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FRANKLIN TEIXEIRA REGIS

Aplicação das metodologias analíticas CLUE-DAD e NIR para a padronização de soluções extrativas das folhas, cascas e raiz de *Ptychopetalum olacoides* Benth. (Olacaceae)

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal do Amapá para obtenção do Título de Mestre em Ciências Farmacêuticas.

Orientador: Profª. Drª. Lílian Grace da Silva Solon

Co-orientador: Prof. Dr. Gabriel Araújo da Silva

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Programa de Pós-Graduação em Ciências Farmacêuticas
da Universidade Federal do Amapá

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Dedico este trabalho a todos que acreditam que a educação é libertadora.

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Aplicação das metodologias analíticas CLUE-DAD e NIR para a padronização de soluções extrativas das folhas, cascas e raiz de *Ptychopetalum olacoides* Benth. (Olacaceae)

Introdução: Ptychopetalum olacoides Benth é uma planta da família Olacacea, popularmente conhecida como muirapuama e marapuama. É amplamente utilizado na medicina popular tradicional para tratar doenças nervosas, impotência sexual e um suplemento dietético para melhorar as atividades físicas e cognitivas. **Objetivo:** O presente estudo teve como objetivo a padronização de soluções extrativas de folhas, cascas e raiz de Ptychopetalum através da aplicação de metodologias analíticas CLUE-DAD e NIR. **Metodologia:** O método cromatográfico foi realizado em coluna C18 com partículas de núcleo sólido de 2,7 µm. Ácido fórmico 0,1% (A) e acetonitrila (B) foram usados como fase móvel a 0,2mL.min-1 e o volume de injeção foi de 3µL. As amostras foram analisadas em um espectrofotômetro de infravermelho próximo (MPA, Bruker Optics, Ettlingen, Alemanha), através do compartimento da amostra líquida. **Resultados e discussões:** O delineamento experimental mostrou que a casca e a raiz das folhas podem ser padronizadas quantitativamente por CLUE e qualitativamente pelo NIR. O método NIR foi desenvolvido para determinar qualitativamente compostos fenólicos em extratos de muirapuama com tratamento mínimo de amostra e sem uso de solventes e reagentes. Pode-se distinguir entre folhas, cascas ou amostras de raízes, sendo indispensável na correta identificação como parte da planta na qual a solução extrativa foi preparada. **Conclusão:** Este trabalho mostra que as metodologias são inovadoras no campo do controle de qualidade e desenvolvimento farmacêutico de fitoterápicos.

Palavras-Chave: *Ptychopetalum olacoides*; flavonóides; CLUE; infravermelho próximo; métodos analíticos.

Agradecimentos: UNIFAP, UEAP, IEPA, UFRN, NUPLAM e CNPq.

ABSTRACT

Application of UHPLC-PDA and NIR analytical techniques for standardization of extractive solutions of leaves, bark and root of *Ptychopetalum olacoides* Benth. (Olacaceae)

Introduction: *Ptychopetalum olacoides* Benth is a plant of the family *Olacaceae*, popularly known as muirapuama and marapuama. It is widely used in traditional folk medicine to treat nervous diseases, sexual impotence and as a dietary supplement to improve physical and cognitive activities. **Objective:** The present study had as objective, the standardization of extractive solutions of leaves, bark and root of Ptychopetalum olacoides through application of UHPLC-DAD and NIR. **Methodology:** The chromatographic method development was performed on C18 column packed with 2.7 µm solid core particles. Formic acid 0.1% (A) and acetonitrile (B) were used as mobile phase at 0.2mL.min^{-1} and the injection volume was 3 µL. Samples were analyzed in a near infrared spectrophotometer (MPA, Bruker Optics, Ettlingen, Germany), through the liquid sample compartment. **Results and discussions:** The experimental design showed that leaves bark and root can be quantitatively standardized by UHPLC and qualitatively by NIRS. A NIRS method was developed for qualitatively determining phenolic compounds in muirapuama extracts with minimal sample treatment and no use of solvents and reagents. It could distinguish among leaves, bark or root samples being indispensable in the correct identification as the part of the plant in which the extractive solution was prepared. **Conclusion:** This present work shows that the methodologies are innovative in the field of quality control and pharmaceutical development of phytomedicines.

Keywords: *Ptychopetalum olacoides*; flavonoids; UHPLC; Near infrared; analytical methods.

Acknowledgements: UNIFAP, UEAP, IEPA, UFRN, NUPLAM e CNPq.

1 INTRODUÇÃO

O Brasil é um país de grande tradição no uso de matérias vegetais, por causa de sua grande biodiversidade o que oferece grandes possibilidades de tratamento para diversas enfermidades.

A biodiversidade e o potencial econômico da flora brasileira, desde 1886, já eram descritos em inventários, testemunhando a sua riqueza em plantas produtoras de frutos alimentares, resinas, óleos, gomas, aromas e, principalmente, o potencial medicinal (JACOBSON et al., 2005).

Além de sua reconhecida riqueza natural, a Amazônia abriga expressivo conjunto de povos indígenas e populações tradicionais que aprenderam, ao longo do tempo, como conviver com ambientes diversificados (AMOROZO, 1996). Este conjunto é detentor de um vasto conhecimento sobre as plantas e seu ambiente. Este conhecimento tem passado de geração em geração por via oral, estando intimamente interligados com a necessidade dos povos em aplica-los em seu proveito (RODRIGUES e CARVALHO, 2001).

Nesse contexto, a muirapuama (*Ptychopetalum olacoides* Benth) é uma planta medicinal usada no tratamento de doenças do sistema nervoso. Muito utilizada também por causa de seus efeitos afrodisíacos (SIQUEIRA et al., 2007). Sua utilização era feita por meio de uma garrafada, que é uma mistura da matéria vegetal com cachaça, produzida para tomar uma dose antes das refeições (PIATO et al., 2010).

O aumento no consumo de plantas medicinais pode ser associado ao fato de que as populações questionam os perigos do uso irracional dos medicamentos alopáticos associados a seus custos dispendiosos (TOMAZZONI et al., 2006), pela preferência dos consumidores pelo produto natural com confirmação científica e o desenvolvimento de novos métodos de controle de qualidade (CAÑIGUERAL et al., 2003; MELO et al., 2007).

A padronização de uma solução extrativa é indispensável para garantir a qualidade e a reprodutividade das características químicas, farmacológicas, tecnológicas de preparações vegetais. Entre os diversos fatores que influenciam o grau de extração de substâncias de uma droga vegetal estão, as técnicas de extração empregadas, a natureza do solvente utilizado, a solubilidade das substâncias de interesse no solvente, a relação droga/solvente e o tempo de extração (LIANG et al., 2004).

A falta da padronização interfere na reprodutibilidade dos fitoterápicos, pois estes podem conter ou não o princípio ativo ou os compostos tóxicos em quantidade adequada dependendo do plantio e da época do ano. É reconhecido que dados de eficácia e segurança de várias plantas medicinais ainda não suficientes para dar suporte ao seu uso, por vezes devido à falta de metodologias adequadas de avaliação dessas plantas (BARATA, 2005; SPRINGFIELD et al., 2005).

Para isso, pode-se considerar a aplicação de metodologias analíticas UHPLC-DAD e NIR, que sempre foram destaques na química analítica pela sua capacidade de oferecerem análises qualitativas e quantitativas em amostras ambientais, farmacêuticas, biológicas e nos alimentos (BEDNER et al., 2008). Além disso, são técnicas que viabilizam processos e produtos de maneira a evitar ou minimizar o impacto negativo causado ao homem e ao meio ambiente – química verde (SOUSA-AGUIAR et al., 2014).

Nesse contexto, este trabalho propõe a padronização de soluções extrativas de muirapuama (*Ptychopetalum olacoides* Benth), através das metodologias analíticas UHPLC-DAD e NIR, resultando em derivados vegetais de maior uniformidade, importantes na produção de fitoterápicos seguros e eficazes.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Padronizar *soluções extrativas d*e m*uirapuama (Ptychopetalum olocoides Benth) através d*a aplicação de metodologias analíticas verdes CLUE-DAD e NIR.

2.2 OBJETIVOS ESPECÍFICOS

- Revisar as atividades farmacológicas, compostos químicos e patentes de muirapuama *(Ptychopetalum olocoides Benth)*;
- Selecionar as melhores condições do processo extrativo para cada parte da planta;
- Avaliar a influência do solvente, tamanho de partícula e tempo de extração na concentração de flavonoides totais em raiz, cascas e folhas, empregando planejamento fatorial;
- Otimizar o método de separação cromatográfico por CLUE;
- Investigar similaridades dos extratos obtidos de diferentes partes da planta com a mesma graduação alcoólica através do NIR;
- Avaliar a capacidade do NIR de encontrar similaridades dos extratos obtidos com diferentes graduações alcoólicas com a mesma parte da planta;

3 REFERENCIAL TEÓRICO

3.1 Plantas medicinais

Desde os primórdios da civilização o poder curativo das plantas vem sendo utilizado no combate às doenças, mesmo que incialmente tudo fosse feito de maneira empírica. Porém, o que antes era apenas sabedoria popular virou estudo de muitos pesquisadores e os princípios ativos desses vegetais passaram a ser utilizados na fabricação de medicamentos (BADKE et al., 2011).

Plantas medicinais, segundo Lopes et al. (2005), são os vegetais que quando administrados de qualquer forma ou via ao homem ou ao animal desempenham ação terapêutica. O tratamento em que se utiliza as plantas medicinais é chamado de fitoterapia e os medicamentos que as possuem como matérias-primas são chamados de fitoterápicos.

De acordo com o Boletim da Organização Mundial de Saúde (1998), planta medicinal é:

> Todo e qualquer vegetal que possui, em um ou mais órgãos, substâncias que podem ser utilizadas com fins terapêuticos ou que sejam precursores de fármacos semissintéticos (BULLETIN OF THE WORLD HEALTH ORGANIZATION, 1998).

3.1.1 Breve histórico

A utilização de produtos naturais com fins medicinais surgiu junto com a humanidade, mas não se sabe exatamente a data certa de quando esses recursos começaram a ser utilizados, pois existem muitos registros conhecidos em vários períodos diferentes, além dos que ainda não foram encontrados ou dos que não se tem registro.

> O conhecimento sobre as plantas medicinais sempre tem acompanhado a evolução do homem através dos tempos. Remotas civilizações primitivas se aperceberam da existência, ao lado das plantas comestíveis, de outras dotadas de maior ou menor toxicidade que, ao serem experimentadas no combate às doenças, revelaram, embora empiricamente, o seu potencial curativo. Toda essa informação foi sendo, de início, transmitida oralmente às gerações posteriores e depois, com o aparecimento da escrita, passou a ser compilada e guardada como um tesouro precioso (ARAÚJO et al., 2007, p. 45).

Segundo Simões, Schenkel e Simon (2001) e Vale (2002), os primeiros registros encontrados sobre plantas medicinais são de 2838-2698 a.C., quando o imperador chinês Shen Nung inventariou 365 ervas medicinais e venenosas que eram usadas através de inspiração taoísta de Pan Ku, o deus da criação.

Duarte (2006), considera como primeiros registros aqueles datados em 500 a.C., no texto chinês que relata nomes, doses e indicações de uso das plantas para tratamento de doenças. Há também os registros encontrados no manuscrito egípcio “Ebers Papirus” de 1500 a.C., que guardavam informações sobre 811 prescrições e 700 drogas.

De acordo com Helfand e Cowen (1990) há documentos que relatam o uso de vegetais para fins medicinais desde 4000 a.C.

Coan e Matias (2013) relatam que os egípcios, assírios e hebreus cultivavam as plantas medicinais, em 2.300 a.C., e delas produziam vermífugos, purgantes, cosméticos, diuréticos e produtos líquidos e gomas para embalsamar múmias.

A medicina chinesa utiliza, desde 2500 a.C. até os dias de hoje, plantas medicinais para o tratamento de várias doenças (SCHENKEL, GOSMAN e PETROVICK, 2003).

Mesmo com toda evolução na medicina, ainda é possível ver uma grande parte da população, principalmente em países em desenvolvimento, utilizar esses vegetais na manutenção da saúde e alívio das enfermidades (SOUZA e FELFILI, 2006), devido ao alto custo dos medicamentos sintéticos e à facilidade de se encontrar esses vegetais na natureza (VASCONCELOS, ALCOFORADO e LIMA, 2010).

3.2 *Ptychopetalum olacoides* Benth

São plantas angiospermas pertencentes a família *Olacaceae,* do gênero *Ptychopetalum,* na região do Amazonas. E é encontrada nos domínios fitogeográficos da Amazônia em floresta ombrófila (ROSSI, 2015).

Segundo Tang et al. (2008), essa planta é conhecida como muirapuama ou marapuama e é utilizada para o tratamento de doenças crônicas degenerativas do sistema nervoso. É muito utilizada também por causa de seus efeitos afrodisíacos e está incluída em vários suplementos dietéticos e multivitamínicos, disponíveis pelo mundo para melhorar a performance cognitiva, sexual e física (SIQUEIRA et al., 2007).

As cascas e raízes, conforme a Figura 1 e 2, respectivamente, são geralmente preparadas em destilado obtido a partir da cana-de-açúcar ou vinho e vendido como garrafadas - jargão local para dar nome a mistura feita de material vegetal com vinho ou

cachaça. Produzido para ser consumido antes das refeições com uma dose por volta de 60 mililitros (PIATO et al., 2010).

Figura 1 – Casca de Muirapuama

Fonte: Autoria própria, 2019.

Figura 2 – Raiz de Muirapuama

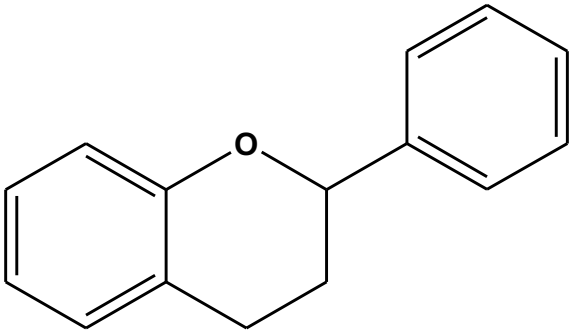
Fonte: Autoria própria, 2019.

3.3 Flavonóides

Os flavonoides são ácidos fracos e, como são compostos polares ou moderadamente polares, são solúveis em etanol, metanol e butanol e combinações de solventes em água. Podem sofrer degradação em meio alcalino na presença de oxigênio (POZZI, 2007).

Mais de 4.000 estruturas de flavonoides já foram identificadas em fontes vegetais, caracterizando um dos mais importantes grupos do reino vegetal. A estrutura básica é composta por dois anéis de benzeno ligados através de um anel de pirano (Figura 1). Suas maiores classes são os flavonóis, flavonas, flavanonas, catequinas, antocianinas, isoflavona, diidroflavonois e chalconas (BECHO et al., 2009).

Figura 3 – Estrutura básica dos flavonoides



Fonte: Autoria própria, 2019.

A formação de radicais livres é uma etapa chave no desenvolvimento de câncer e doenças coronárias devido ao ataque às biomoléculas (lipídios, proteínas, ácido desoxirribonucleico (DNA)) ou às biomembranas. Os flavonoides atuam como potentes antioxidantes, prevenindo a formação de radicais livres (ROBARDS e ANTOLOVICH, 1997).

3.4 Extração de plantas medicinais

Muitos pesquisadores têm estudado o processo de extração de princípios ativos aplicados a plantas medicinais. O objetivo destes estudos é o desenvolvimento de metodologias adequadas para avaliar a influência dos parâmetros de extração de compostos ativos obtidos a partir de marcadores de fitoterápicos (NORIEGA et al., 2012).

A operação extração significa a retirada de um ou mais constituintes a partir de uma matéria-prima natural, sendo realizada frequentemente com auxílio de solventes líquidos. Uma das formas mais aceitas de classificar as operações de extração é segundo a sua eficiência, permitindo reconhecer dois tipos: operações de extração parcial (sem esgotamento dos constituintes de interesse) e operações de extrações exaustivas (com esgotamento da matéria-prima) (SIMÕES et al., 2017).

A escolha do método de extração é considerada uma das etapas mais críticas das pesquisas envolvendo plantas medicinais. A eficiência da operação depende de diversos fatores, tais como as características do material vegetal, o tipo de solvente e a temperatura de extração (COPPA et al., 2017).

3.4.1 Ultrassom

O ultrassom é definido como o conjunto de ondas eletromagnéticas com frequência de superior a 20 kHz. A extração por ultrassom usa a alta potência que produz cavitação acústica conduzindo à desestruturação celular e tecidual, permitindo, assim, o contato imediato dos componentes com o solvente. Como vantagens da técnica, ressaltam-se a efetividade de extração, a economia de energia e solvente e, face às temperaturas moderadas no meio, a menor chance de degradação de substâncias termossensíveis (ESCLAPEZ et al., 2011).

