *C. reticulate* chromatography oleoresin revealed b-bisabolene, Trans-A-bergamotene, b-selinene, a-selinene, ent-agathic-15-methyl, ent-copalic and entpolyalthic. Alpha-humulene, caryophyllene, alpha- and beta-selinene, and alpha-bisabolene.

Sequiterpenes are commonly found in copaiba oil and alpha-copaene, beta-caryophyllene, beta-bisabolene, alpha and beta-selinene, alpha-humulene and and omega-and-amine-cadinene. All of them have been described in several oils in studies (Veiga Junior e Pinto, 2002).

According to Simões et al. (2016) copalic acid diterpene, presents in copaiba resin oil, showed activity against S. mutans strains. Besides that, the oxide provides of beta-caryophyllene acts directly on inhibition of fungi.

In antimicrobial assays using 19 strains of oral pathogens, Bardaji et al. (2016) proved that copaiba oil has good antimicrobial activity in microorganisms involved in caries and periodontal diseases, such as *Streptococcus sobrinus, Streptococcus mitis, Streptococcus mutans, Streptococcus sanguinis, Lactobacillus casei, Streptococcus salivarius, Enterococcus faecalis, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum ,Actinomyces naeslundii, Actinomyces Prevotella buccae, Prevotella nigrescens,Bacteroides fragile and Bacteroides thetaiotaomicron.*

Some authors claims that copaiba oils have advantages over chlorhexidine because it is composed of several different substances that may have different interactions with the bacterial cell, reducing the resistance of *S. mutans* (Simões *et al.*, 2016).

Simões *et al*. (2016) evaluated the antimicrobial activity of a copaiba oil gel against *S. mutans, S. sanguinis, S. oralis, S. mitis, S. constella*tus and *S. salivarius*, presents in dental biofilm. According their study Copaíba oil presents antibacterial activity against the strains and may be used to control the dental biofilm.

Several *in vitro* studies have investigated copaifera oil toxicity. Some authors have analyzed the cytotoxic activity of resin oil and found that oil was poorly cytotoxic for macrophages. Some studies demonstrate cytotoxicity in macrophages concentrations of 500 mg/ml didn't change the viability of macrophages (Santos *et al.*, 2011).

Bardaji *et al*. (2016) showed that oil resin *C. reticulata* at concentrations up to 39mg/mL reduced cell viability, significantly and the IC50 value was 51.85 ± 5.4mg/mL.

Veiga Junior e Pinto (2002) evaluated oil resin concentrations of 5, 50, and 500mg/ml, did not alter the viability of macrophages. Other studies have also investigated in vitro as in vivo studies have reported low toxicity.

The opposite ideas about copaiba oil cytotoxicity among different authors can be explained by the different composition of oleoresins whereas the location and time of sample collection as well as the method of extraction. However, low oil concentrations can be used to inhibit some bacterial strains (Veiga Junior e Pinto, 2002).

Conclusion

The incorporation of copaiba oil at 0.5% increased the antibacterial effect of the experimental sealer in 24h compared to the sealer without oil and commercial sealer. However the radiopacity developed has proved was not accordance to ISO 6876 standars applied. Thus, is necessary more studies in this area such as cytotoxicity assay and adjustments in this formulation to improve the physicochemical characteristics.

Author disclosure statement

There is no conflicts of interests.

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Table 1- Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude copaiba oil against the microbial strains tested

Copaiba oil	***S. mutans***	***S. aureus***	***E. faecalis***	***C. albicans***	***C. parapsilosis***	***C. famata***
			MIC/MBC (µg/ml)			
	4.88	19.53	4.88	4.88	4.88	4.88
	4.88	2,500	4.88	4.88	4.88	4.88

