PROGRAMA DE PÓS-GRADUAÇÃO EM ALIMENTOS E NUTRIÇÃO - PPGAN
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE – CCBS
UNIVERSIDADE FEDERAL DO ESTADO DO RIO DE JANEIRO – UNIRIO

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Bauhinia forficata L.(pata-de-vaca) - caracterização química e avaliação do potencial bioativo de amostras in natura e comerciais.

Bauhinia forficata L.(pata-de-vaca) - chemical characterization and evaluation of the bioactive potential of in natura and commercial samples.

RIO DE JANEIRO

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Orientador: Prof. Dr. Ricardo Felipe Alves Moreira
Co-orientador: Dr. Leilson de Oliveira Ribeiro

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Aprovada em: 28/04/2022

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29/04/2022 17:01:49 - Assinatura realizada por bethfai@yahoo.com.br, IP 201.17.126.3
29/04/2022 15:42:42 - Assinatura realizada por fernandakamp@gmail.com, IP 200.20.215.129
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PV895

Przytyk Jung, Eliane
Bauhinia forficata L.(pata-de-vaca) - caracterização química e avaliação do potencial bioativo de amostras in natura e comerciais. / Eliane Przytyk Jung. -- Rio de Janeiro, 2022.
163

Orientador: Ricardo Felipe Alves Moreira.
Coorientador: Leilson de Oliveira Ribeiro.
Tese (Doutorado) - Universidade Federal do Estado do Rio de Janeiro, Programa de Pós-Graduação em Alimentos e Nutrição, 2022.

1. Bauhinia forficata. 2. óleos essenciais. 3. infusões. 4. atividade antioxidante. 5. enzimas digestivas. I. Alves Moreira, Ricardo Felipe, orient. II. de Oliveira Ribeiro, Leilson, coorient. III. Título.

Dedico este trabalho aos meus filhos, Henrique e André
por serem a mola que impulsiona a minha vida.

AGRADECIMENTOS

A Deus por ter me concedido saúde, inspiração, aprendizado e crescimento durante essa jornada.

Ao meu orientador Ricardo, por ter me aberto sua porta em um momento de desespero e pressa.

Ao meu co-orientador Leilson, que ao longo dessa jornada se tornou um grande amigo e parceiro de trabalho.

À minha família, mãe e pai (*in memoriam*), por terem sempre me mostrado que o conhecimento é a nossa maior riqueza e que a formação intelectual deve estar acima de tudo, pois essa ninguém nos rouba.

Aos amigos do PPGAN, principalmente Thiago, Pryscila e Cátia, parceiros de seminários, aulas e muitas risadas.

Aos companheiros de trabalho do INT, principalmente ao pessoal do LAQOI, Claudete, Alex e Vivianne, pela compreensão com meus sumiços repentinos. Bia, pelo apoio nas análises. E Natália, pela parceria no LC.

Aos meus amados filhos Henrique e André, por serem a luz na minha vida, iluminando o meu caminho sempre!

E ao meu melhor amigo, marido e parceiro de vida, Felipe. Quando eu caí, você me levantou. Quando achei que não conseguiria dar conta de tudo, você estava lá me fazendo acreditar que eu era capaz!!! Love you!!!

“Olha lá quem vem do lado oposto
E vem sem gosto de viver
Olha lá que os bravos são escravos
Sãos e salvos de sofrer
Olha lá quem acha que perder é ser
Menor na vida
Olha lá quem sempre quer vitória
E perde a glória de chorar
Eu que já não quero mais
Ser um vencedor
Levo a vida devagar
Pra não faltar amor
Olha você e diz que não
Vive a esconder o coração
Não faz isso, amigo
Já se sabe que você
Só procura abrigo
Mas não deixa ninguém ver
Por que será?
Eu que nunca fui assim
Muito de ganhar
Junto as mãos ao meu redor
Faço o melhor que sou capaz
Só pra viver em paz”