3.5 Técnicas instrumentais de análise

A produção farmacêutica começou através da confecção de produtos artesanais, com o aumento da demanda sua atividade evoluiu para uma fase industrial. A avaliação de qualidade dos produtos acarretou vários problemas que despertaram a necessidade de uma regulamentação oficial para assegurar a qualidade dessa classe de produtos.

Para isso a Agência Nacional de Vigilância Sanitária criou a Resolução da Diretoria Colegiada (RDC) n° 13 (ANVISA, 2013) estabelecendo os requisitos mínimos para padronizar a verificação do cumprimento das boas práticas de fabricação de fitoterápicos.

Esse controle de qualidade deve ser feito através de análises disponíveis na Farmacopeia Brasileira ressaltando sua execução (BRASIL, 2010).

Muitas técnicas instrumentais, cromatográficas, eletroquímicas e espectroscópicas, de análise têm sido desenvolvidas e apresentaram grandes vantagens sobre as técnicas convencionais (WILLARD et al., 1988; SHARMA, 2000). As técnicas cromatográficas são utilizadas para separar, identificar e quantificar múltiplos compostos em amostras complexas. As técnicas espectroscópicas são em sua maioria não destrutivas, rápidas e confiáveis, e capazes de medir a quantidade de radiação produzida ou absorvida pelas espécies moleculares, por meio das vibrações das ligações químicas (SETTLE, 1997; SKOOG et al., 2014; KUMAR, 2015). As técnicas eletroquímicas fornecem informações a respeito da transferência de elétrons através de potenciais químicos, reações eletroquímicas como eletrólise, cinética de reações, etc. (SETTLE, 1997; SKOOG et al., 2014). Estas técnicas coletam uma grande faixa de informação de

dados com muita facilidade e rapidez (FERREIRA et al., 1999; CHRISTIAN; DASGUPTA; SCHUG, 2014). Os dados obtidos muitas vezes são complexos, possuem inúmeras variáveis, portanto, são dados multivariados e podem ser tratados por ferramentas estatísticas aplicadas à área de química (quimiometria) (HOLLER; SKOOG; CROUCH, 2009; MILLER; MILLER, 2010).

3.5.1 Cromatografia líquida de ultra-eficiência (CLUE)

As técnicas cromatográficas são conhecidas desde o início do século, oriundas das experiências de Tswett com a separação de um extrato de planta em uma coluna de carbonato de cálcio, usando éter de petróleo como fase móvel. No entanto, começaram a ser realmente usadas na década de 30, quando Kuhn e Lederer “redescobriram” e aperfeiçoaram a cromatografia em coluna (MALDANER; CRISTINA; FONTES, 2012)

A cromatografia moderna surgiu a partir de Martin e Synge. Em 1941 eles concluíram que para o melhor desempenho da cromatografia líquida, era necessária uma fase estacionária com partículas bem pequenas e, por sua vez, era essencial alta pressão para forçar a fase móvel a passar pela coluna. Como resultado disto surgiu uma técnica denominada cromatografia líquida de alta eficiência (CLAE) ou *high performance liquidchromatography* (HPLC) (CIOLA, 1998). Desde então o avanço foi gradual e atingiu um alto nível de sofisticação devido ao revolucionário desenvolvimento tecnológico dos equipamentos. Com isso, tornou-se possível preencher colunas com partículas de pequeno tamanho, necessárias para alta resolução e, também, adquirir equipamentos que funcionam nas altas pressões necessárias para obter uma boa velocidade de eluição(DOLAN, 2013). Nos últimos anos ocorreu o desenvolvimento de vários detectores espectrofotométricos que operam em comprimentos de onda variável e houve um aumento na utilização dos detectores por fluorescência, detectores eletroquímicos, e por fluorescência induzida por laser, bem como acoplamento com o espectrômetro de massas. Com estes, tornou-se possível a detecção da maioria dos compostos e a análise de traços em amostras complexas, como sangue, urina, solo, alimentos, petróleo, e etc (ABRAHAM; IBRAHIM; ZISSIMOS, 2004; DOLAN, 2013; YOSHIE; DE, [s.d.]).

O alto nível de desenvolvimento e expansão alcançado pela CLAE, ao longo das últimas décadas, deve-se principalmente a sua vasta aplicabilidade, que engloba o desenvolvimento de novos produtos, o controle da qualidade, determinação da composição ou formulação de um produto e as possíveis contaminações e produtos de degradação (MALDANER; CRISTINA; FONTES, 2012). Dentro deste contexto estão

envolvidas as indústrias farmacêuticas, alimentícias, agropecuárias e químicas, as agências reguladoras que tratam do meio ambiente e dentre outras. A ampla aplicabilidade da CLAE foi o que impulsionou e continua impulsionando as pesquisas em busca do aprimoramento desta técnica, principalmente referente ao desenvolvimento de novas metodologias analíticas. Hoje a CLAE pode ser considerada uma técnica de análise (separação, confirmação e quantificação) bem difundida e empregada, em conseqüência das colunas cromatográficas e equipamentos de alta tecnologia que se encontram disponíveis no mercado (JIN et al., 2008).

A cromatografia líquida tem sido beneficiada nos últimos anos por diversas inovações. O principal aprimoramento refere-se ao desenvolvimento de novas partículas de fases estacionárias, capazes de gerar colunas mais seletivas, eficientes e estáveis química e mecanicamente(HEATON; MCCALLEY, 2014; MCCALLEY, 2011).

Estas novas colunas não foram compatíveis com os sistemas cromatográficos convencionais e seu uso só se tornou possível com o desenvolvimento da cromatografia líquida de ultra eficiência (CLUE) ou a UHPLC (*Ultra High Performance Liquid Chromatography*) (AUTHORS; GUILLARME; VEUTHEY, [s.d.]; MALDANER; CRISTINA; FONTES, 2012). Em 2004 a *Waters Corporation* lançou o primeiro equipamento comercial capaz de operar em pressões acima de 15000 psi, denominado de *Acquity ultra performance liquidchromatography system* (UPLC®). Logo em seguida, outros dois fabricantes, a Jasco e a Agilent, lançaram seus instrumentos de CLUE, o Xtrem LC® (X-LC), também com capacidade de trabalhar em pressões acima de 15000 psi e o 1200 *Series Rapid Resolution LC System*® capaz de trabalhar em pressões acima de 9000 psi, respectivamente (SWARTZ, 2009). As principais modificações encontradas em um sistema de CLUE quando comparadas à CLAE são: capacidade de trabalhar em pressões muito altas, volumes internos muito menores devido às modificações que ocorreram nas conexões, alça de amostragem, cela do detector e bombas. As celas do detector não possuem dispersão e atingem alta taxa de aquisição, melhorando o sistema de controle e de dados. O equipamento também possui colunas resistentes para trabalharem a altas pressões e com baixo volume morto, injetores com precisão na faixa de volumes muito pequenos (microlitros) o que implica em menor tempo de análise (separações ultra-rápidas, em menos de 1 minuto) e uma diminuição considerável no consumo de solventes (MALDANER; CRISTINA; FONTES, 2012).

A cromatografia líquida de alta eficiência (CLAE) é uma técnica bastante consolidada e exigida pelas agências regulatórias, aplicando-se em diversas determinações dentro do campo farmacêutico, como identificação e quantificação de

fármacos, metabólitos, impurezas e produtos de degradação. Entretanto, possui como enorme desvantagem o alto consumo de solventes tóxicos usados rotineiramente, gerando resíduos prejudiciais à saúde do analista e ao meio ambiente (MALDANER; CRISTINA; FONTES, 2012). Nesse sentido, o CLUE está ganhando popularidade e aceitação nos laboratórios analíticos, uma vez que foi desenvolvido com o objetivo principal de diminuir o tempo de análise e o consumo de solventes, garantindo a mesma confiabilidade analítica da CLAE. Em vista das enormes vantagens e da expansão do emprego do CLUE nas análises de rotina, esta técnica tem sido constantemente alvo de pesquisas, seja na melhoria do equipamento ou no desenvolvimento de métodos cromatográficos cada vez mais rápidos, eficientes e de menor custo. Por gerar menos resíduo que a técnica anterior, o CLUE também tem atendido aos princípios da química verde (NOV; MATYSOV; SOLICH, 2006).

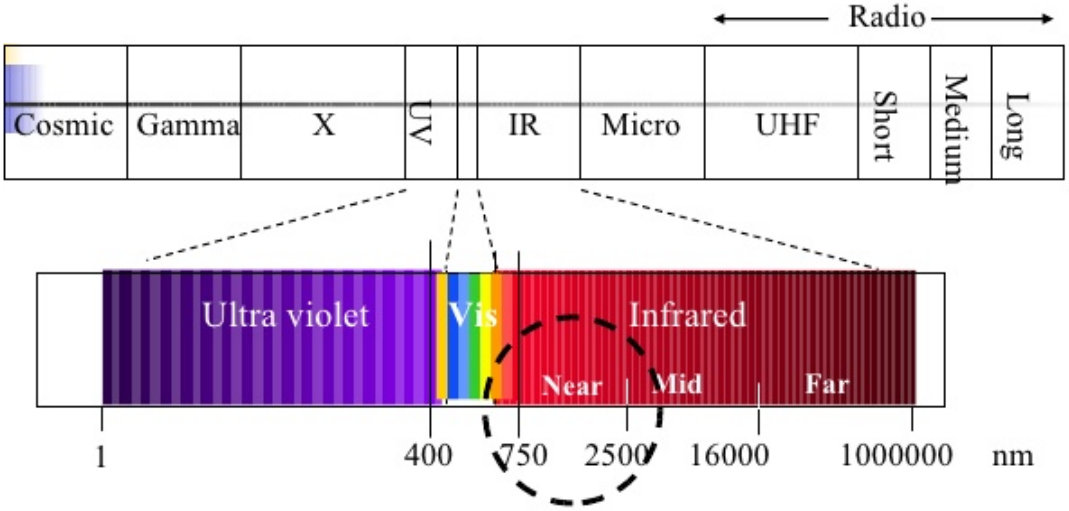
3.5.2 Infravermelho próximo (NIR)

A busca crescente por melhoria da qualidade de produtos e racionalização da produção nas várias áreas da indústria tem levado à substituição gradual de técnicas analíticas conservadoras demoradas e procedimentos de controle inespecíficos por ferramentas analíticas específícas e compatíveis com o ambiente (BURNS e CIURCZAK, 2008).

Nesse sentido, as espectroscopias, em geral, têm tido grande destaque nos últimos anos na indústria farmacêutica, principalmente voltadas ao controle de qualidade e monitoramento de processos, sendo recente o seu uso no controle de fitoterápicos. Os métodos espectroscópicos de análise baseiam-se na interação da radiação com a matéria, através da medida da quantidade de radiação produzida ou absorvida pelas moléculas ou espécies atômicas de interesse (SKOOG e LEARY, 2006). Dentre as existentes, o infravermelho próximo possui características que a destaca.

A região do infravermelho próximo compreende a faixa do espectro eletromagnético (~780 a 2500 nm), situada entre o visível e o infravermelho médio, conforme observado na Figura 4. As absorções na faixa NIR são baseadas em sobretons e combinação de vibrações da molécula investigada. Devido à sua probabilidade de transição inferior, as intensidades geralmente diminuem por um fator de 10 a 100, a cada nível a partir da base para o próximo sobretom (BURNS e CIURCZAK, 2008).

Figura 4 - Região do infravermelho próximo no espectro eletromagnético (~750 a 2500 nm)



Fonte: Ciurczak, 2015.

Na região do NIR, as moléculas sofrem transições vibracionais e rotacionais podendo passar diretamente de um determinado nível energético para dois ou mais níveis de maior energia. As bandas de absorção mais proeminentes que ocorrem na região do NIR estão relacionadas a sobretons e combinações de vibrações fundamentais de grupos funcionais –CH, -NH, -OH e -SH (JAMRÓGIEWICZ, 2012).

O NIR pode ocorrer de três modos: transmitância, reflectância difusa e transflectância. O segundo mede a reflectância e o terceiro mede a combinação entre a transmitância e reflectância. Os modos de transmitância e transflectância são em geral utilizados para amostras líquidas e semissólidas, enquanto que a reflectância difusa é utilizada para amostras sólidas, na qual a radiação não é capaz de atravessar a amostra e ser captada por um detector que fique a 180° da fonte de emissão (COSTA, 2014).

Os procedimentos utilizando a região do NIR são menos trabalhosos daqueles os quais utilizam a região do MIR e são semelhantes aos da região do ultravioleta e visível. A absorvância de um líquido ou solução pode ser rapidamente mensurada utilizando cubetas de quartzo ou safira de dimensões variáveis ou utilizando sondas de fibra ótica. Devido a capacidade de absorver grupos O-H, N-H e C-H fortemente, recomenda-se utilizar solventes os quais não possuam esses grupamentos ou que sua absorção seja mínima para não interferir no sinal produzido pela molécula de interesse (PASQUINI, 2003).

A espectroscopia do NIR pode ser utilizada em determinações qualitativas e quantitativas de espécies moleculares de todos os tipos. Devido à isso ela é amplamente

aceita pela indústria farmacêutica como um método para o controle físico-químico das amostras, proporcionando medição direta de amostras sólidas com uma análise rápida e precisa, necessitando de pouca ou nenhuma manipulação da amostra (BLANCO e ALCALÁ, 2006b).

Das técnicas analíticas disponíveis para o desenvolvimento de métodos qualitativos e quantitativos na indústria farmacêutica, o NIR é provavelmente a mais bem-sucedida. Na literatura, constam diversos trabalhos voltados para a indústria farmacêutica (COSTA, 2014), tais como: na análise qualitativa e quantitativa de diferentes classes de compostos, e em diferentes áreas, tais como produtos farmacêuticos (NEVES, et al., 2012), alimentos (INACIO, DE MOURA e DE LIMA, 2011), ambiental (GELADI et al., 1999), clínica (SAKUDO, et al., 2009), entre outros. NIR mede as transições de ligações moleculares de vibração. Uma ampla gama de moléculas (principalmente compostos orgânicos) absorve no intervalo de NIR e os seus grupos funcionais, tais como OH, CH, NH e C=O aparecem como os mais fortes. As amostras geralmente são medidas sem quaisquer pré-tratamentos, o que torna NIR uma técnica rápida, não invasiva e não destrutiva (MARQUES et al., 2014).

Outras aplicações do NIR descritas na literatura estão relacionadas com o monitoramento do processo de revestimento de comprimidos e na determinação de seu ponto final (DE BEER et al., 2011; MOLTGEN et al., 2012).

Segundo Pasquini (2003) a utilização do NIR em métodos análiticos para a determinação de proteínas em amostras de carne, soja e milho, como forma alternativa ao método compendial (Kjeldhal) ganhou ênfase e espaço, uma vez que após a construção do método por NIR, ele é capaz de obter o sinal sem destruir as amostras, gerar o resultado em menos de um minuto e não produzir resíduos tóxicos ao ambiente.

Além de aplicações na indústria, estudos biológicos também foram descritos, por exemplo, na aplicação da espectrometria de NIR para análise de micro-organismos *Staphylococcus aureus* (MAQUELIN et al., 2003; GRUNERT et al., 2013), que tem sido alvo de estudos nos últimos anos. Amiali et al. (2011) determinaram uma região espectral no infravermelho por transformada de Fourier (FTIR) ou a combinação das regiões que refletem uma característica bioquímica específica de uma cepa de *S. aureus* meticilina-resistente associado à comunidade (CA-MRSA).

Grunert et al. (2013) estudaram a potencialidade da espectroscopia FTIR para diagnóstico diferencial dos tipos de *S. aureus* de polissacarídeo capsular clinicamente mais relevante. Maquelin et al. (2003) realizaram um primeiro estudo clínico prospectivo

em que os patógenos causadores (*Staphylococcus aureus*, *Enterococcus feacalis, Escherichia coli e Pseudomonas aeruginosa*) de infecções do sangue foram identificados por espectroscopia FTIR (MARQUES et al., 2014). Entretanto, o método de análise por NIR possui desvantagens. Essa tecnologia é e sempre será fortemente dependente da existência de bons e aceitáveis métodos de referência, como por exemplo: cromatografia, Karl Fischer, Kjeldahl, contagem de placas, entre outros. Isso ocorre devido à etapa de modelagem ou criação do modelo de calibração, uma vez que o NIR precisa identificar as características espectrais ou as combinações destas referentes ao que está sendo pesquisado, portanto pequenas diferenças no sinal do espectro será o responsável de garantir que tal informação é referente à mudança existente e detectável pelo método de referência. A diferença entre o sucesso e a falha nesta técnica está diretamente dependente da qualidade dos valores de referência associadas às amostras na etapa de modelagem (BLANCO et al., 1998).