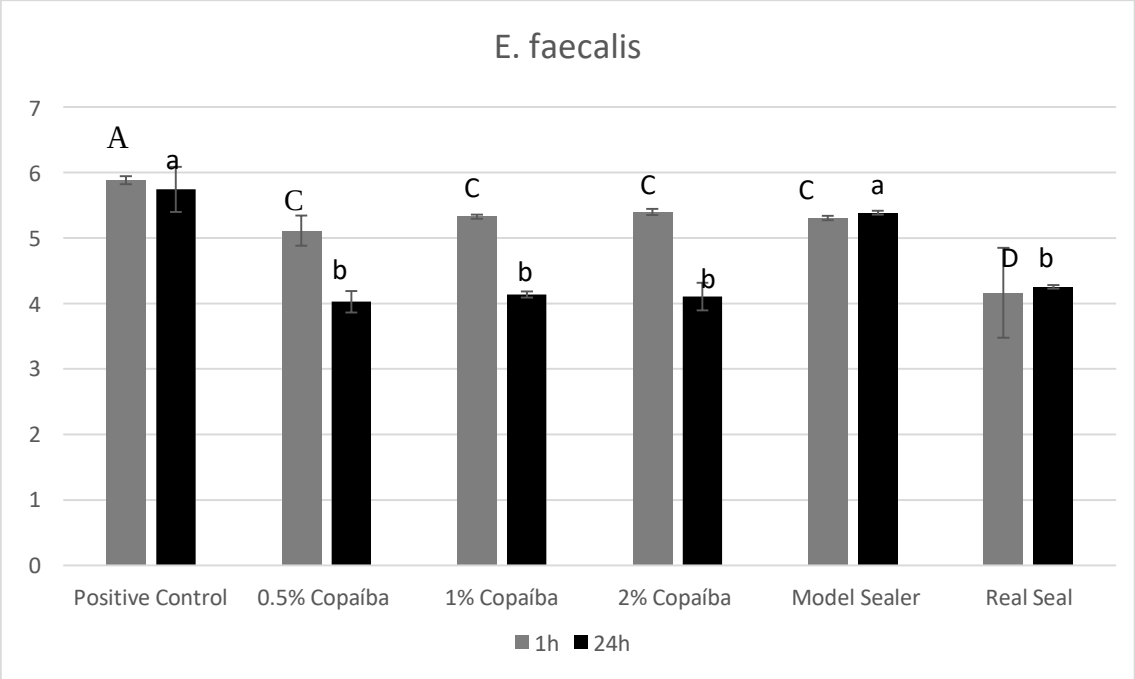

Figure 1 Modified Direct Contact test of experimental materials containing copaiba oil and the commercial reference against *E. faecalis* (mean ±SD). The statistical analyses with significance level of 5%.

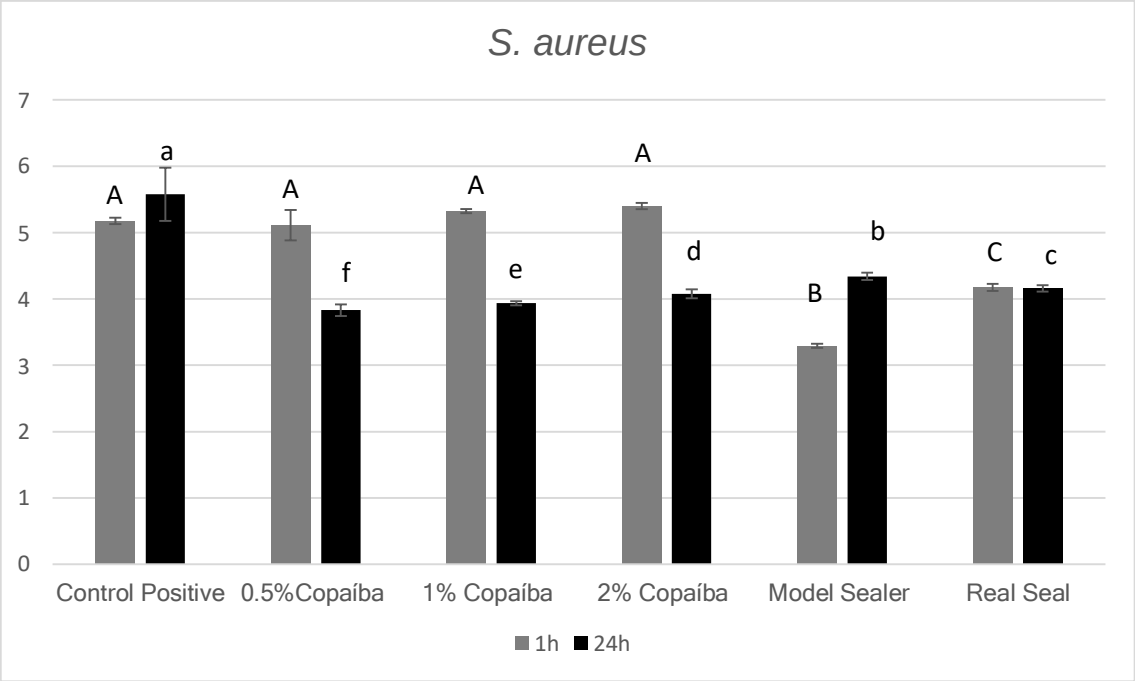

Figure 2-Modified Direct Contact test of experimental materials containing copaiba oil and the commercial reference against *S. aureus* (mean ±SD). The statistical analyses with significance level of 5%.

Table 2- Dimensional alteration, conversion of degree, flow, setting time of experimental endodontic sealer with copaiba oil.