Marcelo Camelo

RESUMO

O gênero *Bauhinia* compreende 300 espécies diferentes distribuídas em florestas tropicais e subtropicais. Infusões de algumas espécies têm sido frequentemente utilizadas na medicina popular para o tratamento de diversas doenças, especialmente a diabetes. A infusão de *Bauhinia forficata* é amplamente utilizada no Brasil, destacando-se seu papel como coadjuvante no tratamento da diabetes mellitus tipo 2. Entretanto, não há dados na literatura quanto à caracterização química e ao potencial bioativo de amostras comerciais. Nesse sentido, o presente trabalho foi direcionado para a caracterização química e avaliação do potencial bioativo de amostras comerciais bem como de amostras botanicamente identificadas. Primeiramente, foi feita uma avaliação quanto à conformidade dos rótulos e peso, frente à legislação vigente. Em geral, as amostras comerciais estavam de acordo com a legislação. Em seguida, os óleos essenciais das amostras foram isolados e seus compostos identificados por CG-EM, com posterior análise quimiométrica, sendo possível identificar 116 compostos presentes nessas frações, sendo estes compostos majoritariamente sesquiterpenos. Onze compostos apareceram em todos os óleos essenciais avaliados, em concentrações relevantes, podendo indicar que as amostras comerciais se tratavam da espécie em questão. As infusões preparadas na concentração de 5% foram avaliadas quanto as características químicas de suas frações volátil e não volátil. Os teores de compostos fenólicos totais e capacidade antioxidante variaram de 1923 a 6355 mg ER/100 g e 482 a 3700 mg ER/100 g, respectivamente, mostrando que as amostras (*in natura* e comerciais de diferentes lotes e marcas) diferiam ($p < 0,05$) com relação a esses parâmetros. Vinte e um compostos não voláteis foram tentativamente identificados nas infusões, sendo o perfil majoritariamente formado por flavonoides glicosilados. Apenas uma amostra comercial apresentou atividade antimicrobiana contra *Staphylococcus aureus* (MBC: 0.39 ER mg/mL). Quanto à inibição da α-amilase, todas as amostras foram capazes de inibir essa enzima digestiva apresentando valores de CI_{50} entre 0,235 e 0,489 mg/mL. A microextração em fase sólida do *headspace* das

infusões seguida da análise por cromatografia gasosa acoplada à espectrometria de massas (HS-SPME/GC–MS) foi aplicada para identificar, pela primeira vez, os compostos voláteis presentes nessas bebidas. Quarenta compostos foram identificados, com destaque para a presença de sesquiterpenóides. Por fim, foi realizada uma otimização da recuperação dos compostos antioxidantes a partir das foçhas de *B. forficata* botanicamente identificada, com aplicação de um delineamento composto central rotacional, combinando as condições operacionais de temperatura (32-74 ºC), percentual de etanol na solução extratora (13-97%) e a razão sólido-líquido (1:10-1:60; g/mL). Os resultados, avaliados estatisticamente, indicaram que as condições operacionais ótimas para a recuperação dos compostos antioxidantes das folhas da planta foram 73,5 °C, utilizando etanol 51% como solvente e uma razão sólido-líquido de 1:40(g/mL). Assim como nas infusões, esse extrato foi principalmente composto de derivados glicosilados do kaempferol e da quercetina. Dessa forma, apesar de terem sido verificadas diferenças entre as amostras aqui avaliadas, a *B. forficata* apresenta-se como uma fonte de compostos bioativos, que pode aumentar a ingestão de compostos antioxidantes pela população que faz uso de suas infusões domésticas, além de poder ser utilizada na elaboração de fitoterápicos por meio do extrato antioxidante aqui otimizado. Nota-se que uma padronização das amostras é necessária para garantir que todos os benefícios associados à planta sejam entregues ao consumidor.