A umidade do ambiente deve ser controlada, uma vez que ele é capaz de detectá-la e interferir no espectro resultante do produto a ser analisado. Durante sua aplicação em rotina é necessário inserir novos dados de amostras no modelo de calibração, uma vez que fenômenos físicos ambientais podem interferir ao longo de sua aplicação e, consequentemente o modelo de calibração torne-se desqualificado para o objetivo. Além disso, é necessário que o operador saiba interpretar corretamente os espectros, bem como realizar com precisão e exatidão os tratamentos quimiométricos, seja de pré-processamentos ou de construção de modelos de calibração multivariados, dos espectros para manter respostas adequadas do produto (JAMRÓGIEWICZ, 2012).

4 ARTIGO 1

Artigo submetido para publicação no Journal of Phytochemistry Reviews

***Ptychopetalum olacoides* (muirapuama), a traditional Amazonian "nerve tonic": Patents, phytochemistry and biological activities review**

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Highlights

- *Ptychopetalum olacoides* Benth, native from Amazon, is known for its “aphrodisiac” and “nerve tonic” properties;

- Antidepressant, anxiogenic and anti-stress effects have been related on literature as a main pharmacological activity of this specie;

- Drugs containing *Ptychopetalum olacoides* are the main patented products;

- Its great pharmacological and economic potential can offer the development of new products;

- Forest management is needed to ensure its preservation.

Abstract

Ptychopetalum olacoides Benth is a plant of the family Olacacea, popularly known as muirapuama, murapuama and miratã. It is widely used in Amazonian communities to treat nervous diseases, sexual impotence and to improve physical and cognitive activities. Many scientific papers can be found in the literature regarding the pharmacological importance of this natural product. Antidepressant, anxiogenic and anti-stress effects are the main pharmacological activity of this specie. Nevertheless, to date, none of these studies has presented a systematic review of literature neither a technological mapping of patents. This review aimed to summarize all data from literature and patents involving *Ptychopetalum olacoides* Benth. To that end, a patent research was performed in the international patent database. For literature review online bibliographical databases were used: PubMed, Science Direct, Scopus and Google Scholar. On Patentscope database results showed a total of 27 patents. Chemical compounds and pharmacological activities found in literature were also compiled. The studies also showed that in Amazonia this species is exploited in an extractive way with serious risks to biodiversity. This review presents different technological approaches to apply *Ptychopetalum olacoides* and emphasizes its great pharmacological and economic potential for the development of new products and discusses actions for its cultivation in a sustainable way.

Keywords*: Ptychopetalum olacoides. Muirapuama. Literature review. Patent review. Forest management.*

1. Introduction

Ptychopetalum olacoides Benth is a plant of the family Olacacea, popularly known as Muirapuama, murapuama and miratã. It is widely used in traditional folk medicine to treat nervous diseases, sexual impotence and as a dietary supplement to improve physical and cognitive activities (Siqueira et al., 2003).

Given the importance of herbal medicine in the current market and the growing search for natural products, it was necessary to develop studies to prove which bioactive compounds and what results are seen in each formulation (Falzon and Balabanova 2017).

The present work sought a survey of the published articles that presented results of the activities tested for the hydroalcoholic extracts of *P. olacoides* between the years of 2003 and 2016. All patents were found that involve formulations containing *P. olacoides* as component were listed.

The objective of this study is to serve as a reference for future work involving Muirapuama by informing the results and progress achieved up to the present moment. The main contributions of this work are to provide contextualization data and extension of the significance of new studies, and serve as a basis for a better knowledge of the current literature on the applications of *P. olacoides*.

Given the background, this review was based on a data compilation of the state of the art on literature and patents involving *Ptychopetalum olacoides* Benth between 2003 to the present.

2. Methods

Patent research was performed in the international patent database Patentscope using the following keywords: muirapuama, marapuama, *Ptychopetalumolacoides,* miratã and catuama. For literature review online bibliographical databases were used: PubMed, Science Direct, Scopus and Google Scholar. Non-English articles, reviews, conference articles, thesis, letters and any other reports that are not original were excluded from this systematic review. We imported and saved the data to Mendeley software, deleted the duplicated references both manually.

3. Results and Discussion

3.1.1 Plant extracts and chemical compounds

The use of medicinal plants to treat disease is very common in the Amazon region. *Ptychopetalum olacoides* Benth is generally used as tincture, syrup, tea, infusion, fluid extract or powder. Extracts, which are rich in active metabolites obtained from various parts of the plant, are widely used in scientific research. For use in in vitro and in vivo studies, the various components of plant (roots, bark, leaves and seeds) are submitted to extraction procedure, separation, purification and fractionation that aids in isolating of the bioactive compounds. It is interesting to note that extracts contain high concentrations of bioactive compounds that are similar to those of the infusions and teas, making them attractive for use in scientific research. The principal compounds identified, type of extract and the used parts of plant are presented in Table 1.

Table 1. Principal compounds identified in hydroalcoholics extracts of *P. olacoides*.

Type of extract Used parts	Class	Compounds	Reference
--	Triterpenoid	Lupeol	AUTERHOFF & MOMBERGER, 1971; ITO et al., 1995
Methanolic extract (obtainedatthe end of 2 pre-extractionsfollowed with n-hexaneand ethyl acetate) Sonicador wood/bark	Flavonoid	3-*O*-metilquercetina, 3,4'-*O*-dimetilquercetina, 3,7-*O*-dimetilquercetina.	DUTRA et al., 2017
--	Saturatedfattyacids	Palmicacid Stearicacid	MONTRUCCHIO et al., 2005
	Methylxanthine	Caffeine	
	Triterpenoid	Lupeol	
	Steroid	B-sitosterol	
MethanolicExtract Barks	Cleodanediterpenoid	ptychonolide 20-*O*-methylptychonal-acetal equilibrium mixture of ptychonal hemiacetal ptychonal	(Tang et al., 2008)
MethanolicExtract Barks	--	6a,7a-dihydroxyannonene 7a,20-dihydroxyannonene 7a-hydroxysolidagolactone ptycho-6a,7a-diol	(Tang et al., 2009)
--	--	Total flavonoids (rutinequivalent)	ROLIM et al., 2008

3.1.2 Pharmacological activities

3.1.2.1 Acetylcholinesterase activity

Acetylcholinesterase is responsible for the termination of signal transmission in the colinergic system, due to its hydrolyzing potential. Its substrate acetylcholine is a neurotransmitter with a predominant effect on motor neurons involved in memory formation. So, by decreasing the activity of this enzyme by employment of specific inhibitors, motor neuron disorders such as myasthenia gravis, Lewy body dementia and Alzheimer's disease can be treated (Khan et al., 2018).

The acetylcholinesterase inhibition through of *Ptychopetalumolacoides* ethanol extract (POEE) was the activity with the highest number of studies (five) in the last fifteen years (Da Silva et al., 2004; Da Silva et al., 2007; Figueiró et al., 2010; Figueiró et al., 2012; Siqueira et al., 2003).

In the study developed by Siqueira et al. (2003), the POEE inhibited acetylcholinesterase activity in vitru, dose and time dependent, in all brain structures, 25% in the frontal cortex, 20% in the striatum and 15% in the hippocampus. Significant inhibitions were observed in these same brain areas of aging mice (14 months) after POEE administration (100 mg/kg ip).

Differential effects may be related to the uneven existence of several molecular acetylcholinesterase forms on particular tissues or brain regions, favoring interactions with particular types of acetylcholinesterase (Bisso et al., 1991).

These effects were verified by Figueiró et al. (2010) through the acetylcholinesterase isoforms, G1 and G4. These were obtained by centrifugation of the hippocampus, frontal cortex and striatum. The resulting supernatants were used as the G1 source. The pellet was suspended in 0.5 M potassium phosphate, pH 7.5 and centrifuged. The supernatant was collected and used as the G4 source (Das et al., 2001).

The POEE inhibited G1 and G4 acetylcholinesterase isoforms in hippocampus (66 and 72%, respectively) and frontal cortex (50 and 63%, respectively), while G4 appeared to be selectively inhibited (72%) in the striatum (Figueiró et al., 2010).

In addition, Figueiró et al. (2010) performed histochemical activities and confirmed that the POEE significantly inhibited acetylcholinesterase in the hippocampus CA1 (~33%) and CA3 (~20%) and striatum (~17%). The Western blotting shoed that POEE did not induce significant changes in the acetylcholinesterase immunocontent suggesting that its synthesis is not extensively modified.

In a more recent study, Figueiró et al. (2012) further characterize the acetylcholinesterase inhibitors pattern, through of cytosolic globular monomer (G1) and membrane bound globular tetramer (G4), presents in the POEE. It mostly inhibited G1 in hippocampus (75%), and G4 in frontal cortex (58%) and striatum (75%). A limitation of this study is the lack of clear concentration-response relationships. Kinetic analysis indicated that inhibition in hippocampus was of a competitive nature for G1 but uncompetitive for G4. The nature of inhibition may be determined by the micro-environment surrounding the active site, which at the same time is a contributing factor in determining the selectivity for both substrate and acetylcholinesterase isoforms. As for it, a detailed study of the interaction of G1 and G4 with POEE requires the structural elucidation of the active compound (s).

Besides of these studies, Da Silva et al. (2004) evidenced that a single intraperitoneally administration of PPOE improved memory retrieval in step-down inhibitory avoidance short-term memory (STM) 24 h after training in adult mice. Therefore, aging mice treated with PPOE performed as well as adult mice. Although the molecular mechanism (s)underlying the improvement in memory retrieval are not defined.

The study by Da Silva et al. (2007) aimed to complement the previous study by Da Silva et al. (2004), by now examining the effects of the same extract on short-term memory in adult and aging mice in a shorter time, 3 h. The results also showed that PPOE reverses the retrieval deficit in aging mice, but not object recognitionmemory when tests were performed 3 h after training.

3.1.2.2 Antidepressant activity

Stress is the main trigger of depression that is constant in today's world. It manifests itself as the body's reaction towards a stimulus, displayed in the form of mental, physical and/or an emotional response. Depression is initiated through stress and stressful situations and cause a person to suffer and function poorly in everyday life, presenting anxiety, loss of appetite, lack of energy, disturbance in sleep and suicidal thoughts (Martins, J; Sukumaran, B., 2018).

In their studies, Piato et al. (2007) verified the effects of POEE in the unpredictable chronic mild stress (UCMS) in mice that induces behavioral changes that mimic symptoms of human depression. Thus, they found that the POEE, similar to imipramine, a tricyclic antidepressant used in the test for comparisons, prevented changes induced by UCMS.

In the last study, Piato et al. (2009) examined the involvement of dopamine, noradrenaline and serotonin on the antidepressant effects of the POEE. The results demonstrated the possible participation of β-noradrenergic and D_1 dopamine receptorsin the antidepressant activity of POEE.

While Piato et al. (2008; 2009) studied the effects of POEE on the forced swimming and tail suspension tests, Piato et al. (2010) evaluated it for glucose and anxiety. In the end, the POOE did not present anxiolytic effects, but was able to prevent the UCMS-induced anxiety as assessed by the light/dark test and no differences were noted in the glycemia.

3.1.2.3 Antioxidant activity

Free radical formation is associated with the normal nature metabolism of aerobic cells that occur during the respiratory process. These reactive oxygen species can be detoxified by antioxidant substances to maintain the balance that occurs during oxidative stress (COULIBALY, et al., 2014).

After confirmation of free-radical scavenging by POEE in vitro, Siqueira et al. (2007) verified the in vivo antioxidanteffect of POEE. After POEE administration, different brain areas of aging mice were examined in order to measure antioxidant enzyme activities, free-radical production and damage to macromolecules. Finally, the POEE reduced free-radical production in the hypothalamus, leading to significant decrease in lipid peroxidation in the cerebral cortex, striatum and hypothalamus. Moreover, there was a decrease of carbonyl content in cerebellum and striatum. In terms of antioxidant enzymes, the catalase activity was increased in the cortex, striatum, cerebellum and hippocampus, while the glutathione peroxidase activity was increased in hippocampus.

Although the in vitro assay was not cited in the study by Siqueira et al. (2007), it was done by De Vargas et al. (2016). In this study, the POEE presented IC_{50}> 10 µg/mL for the DPPH assay, which characterizes a poorly antioxidant activity. However, IC_{50} < 10 µg/mL for the ABTS assay. Both are colorimetric methods, but the ABTS assay was the most adequate for measuring the antioxidant potential of POEE. In addition to these assays, *Ptychopetalum olacoides* inhibited the formation of free radicals in cells.

3.1.2.4 Neuroprotective effects

The study by Siqueira et al. (2004) used hippocampal slices exposed to oxygen and glucose deprivation (OGD, followed by reoxygenation) to evaluate the neuroprotective properties of PPOE. The slices incubated with PPOE (0.6 µg/mL) during and after OGD exposure had significantly increased cellular viability and prevented the increase of free radicals content induced by OGD. The fact that the neuroprotective effect was observed at the highest dose used implies that the effective doses of active compounds can be lower than those of the extract studied, once it is a complex mixture of compounds, of which only one or a few may be relevant for the observed activity.

3.1.2.5 Antimicrobial activity

The determination of antimicrobial activities of different medicinal plants is of special interest these days due to the current global issue of increasing antibiotic resistance of microorganisms. It is assumed that the drug resistance in pathogenic microorganisms is developing due to indiscriminate use of commercial antimicrobial drugs (Farjana et al., 2014).

In the study by Oliveira et al. (2013), the antimicrobial activity was assayed utilizing twogroups of well-known microorganisms, gram negative and gram positive, pathogenic strains, through the agar diffusion test. The aqueous extract of *Ptychopetalumolacoides* inhibited the growth of *Klebsiellaozaenae* and *Acinetobacter baumannii*, producing halos higher than 100 % (9.25 mm halo) and 45.4 % (5 mm halo) respectively, when compared to halos produced by ciprofloxacin (8 and 11 mm).

3.2 Patents of P.olacoides containing-products

The analyses were conducted based on a search in the international Patentscope database (patentscope.wipo.int), bellowing to WIPO. The selected keywords were "*Ptychopetalumolacoides",* "marapuama", "muirapuama" and "catuama" and the search was made in the title, abstract and full text. For all documents the following aspects were observed: Number and date of deposit; title; abstract; assigneecountry; authors and technological focus. After the analysis, the patents were categorized in groups according to the technological use of *P. olacoides* (Table 2).

Table 2. Published patents and patents granted, between 2003 and 2018, with the keywords "muirapuama", "marapuama", "*ptychopelatum olacoides*" or "catuama" found in Patentscope database.

Patent No.	Country	Inventor (s)	Product	Application
PI 1100582-3 A8	Brazil (BR)	ELAINE ELISABETSKY / ADRIANA LOURENÇO DA SILVA / ÂNGELO LUIS STAPASSOLI PIATO / CARLOS ALEXANDRE NETTO / IONARA RODRIGUES SIQUEIRA / MICHELI FIGUEIRÓ / MATILDE ACHAVAL ELENA / LISIANE PORCIÚNCULA / DOMINGOS SÁVIO NUNES	Drugs	Central Nervous System
PI 0605812-4 A2	Brazil (BR)	Maria Del Carmen VelazquezPereda / Samara Eberlin / Cecilia Nogueira / Marcos Roberto Rossan / Marcio AntonioPolezel	DrugsandCosmetics	Skin
PI 0205432-9 A2	Brazil (BR)	Adriana Lourenço da Silva / Elaine Elisabetsky /Carlos Alexandre Netto / Ionara Rodrigues Siqueira	Drugs	Central Nervous System

PI 0307647-4 A2	Brazil (BR)	Elaine Elisabetsky / Carlos Alexandre Netto / Adriana Lourenço da Silva / Ionara Rodrigues Siqueira / Domingos Sávio Nunes	Drugs	Central NervousSystem
20170135369	United States (US)	Jose Luis ROJANO JORGE/ Jordi CLARAMONTE CALLEJON	Chewinggum	-
202017003961	Germany (DE)	König Herbert	Meat products	-
20201700324	Germany (DE)	König Herbert	Sausages	-
37635	MA	EL ISMAILI EL IDRISSI LALA MERIEM	Micronizedpowder	Sexual dysfunction
20201600467	Germany (DE)	KönigSwann	Spirits	-
202016004929	Germany (DE)	KönigSwann	Energy drinks	-
202016003812	Germany (DE)	KönigSwann	Soft drinks	-

2020160020 2	Germany (DE)	KönigSwan n	Alcoholicbev erages	-
2014121785	Russia (RU)	БУХВАЛЬД-ВЕРНЕР Сибилле/ ФУДЖИИ Хаджиме	Drugs	Central Nervous System
2014121051	Russia (RU)	БУХВАЛЬД-ВЕРНЕР Сибилле/ ФУДЖИИ Хаджиме (	Drugs	Muscular System
WO2015189 445	WO	ROJANO JORGE, Jose Luis/ GUTIÉRREZ HERNÁNDEZ, Francisco	Chewinggum	-
2020130159 46	Brazil (BR)	NEMIAS CIGANO DE SOUZA	Animal feed	-
249556 5	ES	ROJANO JORGE José Luis/ CLARAMONTE CALLEJON Jordi	Chewinggum	-
1020090126 033	Korea (KR)	PARK, HYUN SEOK/ LEE, BONG HWAN/ CHANG, YUN HEE	Drugs	Weight loss

20080260806	United States (US)	Miller Risa	Cosmetics	Skin
2005350391	Japan (JP)	UENO HIROKO	Drugs	Central Nervous System
6746695	United States (US)	Martin, Michael Z.	-	Methods of extracting and purifying bioactive substances
2001297991	Australia (AU)	Andre, Eunice	Drugs	Antioxidantandcerebral vasodilatoragent
2001297993	Australia (AU)	Batista Calixto, Joao	Drug	Antithromboembolicagent
2001297992	Australia (AU)	Batista Calixto, Joao	Drug	Antidepressant agent and in anxiety disorders
WO/2002/096442	WO	SILVA FILHO, Osvaldo	Drug	ANTIDEPRESSANT AGENT AND IN

				ANXIETY DISORDERS
2001322941	Japan (JP)	HOSHINO HIROSHI	Cosmetics	Skin
2000119187	Japan (JP)	YOSHIMURA HIROYUKI	Drugs	Central Nervous System

3.3 The increasing development of new products and the strategy of sustainable cultivation in the Amazon Forest

The growing number of products developed each year containing muirapuama draws attention to the exploitation of this plant. A study carried out by the Brazilian Agricultural Research Corporation (EMBRAPA) (2001) has reported that the young plants of *Ptychopetalum olacoides* are eliminated for root use and that this type of exploitation involves serious risks of diminishing genetic diversity. In the state of Amazonas, Brazil, the wood of adult plants is explored, also exposing this species to serious extinction risks. In the state of Pará, northern Brazil, the seeds of the muirapuama are commercialized, showing a more sustainable form of exploitation. Measures are necessary to avoid the extinction of this species in the forest. Interest of communities and national governments in small and large-scale industrial development based on local / national biodiversity resources are also necessary.