Group	Dimensional stability	Flow	Setting time	Conversion of Degree
Copaíba 0.5%	5.31 (3.16)[a]	26.87 (1.28)[a]	72	87.48 (2.03)[b]
Copaíba 1%	5.40 (2.69)[a]	25.91 (1.60)[a]	72	90.79 (0.46)[b]
Copaíba 2%	5.02 (1.64)[a]	22.36 (3.21)[a]	72	97.32 (2.32)[a]
Model Sealer	7.44 (1.48)[a]	20.89 (0.01)[a]	40	74.53 (0.91)[c]
Commercial Sealer	6.00 (2.68)[a]	20.93 (0.01)[a]	24	53.84 (2.52)[d]

Table 3- Radiopacity of cements with and without copaiba oil

Aluminun Stepwedeg/Sealer	**Radiopacity (in pixel) Average (Standard Deviation)*§**
3-mmAl	75.74 (±1.75)[a]
0.5% Copaíba	46.20 (±1.84)[b,c]
1% Copaíba	47.06 (±2.04)[b]
2% Copaíba	43.15 (±1.62)[c]
Sealer without oil	49.45 (±1.81)[b]

*Significant statistical difference by ANOVA test ($p<0.0001$)

§ Averages with different letters showed statistical difference by Tukey's test ($p<0.05$)

Conclusões

Apesar de existir poucos estudos a respeito da atividade terapêutica da *Butia sp.,* este estudo de revisão sistemática e levantamento tecnológico de espécies desta planta nos permitiu concluir que estas apresentam propriedades medicinais promissoras, predominantemente demonstradas *in vitro*, como atividade antioxidante, anti-inflamatória, antimicrobiana e antineoplásica.

Além disso, os estudos laboratoriais desta pesquisa revelaram que extratos das folhas de *Butia sp*. testados apresentaram elevada atividade antioxidante, destacando-se os extratos cetônicos. Quanto à atividade antimicrobiana, os extratos testados apresentaram elevada atividade antifúngica, porém baixa atividade antibacteriana. Apesar dos extratos etanólicos obterem maiores rendimentos, eles apresentaram os piores resultados, quando comparados com os extratos cetônicos. O óleo de butiá apresentou atividade antifúngica, porém não apresentou atividade antibacteriana. São necessários mais estudos sobre a atividade biológica das folhas de *Butia sp.* pois estes são limitados. A avaliação das folhas e raízes da *Arctium lappa*, apresentaram pouca atividade antibacteriana, porém grande atividade antifúngica, destacando-se os extratos etanólicos.

Adicionalmente a isto, a utilização de sonicador para obtenção dos extratos vegetais aumentou a atividade antimicrobiana, porém não aumentou sua atividade antioxidante.

O óleo de copaíba, quando testado isolado, apresentou boa atividade antimicrobiana. No entanto, quando incorporado ao cimento experimental, sua atividade antimicrobiana frente a cepas de *S. aureus,* no tempo de 1 hora, não superou o cimento comercial, porém em 24h foi superior ao cimento comercial Real Seal® em todas as concentrações testadas, destacando-se o cimento experimental contendo 0,5% de óleo que revelou ser o mais antimicrobiano. Logo, os cimentos endodônticos com óleo de copaíba são promissores materiais odontológicos, porém são necessários estudos complementares, como citotoxidade, para confirmar este estudo.

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Anexos

ANEXO 1 – Normas de publicação do periódico *Phytotherapy Research*

Phytotherapy Research

Edited By: Elizabeth M. Williamson, Angelo A Izzo, Sung Hoon-Kim and Rajan Radhakrishnan

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Journals: Wright CW, Phillipson JD. 1990. Natural products and the development of selective antiprotozoal drugs. *Phytother Res* **4:** 127-139.
Books: Wagner H, Bladt S. 1996. *Plant Drug Analysis: A Thin Layer Chromatography Atlas* (2nd edn). Springer-Verlag: Berlin Heidelberg.
Chapters in Books: Kips RH. 1985. Environmental aspects. In *Pesticide Application: Principles and Practice* , Haskel PT (ed). Oxford University Press: Oxford; 1-34.
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Anexo 4- Normas para publicação do periódico *International Endodontic Journal*

International Endodontic Journal

Edited By: PMH Dummer

Impact Factor: 2.842

ISI Journal Citation Reports © Ranking: 2015: 12/91 (Dentistry Oral Surgery & Medicine)

Online ISSN: 1365-2591

Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

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The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.
Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *International Endodontic Journal*. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

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Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.
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authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical AssociationDeclaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

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Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

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Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

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5. MANUSCRIPT FORMAT AND STRUCTURE

Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

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Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

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All manuscripts submitted to *International Endodontic Journal* should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate
Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department,

hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.
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• **Methodology**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.
• **Results**: Give the main results of the study, including the outcome of any statistical analysis.
• **Conclusions**: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.
Abstract for Review Articles should be non-structured of no more than 250 words giving details of what was done including the literature search strategy.
Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.
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Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion
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Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.
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When experimental animals are used the methods section must clearly indicate that adequate

measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.
All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

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Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.
Conclusion: should contain a summary of the findings.
Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.
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Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality
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References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the

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Examples of correct forms of reference follow:

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British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* **16**, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). *Blood* **54** (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) *Surgical Endodontics*, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. *Endodontics in Clinical Practice*, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

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Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html
[accessed on 7 November 2003]

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Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

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Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.
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