4. Conclusion

In the present manuscript, we evidence that *Ptychopetalum olacoides* possesses various pharmacological activities, indicating its potential in the treatment of some disorders. In addition, it is consistent with its medical use by traditional communities in the Amazon region. Considering the previous studies, our study reinforces the need to isolate its compounds in order to determine its mechanisms of action and selectivity. Thereat, the extracts will be produced with more safety and efficacy to ensure better clinical results.

The growing number of products developed each year containing muirapuama draws attention to the exploitation of this plant. this review showed the potential pharmacological application of muirapuama, reinforcing the need for further studies for this plant. In addition, most of the products found in the patents are for pharmaceutical use. Due to its great commercial importance, this species has been extensively explored in the Amazon. Sustainable forest management measures are needed to make this specie more accessible, more studied and more products can be developed and patented from it.

5. Acknowledgements

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5 ARTIGO 2

Artigo a ser submetido para publicação no Microchemical Journal

Application of ultra-high performance liquid chromatography and near infrared spectroscopy techniques for standardization of extractive solutions of leaves, bark and root of *Ptychopetalum olacoides* Benth. (Olacaceae)

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1. Introduction

Ptychopetalum olacoides Benth is a plant of the family Olacacea, popularly known as muirapuama, murapuama and miratã. It is widely used in Amazonian communities to treat nervous diseases, sexual impotence and to improve physical and cognitive activities. Many scientific papers can be found in the literature regarding the pharmacological importance of this natural product. Antidepressant, anxiogenic and anti-stress effects are the main pharmacological activity of this specie (Falzon and Balabanova 2017). This specie is generally used as tincture, syrup, tea, infusion, fluid extract or powder extracts, which are rich in active metabolites obtained from various parts of the plant.

To transform a tradicional product into a phytomedicine it is necessary the validation of quality control techniques in order to characterize and quantify the most important compounds of the plant or the active substances. This enables possible the standardization of the vegetable specie, intermediate product as well as phytopharmaceutical with a concentration of active substances within a straight interval, which may be used as a reference of quality by the pharmaceutical industry (Schmidt, 1990).

For the development of a phytopharmaceutical product which meets all legal criteria, the standardization of extractive solutions represents an indispensable step (Petry, 2001). The technological transformation of vegetable drugs is required in the use of extraction operations in order to remove the substances or active fractions of interest in these vegetable drugs, using a solvent or a mixture of solvents which are both toxicologically safe (Schmidt, 2003). There are few studies concerning standardization of extractive solutions from medicinal plants from Amazon, to be submitted to extractive processes which could compromise quality, yield, efficiency and even the safety of the intended usage (Cortés-Rojas et al, 2013). The main factors which affect efficiency of the extraction process are related to the vegetable drug (quantity, granulometry), extracting solvent or mixture of solvents (selectivity or quantity) and extraction conditions (plant:solvent ratio, agitation, extraction time and temperature) (Pandey, 2014). The total flavonoids contents in extractive solutions represents an important parameter in evaluating the efficiency of the extractive process, and is frequently based on the complexation of the flavonoids and analyzed by spectrophotometer (Marinova et al., 2005). Modern analytical techniques that meet the requirements of green analytical chemistry are in high use. Besides being fast techniques, spend little or no solvent during the analysis, and are still very economical when compared to the conventional analytical techniques (Armenta et al., 2008). An example of this is ultra-high performance liquid chromatography (UHPLC) and near infrared spectroscopy (NIRS). According to the advancement of electronics, the use of infrared spectroscopy is allowing accurate calculations by using algorithms that allow a safe analysis with no chemical residue to the environment (Moros et al., 2010). Besides NIRS presents the characteristics of high speed, non-destructiveness, high precision, and reliable detection data, providing a valid alternative to classical chromatographic approaches.

The objective of the present study is to apply green analytical techniques, UHPLC and NIRS, to select the best extractive process conditions, evaluating the influence of the extraction method, extracting solvent, granulometry and time of extraction, on the total flavonoids content found in *P. olacoides* extractive solutions, by employing factorial design, as a first step in the development of a phytomedicine according to the international guidelines of World Health Organization (WHO) (Akerele, 1993).

2. Material and Methods

2.1. Materials and Chemicals

Leaves, bark and roots of *P. olacoides* were collected from Porto Grande, a town localized in Amapa State, Brazil. Specimens were identified by Federal University of Pará (UFPA) herbarium (under the number: 169916) and then they were registered to *SisGen* (*National Management System of Genetic Heritage and Traditional Knowledge,* n° AF451C5). The samples were dried and crushed to a powder in an electric grinder and then passed through a mesh sieve. Reference compound, quercetin, was obtained from Sigma-Aldrich® (purity >96%). HPLC grade methanol and acetonitrile were purchased from J.T.Baker® (USA). Ethanol 95% and formic acid were purchased from VETEC® (Brazil). Ultrapure water was obtained from a Milli-Q Integral Water Purification System (Millipore, Bedford, MA,USA).

2.2. Extractive solutions preparation

The leaves, bark and root were dried in an oven at 45 ± 1°C, for 7 days. The dry matter was ground in a crusher and strained through cloth with openings of 500, 425~150 and 150~63 μm. These were performed at 60 vibrations per second for 15 minutes, using a straining device. Vegetable drug materials were classified under three granulometric categories: Fraction I (63 < ø < 150 μm), Fraction II (150 < ø < 425 μm) and Fraction III (ø < 500 μm) extracted by ultrasound (15, 30 and 60 minutes) with 1000 mL of ethanol (50, 70 and 90°GL), at plant:solvent ratio: 1.0:10 (w/v), originating 27 extractive solutions for each part of the plant, in a total of 81 extractive solutions for analysis.

2.2. Sample preparation for instrumental analysis

Each part of plant extract (0.5 mL of 1:10 $g.mL^{-1}$) and quercetin (standard phenolic compound) were diluted in triplicate with methanol. Extracts showed the concentration of 1:1 and quercetin standard curve calibration was dilute to the following concentrations: 100, 75, 50, 25, 10, 5, 2.5 $\mu g.mL^{-1}$. All solutions were filtered through 0.22 μm membrane filter before analysis in UHPLC and NIRS.

2.3. Experimental design and statistical model

In this study, a three level, three variable full factorial design was applied to determine the best combination of variables for total flavonoids content (equivalent to quercertin %) in extractive solutions obtained from leaves, bark and root of *P. olacoides*. Particle size range (μm), ethanol concentration (ºGL) and extraction time (minutes), which were identified to have strong effects on the response in preliminary one-factor-at-a-time experiments, were taken as the variables tested in a 27-run experiment to determine their optimum levels. Independent variables were designated as *x1, x2* and *x3*, and their levels values are shown in Table 1. The polynomial equation used for the three variables is given below (Equation 1):

$$Y = \beta 0 + \beta 1x1 + \beta 2x2 + \beta 3x3 + \beta 11x1^2 + \beta 22x2^2 + \beta 33x3^2 + \beta 12x1x2 + \beta 13x1x3 + \beta 23x2x3 \quad [Eq.1]$$

Where: *Y* is the predicted response; *β0* is model constant; *β1, β2*, and *β3* are the linear coefficients; *β11, β22*, and *β33* are the quadratic coefficients; *β12, β13*, and *β23* are the interaction coefficients; and *x1, x2*, and *x3* are independent variables.

Table 1. Three independent variables used in 3^3 full factorial design.

Factor	Name	Levels		
		-1	0	1
*X*1	Particle size range (μm)	63~150	150~425	>500
*X*2	Ethanol concentration (°GL)	50	70	90
*X*3	Time of extraction (min)	15	30	60

Optimal condition was determined considering total flavonoids content (%) as response. The software STATISTICA® (version 10, Statesoft – Inc., Tulsa, USA, trial version, 2011) was used for experimental design, data analysis and determination of optimal conditions. ANOVA was used for evaluation of significance of independent variables' influence and interactions. Pareto charts were applied to obtain the significance of impact of tested variables on mentioned responses. The correctness of the model was verified by performing ultrasound-assisted extraction of samples at obtained optimal conditions

(particle size range, ethanol concentration and extraction time) in order to obtain maximal total flavonoid content.

2.4. UHPLC analysis

Analyses were carried out using a Shimadzu Prominence UFLC-XR (Shimadzu, Japan), equipped with two LC 20-ADXR solvent delivery units, auto sampler (SIL-20ACXR), degassing unit (DGU-20A3), column oven (CTO-20AC) and a photodiode-array detection (SPD-M20A) set at 340 and 370 nm (scan 200 – 400 nm). The methodology was transferred from HPLC to UHPLC by using the software "*Shimadzu® Method Transfer*" from the original method developed by Zeirak and collaborators (2010). After transfer, method optimization was performed on C18 column packed with 2.7 µm solid core particles (4.6 x 50 mm) from Agilent Technologies®. Formic acid 0.1% (A) and acetonitrile (B) were used as mobile phase at 0.2mL.min^{-1} in gradient mode and the injection volume was 3 µL. The column oven and the auto sampler were maintained at 40 °C and 20 °C, respectively. Chromatograms were integrated using the LC Solution software. Details of gradient mode are shown in Table 2.

Table 2. Optimized UHPLC gradient mode of mobile phase for flavonoid determination

Time (minutes)	A % (Formic acid 0.1%)	B % (Acetonitrile)	Flow rate (mL.min^{-1})
0.00	95	5	0.2
3.00	95	5	0.2
7.00	80	20	0.2
9.00	80	20	0.2
10.00	77	23	0.2
15.00	77	23	0.2
19.00	50	50	0.2
20.00	5	95	0.2
25.00	95	5	0.2
30.00	95	5	0.2

2.5. NIR spectroscopy analysis

Samples were analyzed in a near infrared spectrophotometer (MPA, Bruker Optics, Ettlingen, Germany), through the liquid sample compartment. Each measured spectrum (in triplicate) was the average of 32 scans obtained with a resolution of 16 cm^{-1} and over the range of 12500-4000 cm^{-1}. Then, it was possible to convert the wave number data to wavelength (800 to 2500 nm), which was used for data processing. Spectral measurements were done in an acclimatized room under controlled temperature of 22 ºC, and 60% relative air humidity. The software used to perform the NIRS statistical analysis was Matlab R2014b®, with the PLS_Toolbox 2014 package.

3. Results and discussion

In this work two green analytical methods were applied for the analysis of total flavonoids (expressed as quercetin, using the external standard method) in leaves, bark and root of muirapuama (*Ptychopetalum olacoides* Benth, Olacaceae). Extraction of flavonoids was optimized by experimental design methodology, and quantitative analysis was performed by ultra-high-performance liquid chromatography with photo-diode array detection (UHPLC/DAD) and near infrared spectroscopy (NIRS). Quercetin was chosen as standard for the quantification of total flavonoids in order to propose a method feasible for routine analysis of the flavonoids in the muirapuama extracts. This phenolic compound is widely present in the three parts of the plant (root, bark and leaves) and therefore was chosen to monitor extraction efficiency in standardization.

3.1. Experimental design and UHPLC analysis

Total flavonoid content (TFC%) was obtained from different trials of the experimental design protocol. A Pareto chart of standardized effects of *P. olacoides'* root presented in Figure 1 shows significant effect of particle size range of the raw material (μm) and concentration of ethanol variables (linear). The bar length of each parameters characterizes the absolute importance of the estimated effects. The vertical line represents the limit between the significant and insignificants effects with a 5% risk of error. Three effects are significant at 95% confidence level in the studied experimental domain ($P < 0.05$) as shown in Figure 1.

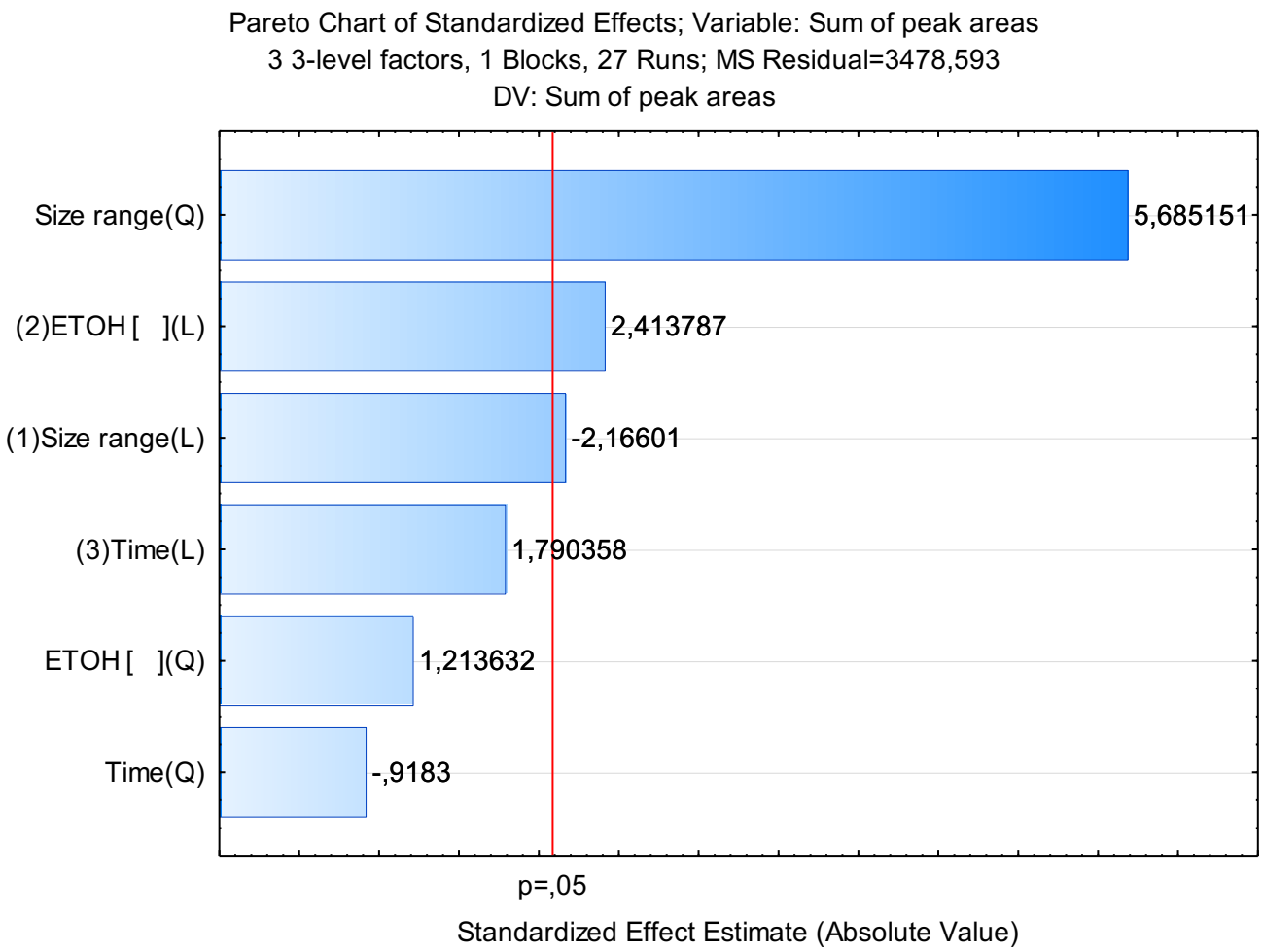

Figure 1. Pareto chart of effects for the total flavonoid content (TFC%) in root of *P. olacoides.*

Table 3 provides the analysis of variance (ANOVA) of the model. The value of the coefficient of determination (R^2) was 0,87. The proficiency of the model is demonstrated if R^2 is equal to 0,75 or higher than this value (HAALAND, 1989). The response surface of the TFC% as a function of particle size range (*x1*) and ethanol concentration (*x2*) is presented in Figure 2 as a 3D response surface plot and as 2D contour plot. Interactions between these two factors shows that both variables presents strong effect on response.

Table 3. Analysis of variance (ANOVA) of the model.

Sources	SS	Df	MS	*F*-value	*F*0.05
Total flavonoid content					
*X*1	1458.00	1	1458.00	1.41553	0.268249
*X*2	60.50	1	60.50	0.05874	0.814600
*X*3	17860.50	1	17860.50	17.34029	0.003147
$X1^2$	8418.69	1	8418.69	8.17349	0.021186
$X2^2$	5544.23	1	5544.23	5.38275	0.048914
$X3^2$	387.92	1	387.92	0.37662	0.556459
Pure error	8240.00	20	1030.00		
Total	43165.33	26			
R-squared 0,87					

SS: *sum of squares,* Df: *degrees of freedom,* MS:*mean square*

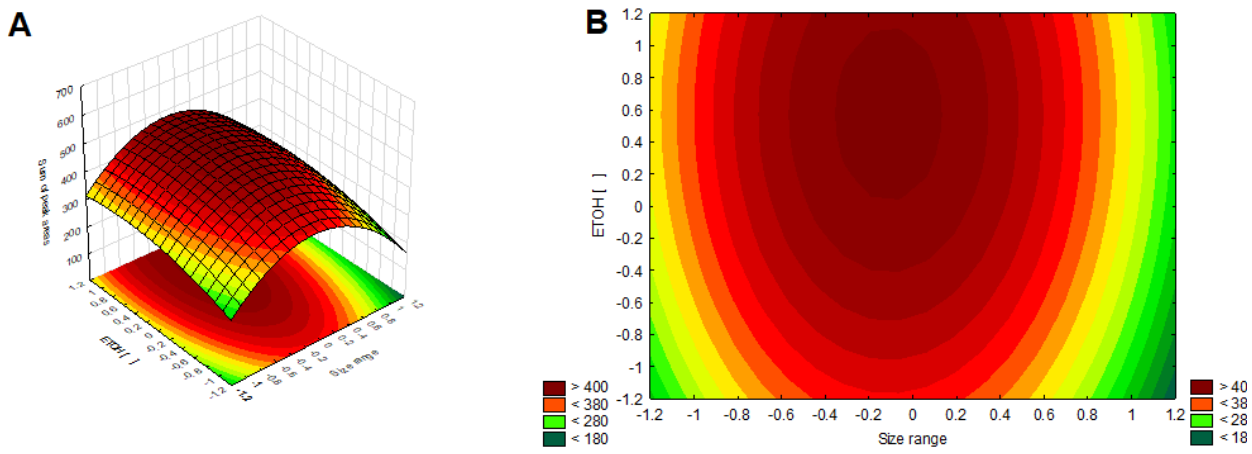

Figure 2. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in root of *P. olacoides* as a function of particle size range (*x1*) and ethanol concentration (*x2*).

It can be observed in Figure 2 that the utilization of ethanol at 70° GL leads to an increase in the total flavonoids contents, which can be attributed to the difference of the dielectric constant of the solvents, resulting in a greater extraction capacity of ethanol at 70° GL.

On the other hand Figure 3 shows that an increase of ethanol concentration (*x2*) has a great influence on the TFC% in any time value. The time values (*x3*) are also observed in Figure 4, showing that *x3* also do not influence the TFC%, regardless of the particle size of the root raw material.

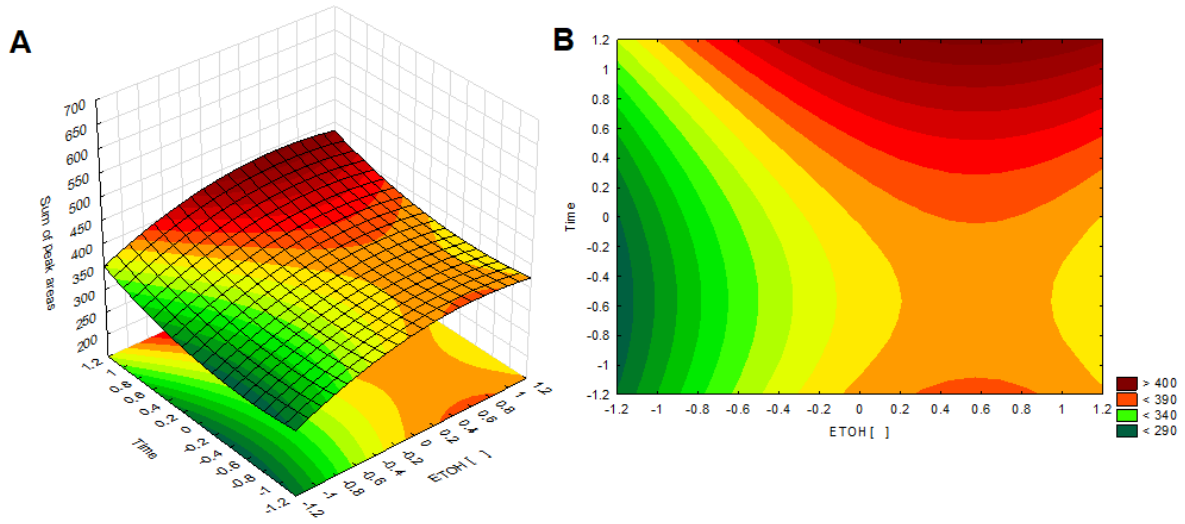

Figure 3. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in root of *P. olacoides* as a function of ethanol concentration (*x2*) and time of extraction (*x3*).

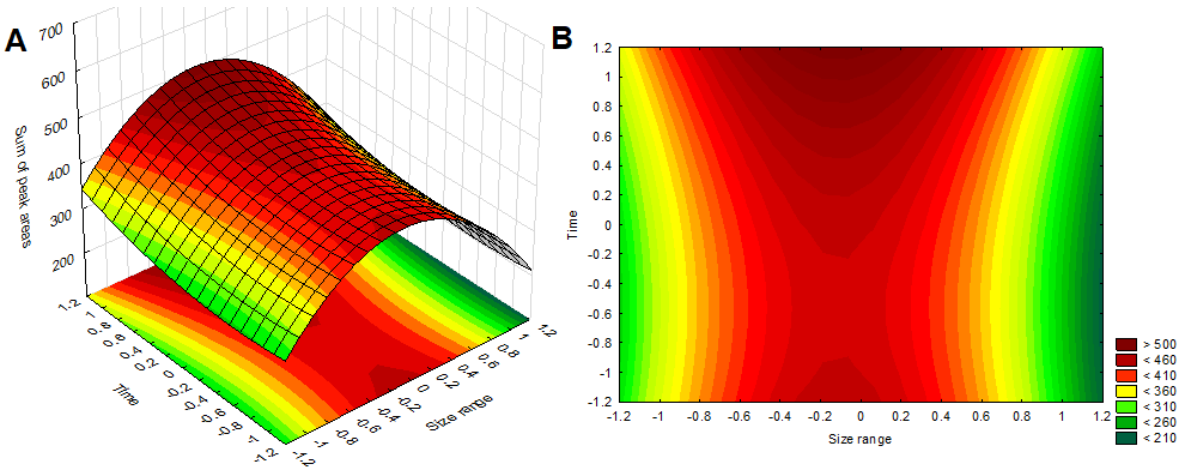

Figure 4. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in root of *P. olacoides* as a function of particle size range (*x1*) and time of extraction (*x3*).

A representative chromatogram, main peak profile and scan spectrum data of root extractive solution is presented in Figure 5. Chromatogram data was obtained by diode-array detector at 340 nm.

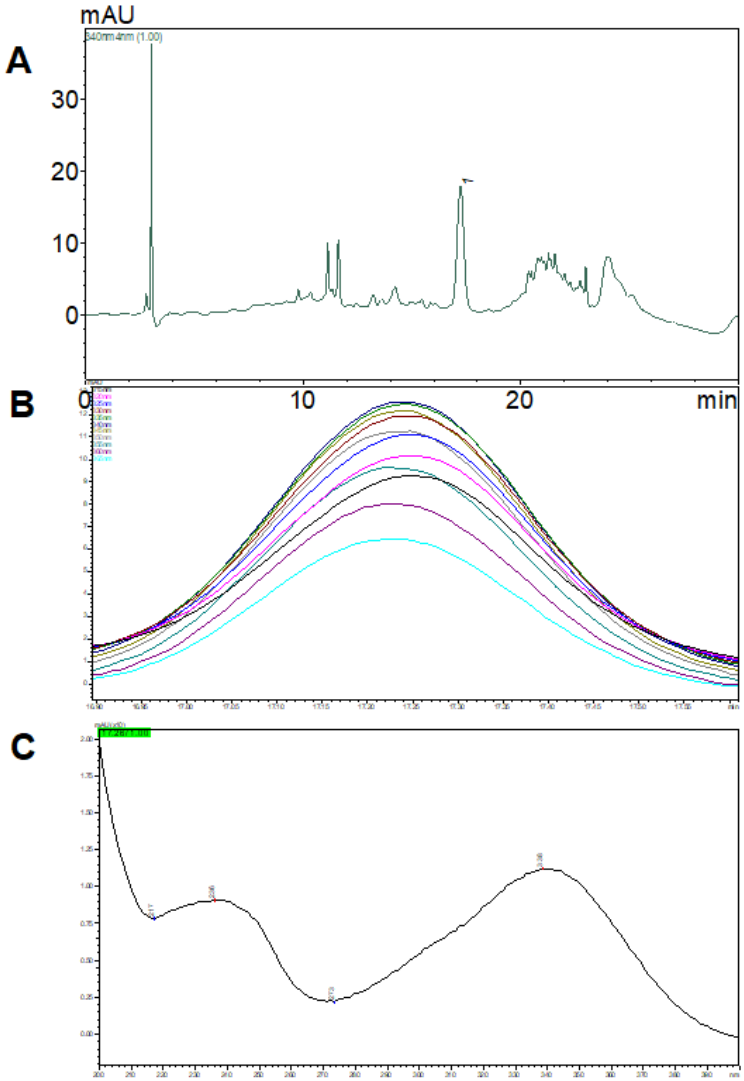

Figure 5. Representative chromatogram of extractive solution obtained from roots of *P. olacoides* at 340 nm (A). Peak profile (B) and spectrum scan of main phenolic compound (*peak 1)* obtained from 200 to 400 nm (C).

Figure 6 shows a pareto chart of standardized effects of *P. olacoides'* bark showing a significant effect of particle size range of the raw material (quadratic) and time of extraction variables (linear). Three effects are significant at 95% confidence level in the studied experimental domain ($P < 0.05$).

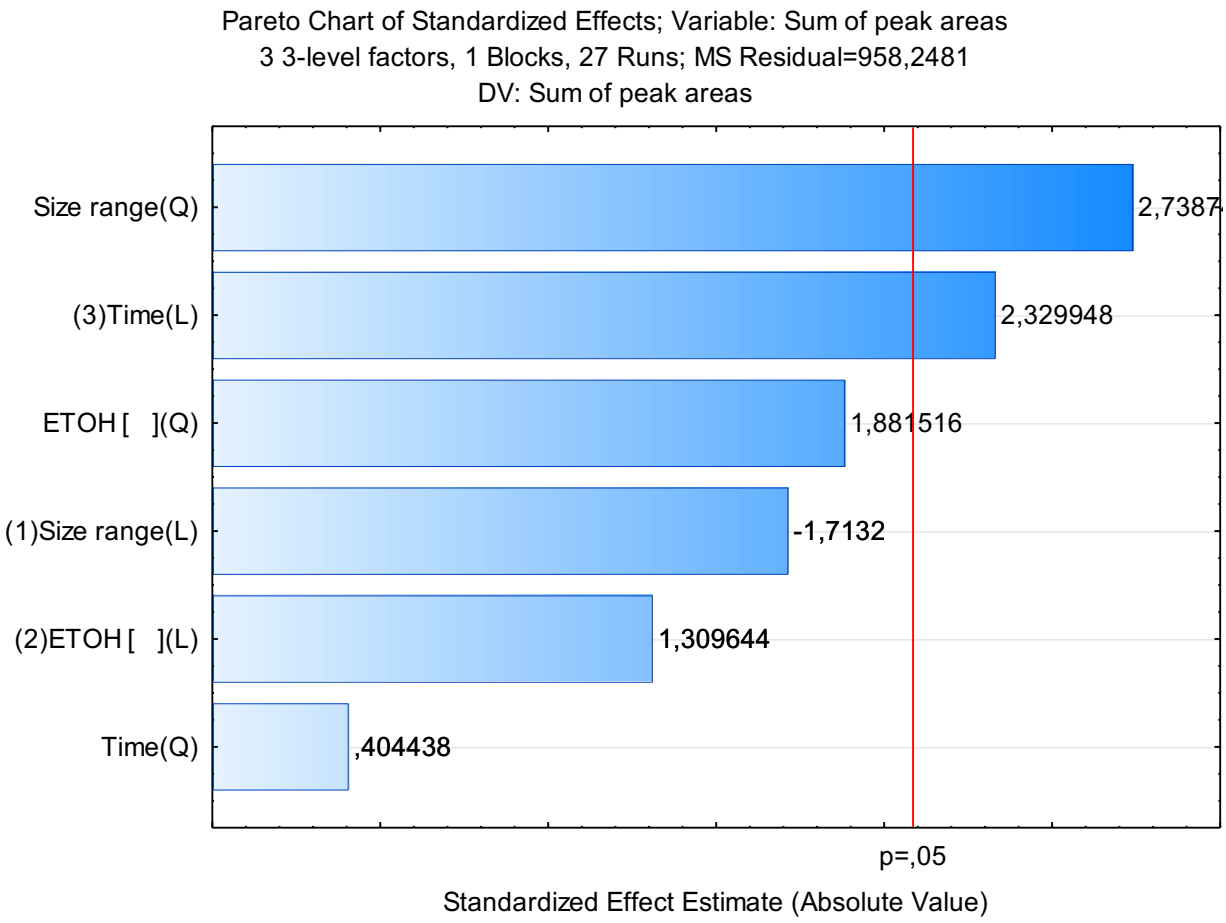

Figure 6. Pareto chart of effects for the total flavonoid content (TFC%) in bark of *P. olacoides.*

Figure 7 shows that the particle size of the bark raw material presents high influence on TFC%. Similar results were found in a published manuscript by Vieito and collaborators (2018) for extracts from *Pinus pinaster* subsp. atlantica.

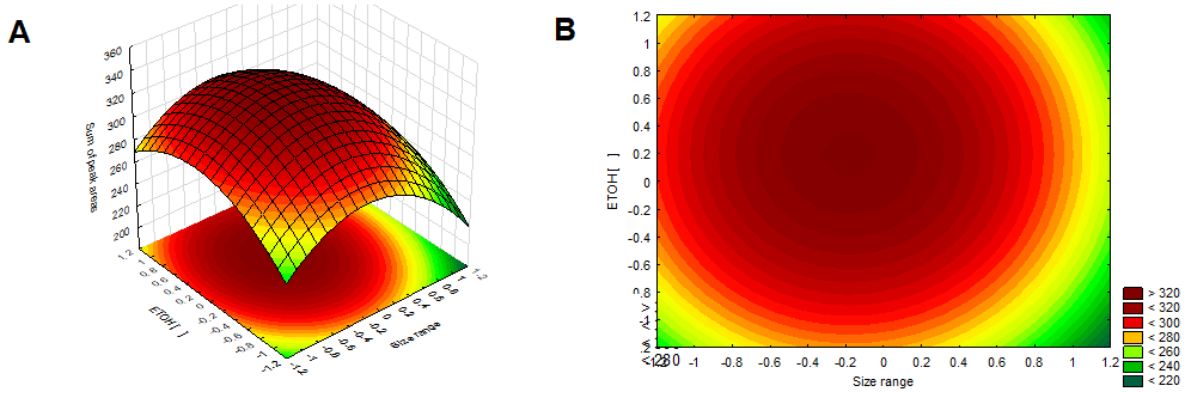

Figure 7. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in bark of *P. olacoides* as a function of particle size range (*x1*) and ethanol concentration (*x2*).

Total flavonoid content from bark parts of *P. olacoides* showed similar results of TFC% from root parts of this plant concerning the influence of ethanol concentration and high influence of particle size of raw material with no interference of extraction time on response, as showed in Figures 8 and 9.

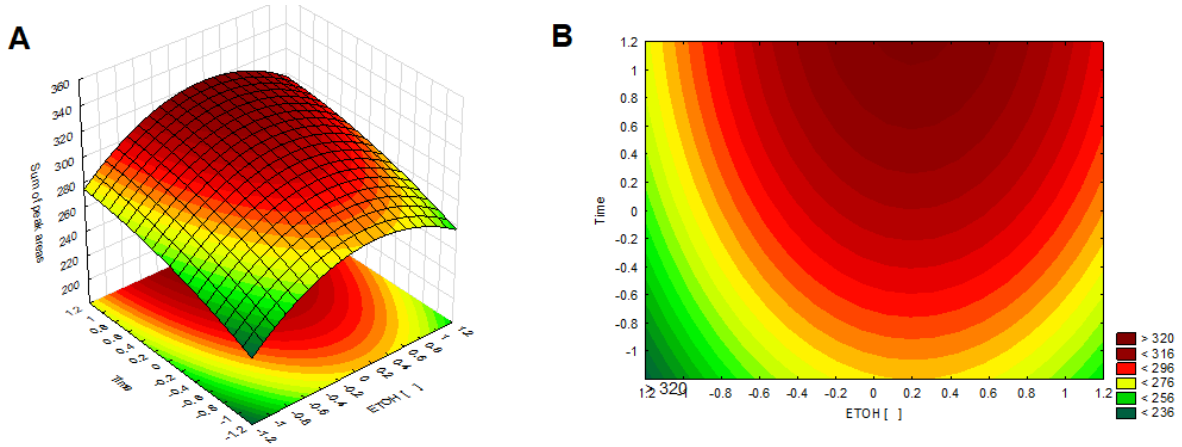

Figure 8. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in bark of *P. olacoides* as a function of ethanol concentration (*x2*) and time of extraction (*x3*).

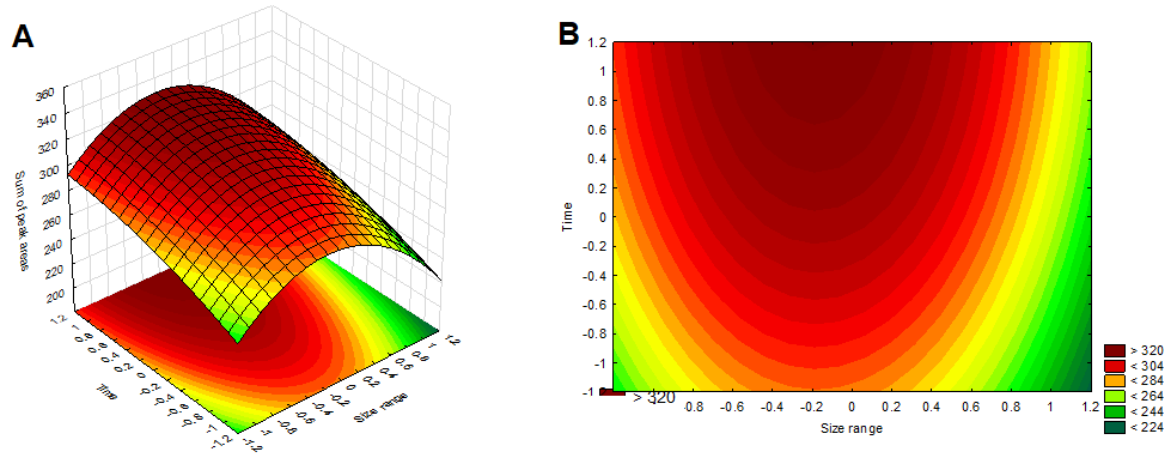

Figure 9. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in bark of *P. olacoides* as a function of particle size range (*x1*) and and time of extraction (*x3*).

A representative chromatogram, main peak profile and scan of bark extractive solution is presented in Figure 10. Chromatogram data was obtained by diode-array detector at 340 nm.

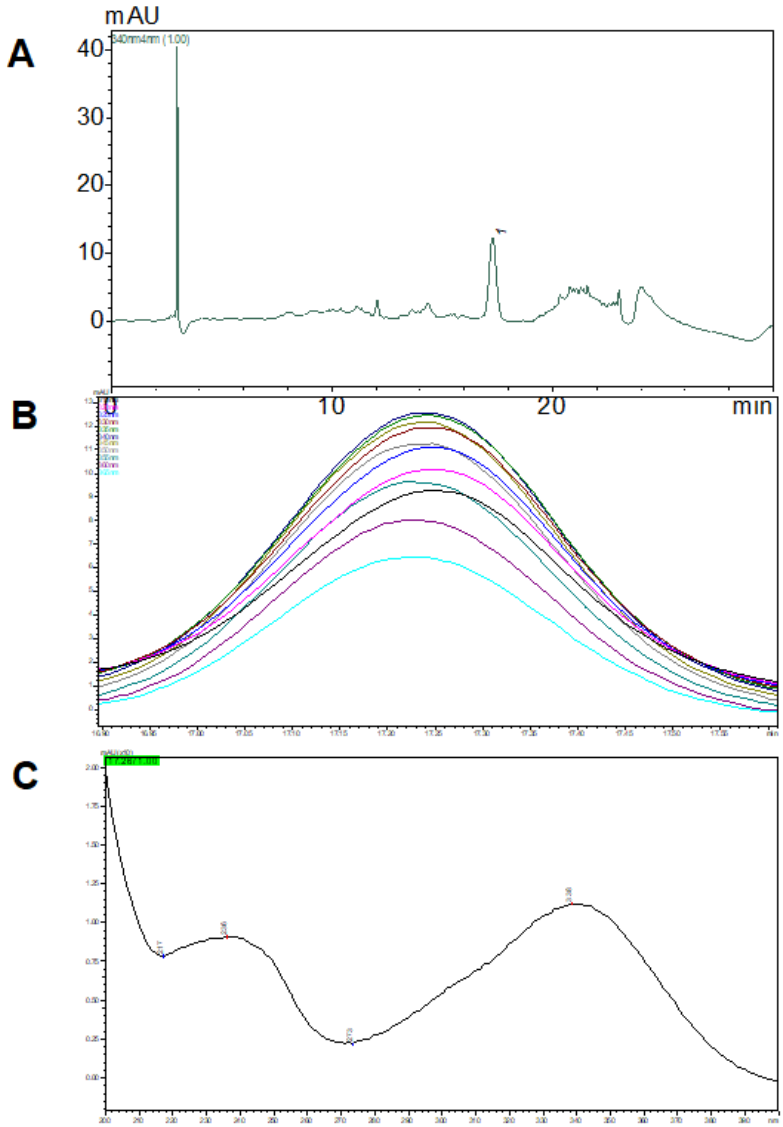

Figure 10. Representative chromatogram of extractive solution obtained from bark of *P. olacoides* at 340 nm (A). Peak profile (B) and spectrum scan of main phenolic compound (*peak 1)* obtained from 200 to 400 nm (C).

Figure 11 shows significant effect of particle size range of the leaves raw material (quadratic effect). The bar length of each parameters characterizes the absolute importance of the estimated effects. Only one effect is significant at 95% confidence level in the studied experimental domain ($P < 0.05$) as shown in Figure 11.

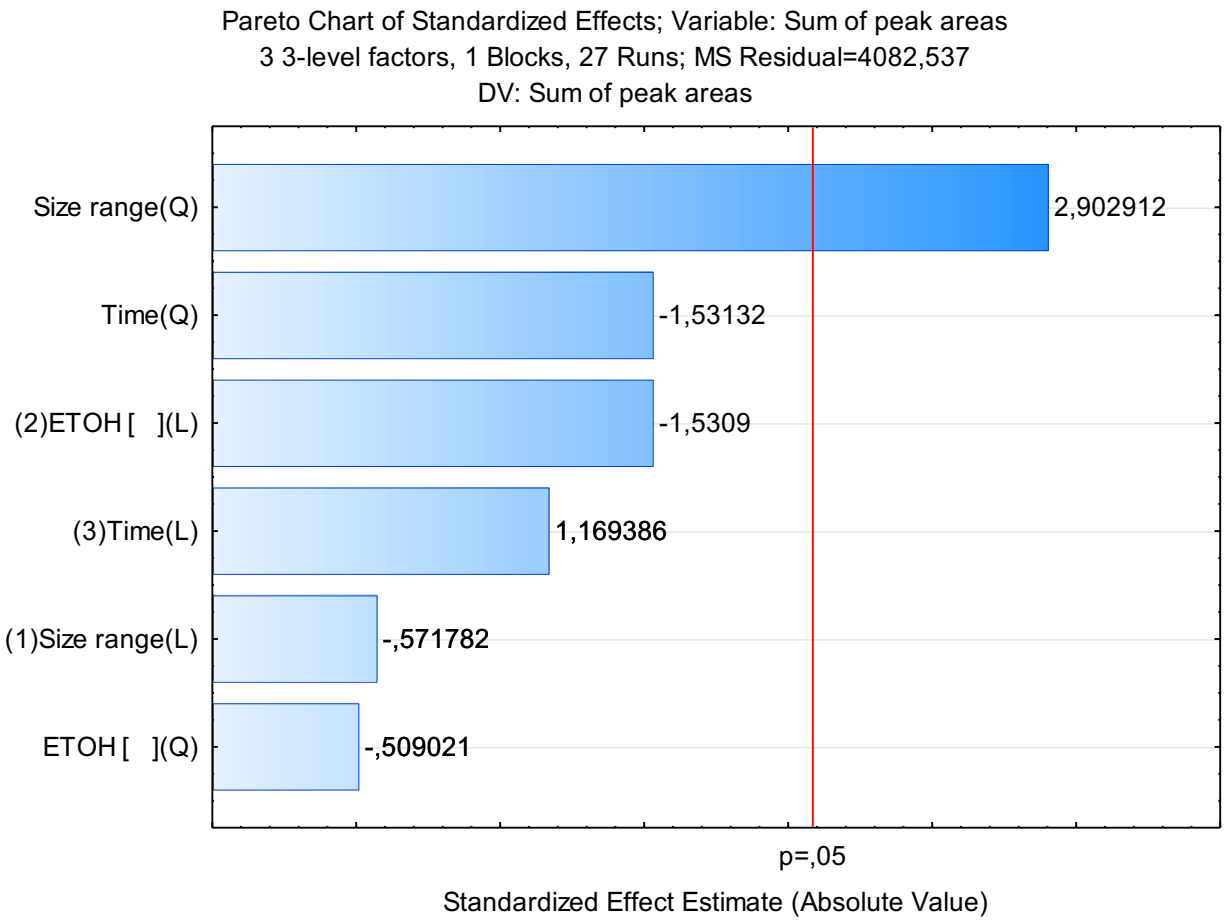

Figure 11. Pareto chart of effects for the total flavonoid content (TFC%) in leaves of *P. olacoides.*

Leaves of *P. olacoides* showed high concentration of total flavonoids (99.67%) when compared with its bark (8.59%) and root (9.93%) parts at the same conditions showed in Figure 12.

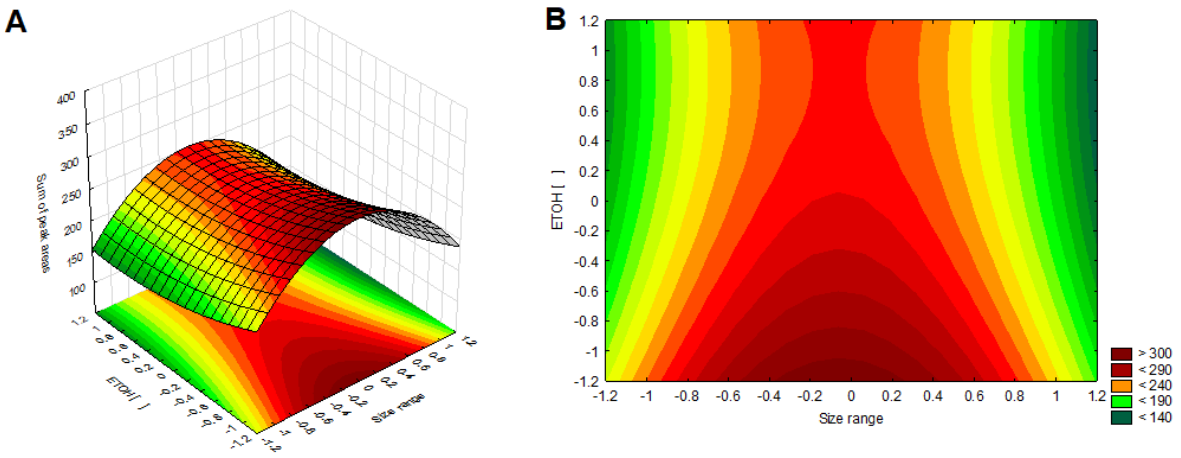

Figure 12. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in leaves of *P. olacoides* as a function of particle size range (*x1*) and ethanol concentration (*x2*).

Similar results found in root and bark extractive solutions were found in TFC% of the leaves extracts as presented in Figure 13 with no influence of extraction time on response.

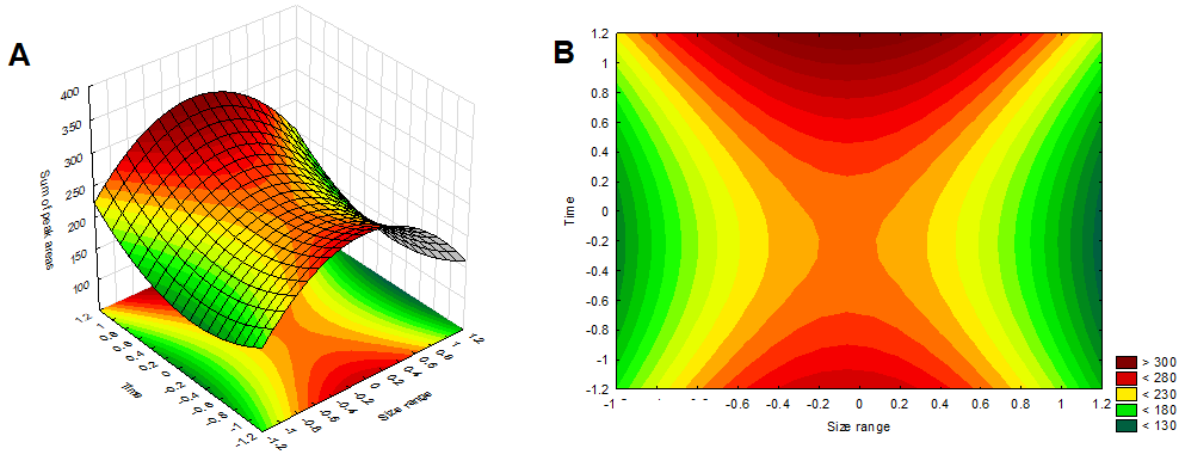

Figure 13. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in leaves of *P. olacoides* as a function of particle size range (*x1*) and time of extraction (*x3*).

Otherwise when investigated the ethanol concentration (*x2*) and time of extraction (*x3*) interactions, as showed in pareto chart, these factors present no significance on response (Figure 14).

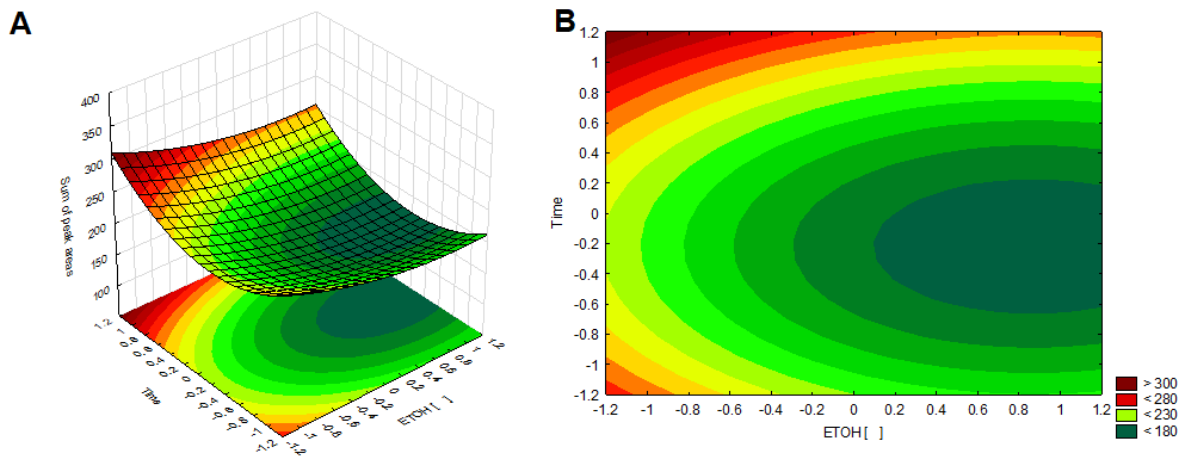

Figure 14. Response surface plot (A) and contour plot (B) of the total flavonoid content (TFC%) in leaves of *P. olacoides* as a function of ethanol concentration (*x2*) and time of extraction (*x3*).

The representative chromatogram of leaves extracts, with main peak profile and scan spectrum is presented in Figure 15. Chromatogram data was obtained by diode-array detector at 370 nm.

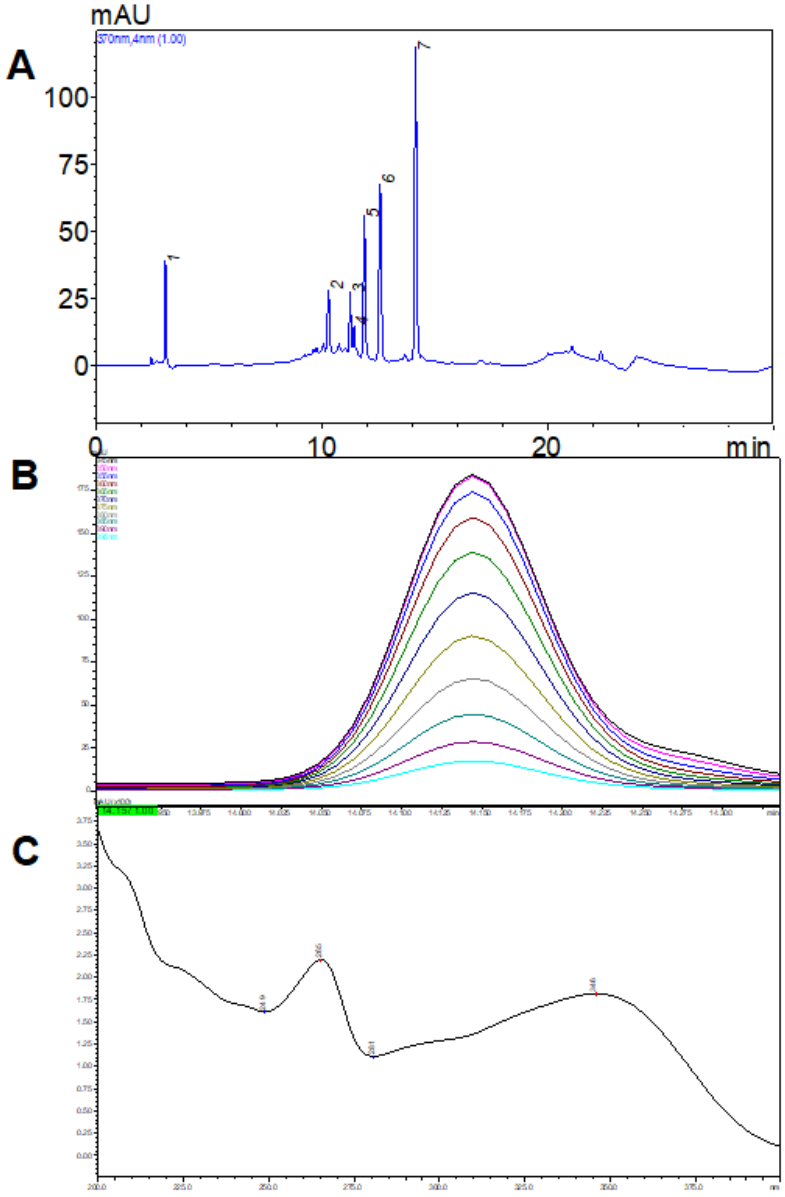

Figure 15. Representative chromatogram of extractive solution obtained from leaves of *P. olacoides* at 370 nm (A). Peak profile (B) and spectrum scan of main phenolic compound (*peak 7)* obtained from 200 to 400 nm (C).

The granulometry of the vegetable drug is considered to be one of the determining factors for the homogeneity and reproductibility of the extractive process. In comparison with the entire drug, the grated or ground matter obviously presents a greater contact surface with the extracting liquid, besides possessing a larger proportion of cells whose walls are ruptured, providing greater exposure of the cellular contents to the solvent (Schmidt,

1990). This fact can explain the factor *x1* (particle size range) as the most significant on the responses for leaves, bark and root of *P. olacoides* reinforcing that he total flavonoids contents was directly proportional to the decrease in particle size of raw material, which benefit the production of extracts richs in flavonoids, which are responsible for the pharmacological activity of the plant. Quercetin standard phenolic compound used in this work is show in Figure 16, which chromatogram was obtained at 370 nm.

UHPLC analysis was able to determine the extractive parameters for standardization of *P. olacoides*. Standard curve calibration of quercetin (n = 3) was linear (y = 0.0153x + 0.0205; R^2 = 0.9997).The conditions selected for obtaining an extractive solution with greater yield on flavonoid extraction were: extraction with ethanol 70°GL, granulometry about 63~150µm and time of 15 minutes. Under these conditions, the selected extractive solution contains 0.697g% of total flavonoids, expressed as quercetin.

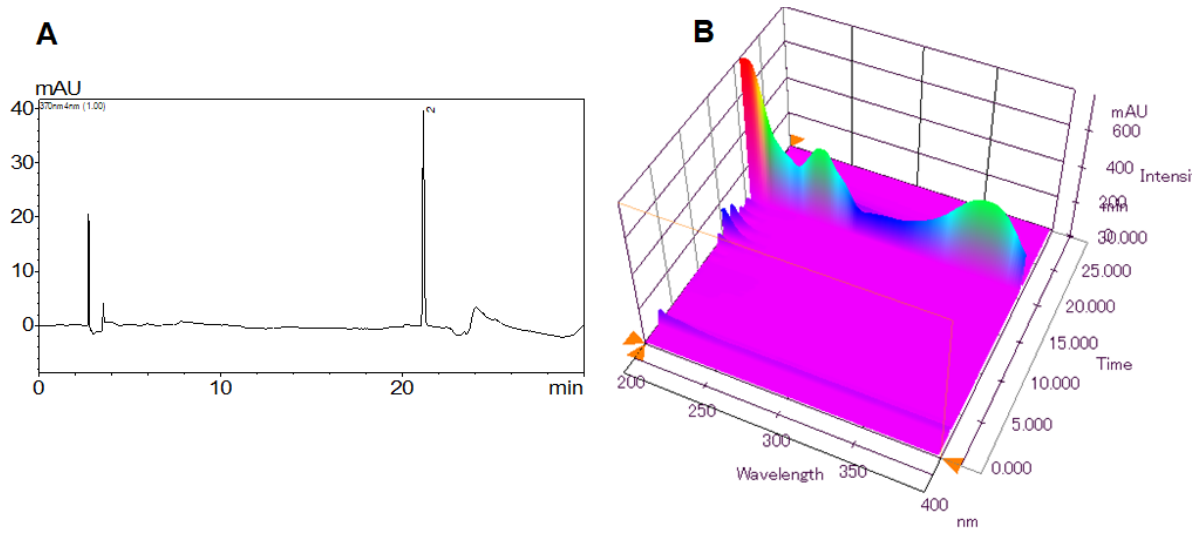

Figure 16. Representative chromatogram of quercetin standard solution (retention time of 21min) at 370 nm (A) and its 3D spectrum scan (B).

Comparing to HPLC conventional analysis reported by Zeirak and collaborators (2010), UHPLC method optimization showed advantages concerning operational costs. It could promote a decrease of analysis time and solvent consumption on mobile phase. Many authors have discussed about advantages of the use of green methodologies from UHPLC in analytical laboratories (Naváková et al, 2006; Xu, Q., 2013; Guillarme D.; Veuthey,J.L., 2012). The main advantage was especially a significant decrease of analysis time allowed by a shorter column (50 x 4.6 mm i.d., 2.7 µm of particle size) which meant also reduction in solvent consumption. In this case, the optimized method is more economically

convenient for routine procedures in quality control of phytomedicines than traditional methods.

3.1. NIRS analysis

For NIRS analysis, spectral pre-processing methods was applied to raw NIR spectral data in order to remove unwanted spectral variations due to baseline drifts, light scattering effects, temperature variations, and systematic noise (Figures 17 and 18).

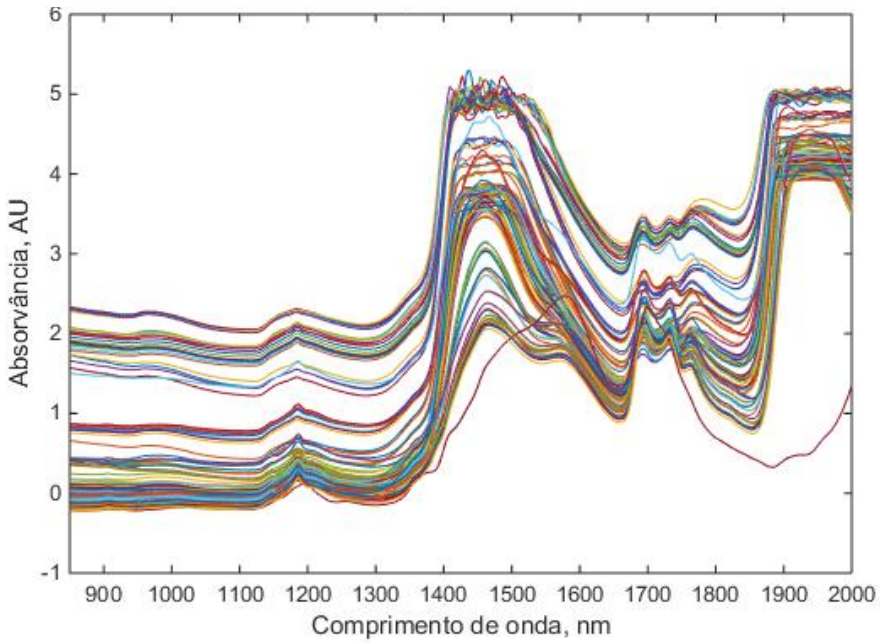

Figure 17. NIR spectral data obtained from 900 to 2000 nm with no mathematical treatment.

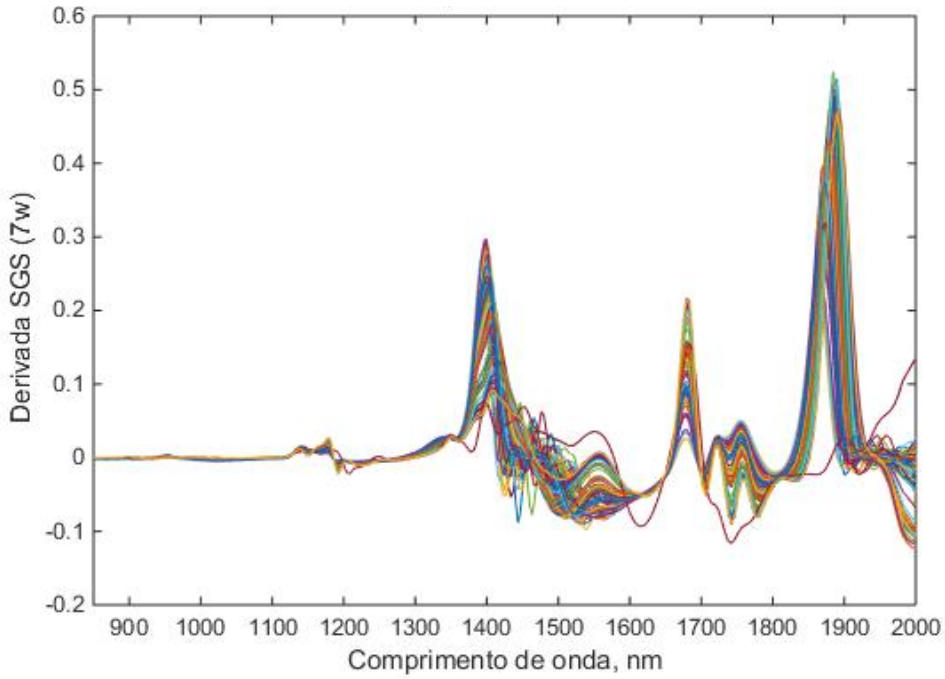

Figure 18. NIR spectral data obtained from 900 to 2000 nm after mathematical treatment.

The most commonly used include the multiplicative scatter correction (MSC), Norris derivative, and Savitzky–Golay (SG) first-order derivative. MSC is a transformation method used to compensate the offset shifts and other interference in the spectral data. The Norris derivative is a procedure to remove the effects of varying pathlengths because of sample scattering. The SG filter, on the other hand, is used to preserve features that are usually 'flattened' by other adjacent averaging techniques. The software to perform the analyzes was Matlab R2014b®, with the PLS_Toolbox 2014 package. Initially, the pre-treatments were: MC, MSC (average), SNV, SGS Smoothing (7w) and SGS Derivative (7w). individual and combined, to obtain a decomposition of the data by Principal Component Analysis (PCA) (Figures 19 - 22) and by Soft Independent Modelling of Class Analogies (SIMCA) (Figures 23 – 26).

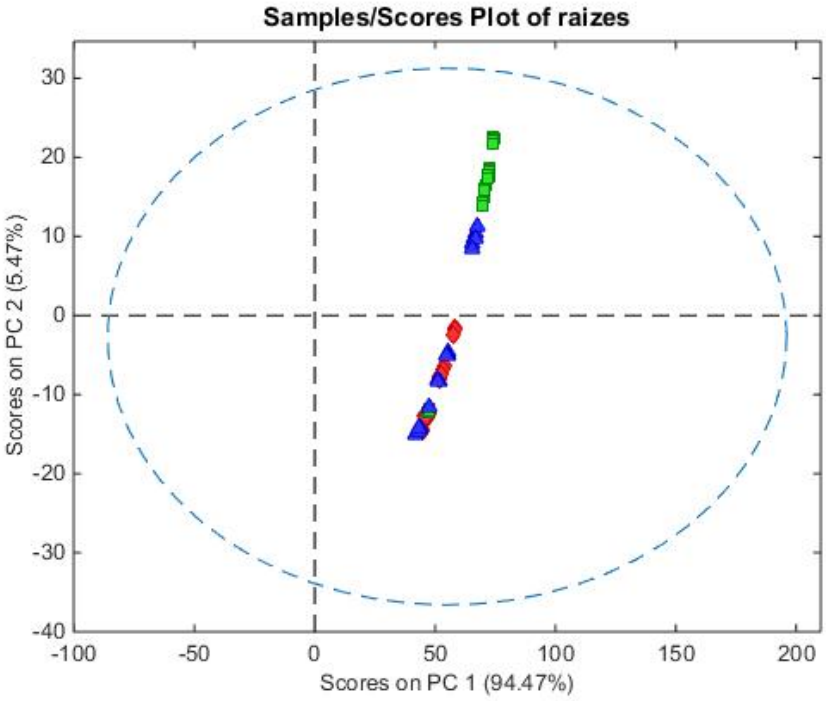

Figure 19. PCA of root extracts without pre-treatment

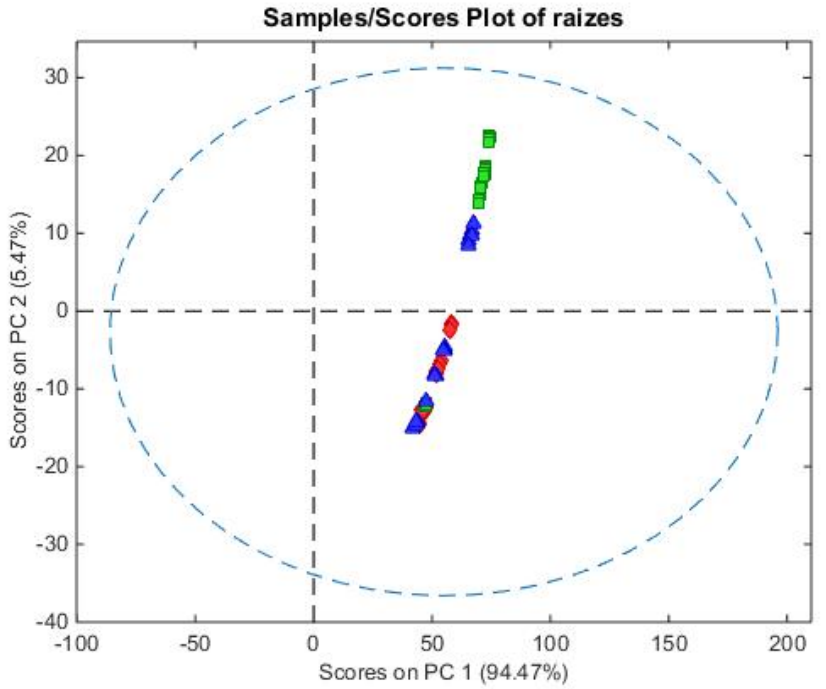

Figure 20. PCA of root extracts with SGS smoothing (7w)

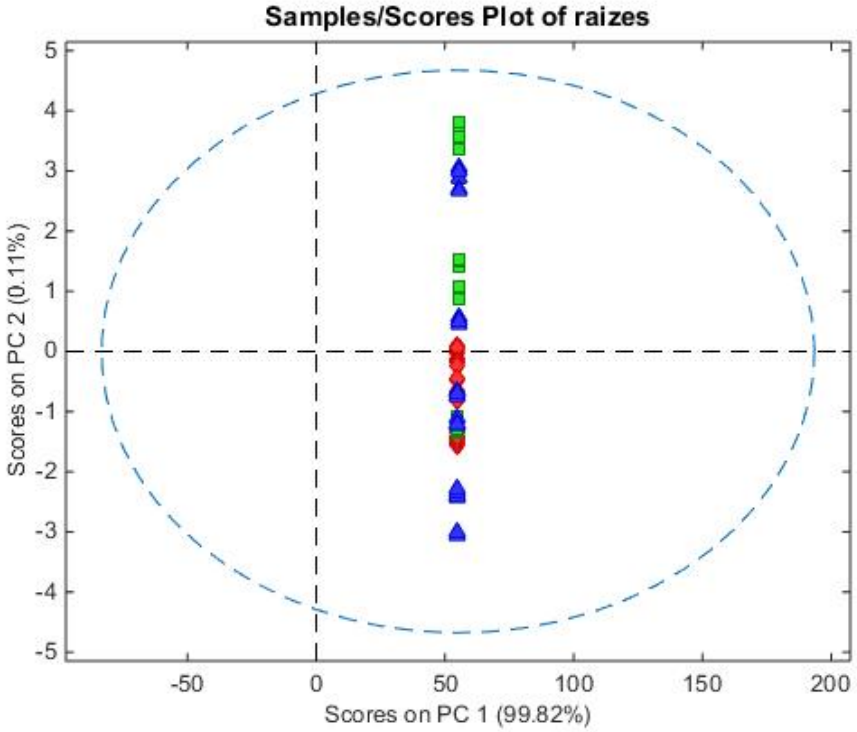

Figure 21. PCA of root extracts with MSC.

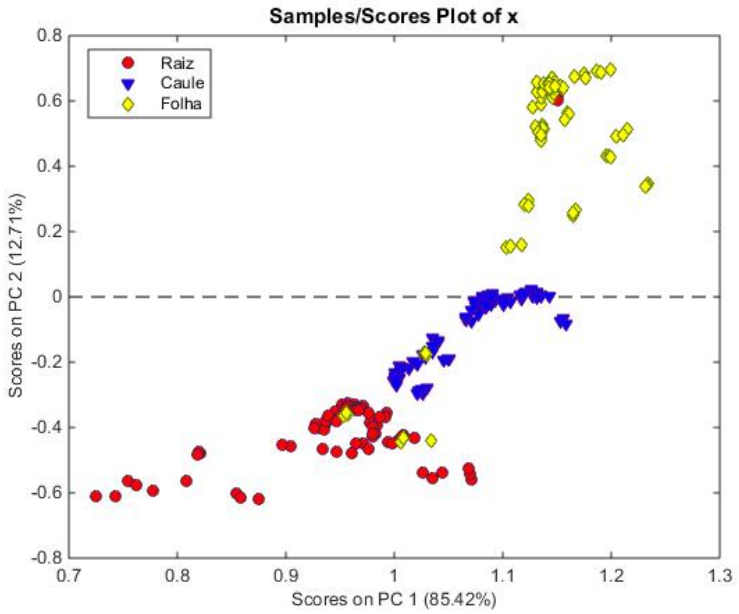

Figure 22. PCA of root, bark and leaves extracts with MSC.

According to these results, it was possible to verify that there were overlaps of the extracts in all pre-treatments inserted in the NIR spectra. The same event occurred for the spectra obtained from the bark and leaves. Most likely the information that alcohol provides to the

spectrum may overlap or interfere with its differentiation. Thus the spectrum were also treated with SIMCA.

In order to create SIMCA, it is necessary to classify known samples and perform in each one a decomposition into subgroups. Thus, as each sample was read in triplicate, it was possible to obtain a quantitative of 213 spectra, being 72 spectra at 50 ° GL, 72 spectra at 70 ° GL and 69 spectra at 90 ° GL. From the total of 213 spectra, 130 were chosen to carry out the construction of the method and 83 to test it, randomly.

Two methods were constructed with the following formatting, both with the derivative SGS (7w):

1) Sort by part of the plant (root, bark and leaves)

2) Classify by ethanol concentration (50, 70 and 90 ° GL)

Results are present in Figures 23 and 24.

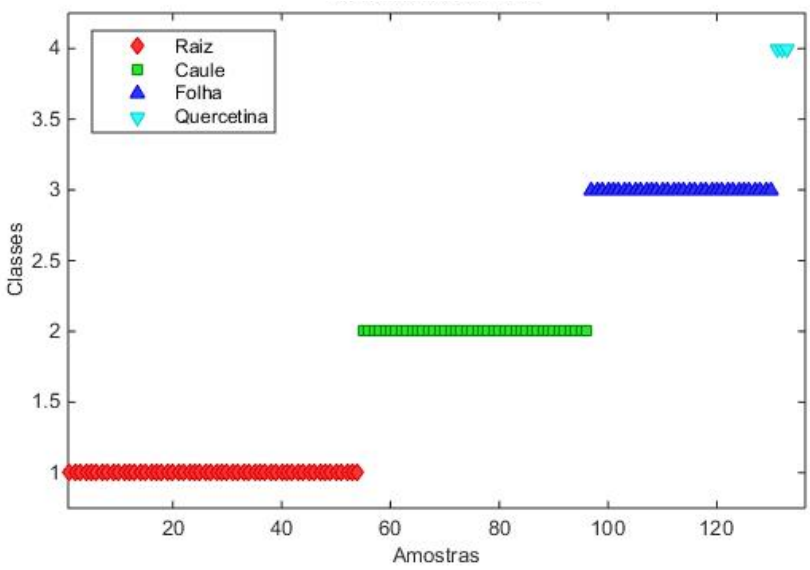

Figure 23. SIMCA model according to plant parts (root, bark and leaves)

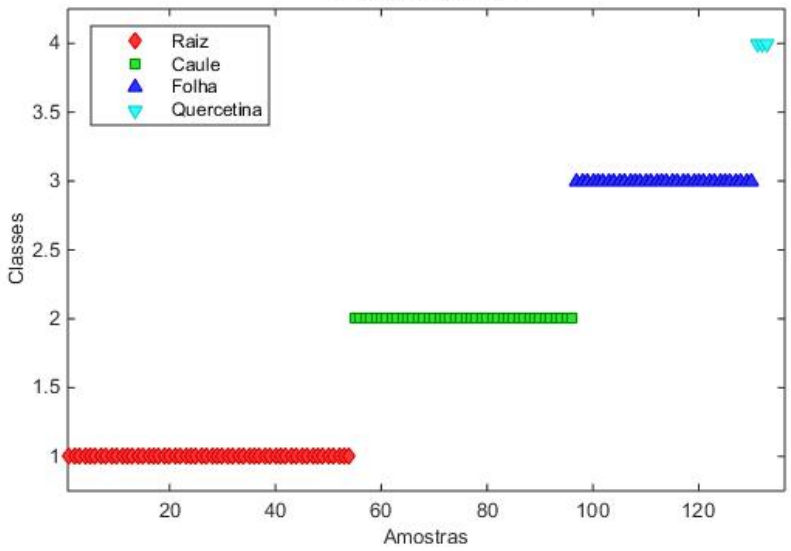

Figure 24. SIMCA model according to ethanol concentration of extractive solutions

After the creation of the models, the random samples (83) were inserted in the method to evaluate if a classification of which part would come from (Figures 25 and 26).

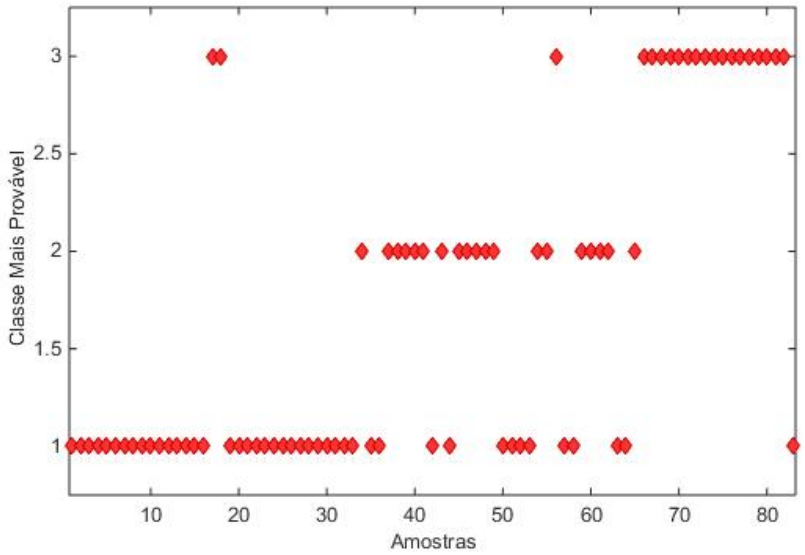

Figure 25. Predicting model classes by plant parts

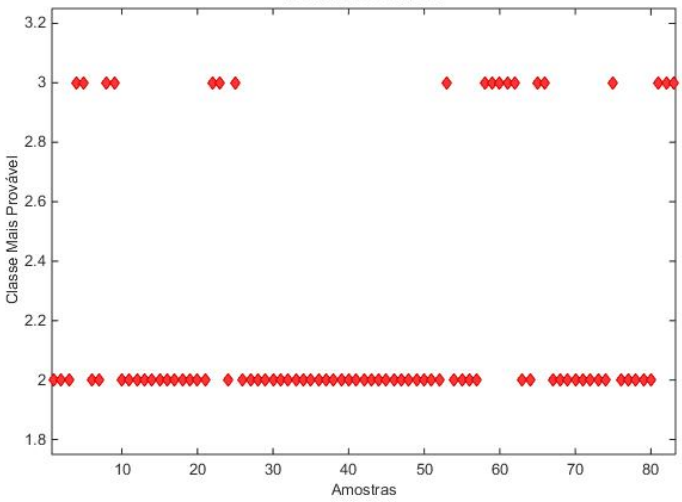

Figure 26. Predicting model classes according to ethanol concentration in extractive solutions

As the samples were known, it was possible to correlate them and calculate the percentage of correctness of each method. For the model created by plant parts, of the 83 samples, 21 were misclassified, resulting in a total of 74.70% method accuracy. For the model created for the ethanol concentration, 54 samples were classified imprecisely, resulting in an accuracy of 34.93%. This result shows that NIRS is not able to accurately quantify TFC% however the method can be applied for qualitative analysis once it can differentiate the parts of the plant by PCA.

Conclusions

Green analytical methods were applied in the analysis of phenolic compounds present in *P. olacoides* extracts. The experimental design showed that leaves bark and root can be quantitatively standardized by UHPLC and qualitatively by NIRS. Both methodologies are able to analyze phytomedicines concerning a safe analysis with a minimum or no residue to the environment. Modern analytical technologies are strongly recommended to be applied in the various field of science. This present work shows that the methodologies are innovative in the field of quality control and pharmaceutical development of phytomedicines.

A NIRS method was developed for qualitatively determining phenolic compounds in muirapuama extracts with minimal sample treatment and no use of solvents and reagents.

It could distinguish among leaves, bark or root samples being indispensable in the correct identification as the part of the plant in which the extractive solution was prepared. The model developed by NIR spectroscopy for *P. olacoides* can be used as an alternative methodology for phytopharmaceuticals analysis.

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6 CONSIDERAÇÕES FINAIS

- A *Ptychopetalum olacoides* apresentou diversas atividades farmacológicas, indicando seu potencial no tratamento de vários distúrbios;
- Várias patentes são registradas no Brasil e no mundo;
- Estudos mais detalhados são necessários para isolar seus compostos e determinar seus mecanismos de ação e seletividade;
- O planejamento fatorial permitiu a padronização da solução extrativa, oferecendo aos usuários de fitoterápicos, segurança, eficácia e qualidade;
- A aplicação das metodologias analíticas CLUE-DAD e NIR foram capazes de analisar as soluções extrativas de forma segura com um mínimo ou nenhum resíduo ao meio ambiente;

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ANEXOS

Anexo 1 – Comprovante de cadastro no SISGEN

Anexo 2 – Normas de publicação da revista *Phytochemistry Reviews*

Anexo 3 – Comprovante de submissão à revista *Phytochemistry Reviews*

Anexo 4 – Normas de publicação da revista *Microchemical Journal*

Anexo 1 – Comprovante de cadastro no SISGEN

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº AF451C5

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**AF451C5**
Usuário:	**Franklin Teixeira Regis**
CPF/CNPJ:	**965.893.102-20**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Ptychopetalum olacoides

Título da Atividade:	**Aplicação das metodologias analíticas CLUE-DAD e NIR para a padronização de soluções extrativas das folhas, cascas e raiz de Ptychopetalum olacoides Benth (Olacaceae).**

Equipe

Franklin Teixeira Regis	**Universidade Federal do Amapá**
Lilian Grace da Silva Solon	**Universidade Federal do Amapá**

Data do Cadastro:	**15/05/2019 21:40:29**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **9:33** de **18/05/2019**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

Anexo 2 – Normas de publicação da revista *Phytochemistry Reviews*

Manuscript submission

Online Manuscript Submission

Springer now offers authors, editors and reviewers of *Phytochemistry Reviews* the option of using our fully web-enabled online manuscript submission and review system. To keep the review time as short as possible (no postal delays!), we encourage authors to submit manuscripts online to the journal‘s editorial
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Anexo 3 – Comprovante de submissão à revista *Phytochemistry Reviews*

Phytochemistry Reviews

Ptychopetalum olacoides (muirapuama), a traditional Amazonian "nerve tonic": Patents, phytochemistry and biological activities review

--Manuscript Draft--

Manuscript Number:	
Full Title:	Ptychopetalum olacoides (muirapuama), a traditional Amazonian "nerve tonic": Patents, phytochemistry and biological activities review
Article Type:	Review Article
Keywords:	Ptychopetalum olacoides. Muirapuama. Literature review. Patent review. Forest management.
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Funding Information:	
Abstract:	Ptychopetalum olacoides Benth is a plant of the family Olacacea, popularly known as muirapuama, murapuama and miratã. It is widely used in Amazonian communities to treat nervous diseases, sexual impotence and to improve physical and cognitive activities. Many scientific papers can be found in the literature regarding the pharmacological importance of this natural product. Antidepressant, anxiogenic and anti-stress effects are the main pharmacological activity of this specie. Nevertheless, to date, none of these studies has presented a systematic review of literature neither a technological mapping of patents. This review aimed to summarize all data from literature and patents involving Ptychopetalum olacoides Benth. To that end, a patent research was performed in the international patent database. For literature review online bibliographical databases were used: PubMed, Science Direct, Scopus and Google Scholar. On Patentscope database results showed a total of 27 patents. Chemical compounds and pharmacological activities found in literature were also compiled. The studies also showed that in Amazonia this species is exploited in an extractive way with serious risks to biodiversity. This review presents different technological approaches to apply Ptychopetalum olacoides and emphasizes its great pharmacological and economic potential for the development of new products and discusses actions for its cultivation in a sustainable way.

Anexo 4 – Normas de publicação da revista *Microchemical Journal*

The Microchemical Journal is a peer reviewed journal devoted to all aspects and phases of analytical chemistry and chemical analysis. *The Microchemical Journal* publishes articles which are at the forefront of modern analytical chemistry and cover innovations in the techniques to the finest possible limits. This includes fundamental aspects, instrumentation, new developments, methods and applications including environmental and clinical analysis. Traditional classical methods such as spectrophotometry and titrimetry as well as instrumentation methods such as flame atomic absorption spectrometry and gas chromatography will be considered, provided they show significant improvements and novelty compared to the established methods. The journal was established in the late 1950's as a journal devoted to the rapidly developing field of microchemistry. As the area of microchemistry has evolved into analyses in microgram masses and microvolumes (and lower), lower detection limits, and more sophisticated and compact instrumentation, the Microchemical Journal has continued to evolve and change with this growing and expanding area, covering now analytical chemical research in its broadest sense.

Types of Paper

The Journal publishes original research papers and reviews. Authors of reviews should check with the Editor prior to submission to ensure that the topic is appropriate for the Journal.

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