Palavras-chaves: *Bauhinia forficata*, óleos essenciais, infusões, atividade antioxidante, enzimas digestivas

ABSTRACT

The genus Bauhinia comprises 300 different species distributed in tropical and subtropical forests. Infusions of some species have been frequently used in folk medicine for the treatment of various diseases, especially diabetes. *Bauhinia forficata* infusion is widely used in Brazil, highlighting its role as an adjunct in the treatment of type 2 diabetes mellitus. However, there are no data in the literature regarding the chemical characterization and bioactive potential of commercial samples. In this sense, the present work aimed to evaluate the chemical profile and bioactive potential of commercial samples and, additionaly, of botanically identified samples. First, an assessment was made regarding the compliance of the labels and weights against the current legislation. In general, the commercial samples were in accordance with the legislation. Next, the essential oils of the samples were isolated and their compounds identified by GC-MS, with subsequent chemometric analysis. It was possible to identify 116 compounds present in this fraction, being most of them sesquiterpenes. Eleven compounds appeared in all the essential oils evaluated, in relevant concentrations, which may indicate that the commercial samples were actually *Bauhinia forficata*. Infusions prepared at a concentration of 5% were evaluated for the chemical characteristics of their volatile and non-volatile fractions. The content of total phenolic compounds and antioxidant capacity ranged from 1923 to 6355 mg RE/100 g, and 482 to 3700 mg RE/100 g, respectively, showing that the samples (*in natura* and commercial samples of different batches and brands) were different ($p<0.05$) regarding these parameters. Twenty one non volatile compounds were tentatively identified in the infusions, the profile being mostly composed of glycosylated flavonoids. Only one commercial sample showed antimicrobial activity against *Staphylococcus aureus* (MBC: 0.39 RE mg/mL). As for the inhibition of α-amylase, all samples were able to inhibit this digestive enzyme, presenting IC_{50} values between 0.235 and 0.489 mg/mL. Solid phase microextraction of the infusion headspace followed by gas

chromatography analysis coupled to mass spectrometry (HS-SPME–GC–MS) was applied to identify, for the first time, the volatile compounds present in the beverages. More than 20 compounds were identified, highlighting the presence of sesquiterpenoids. Finally, an optimization of the recovery of the antioxidant compounds from the botanically identified *B. forficata* samples was carried out through a rotational central composite design, combining the operating conditions of temperature (32-74ºC), percentage of ethanol in the extracting solution (13-97 %) and the solid-liquid ratio (1:10-1:60; g/mL). The results, evaluated statistically, indicated that the optimal operating conditions for the recovery of antioxidant compounds from the leaves of the plant were 73.5°C, using a 51% ethanol solution as solvent and a solid-liquid ratio of 1:40 (g/mL). As with the infusions, this extract was mainly composed of glycosylated derivatives of kaempferol and quercetin. Thus, despite differences between the samples evaluated here, *B. forficata* presents itself as a source of bioactive compounds, which can increase the intake of antioxidant compounds by people who use its infusions. Additionally, it can be used in the elaboration of phytotherapics from the antioxidant extract here optimized. It is noted that a standardization of the samples is necessary to ensure that all benefits associated with the plant are delivered for the consumer.

Keywords: *Bauhinia forficata*, essential oils, infusions, antioxidant activity, digestive enzymes.

SUMMARY

LISTA DE ILUSTRAÇÕES

FIGURAS

LISTA DE TABELAS

INTRODUÇÃO GERAL

A utilização de plantas com fins medicinais, para tratamento, cura e prevenção de doenças, é uma das mais antigas formas de prática medicinal da humanidade, com um contínuo e crescente interesse na área de fitoterapia, principalmente, com relação à identidade, qualidade e eficácia de fitoterápicos, representando um importante campo de estudo em áreas como medicina, biologia, farmácia e química.

Os recentes avanços em tecnologias analíticas e de quimiometria, além de áreas como genômica, proteômica, metabolômica, têm impulsionado estudos na área de produtos naturais, permitindo estudos mais abrangentes do que os baseados nas técnicas de isolamento tradicionais. Destaca-se o interesse nos tópicos de identificação, qualidade, autenticação de substâncias/extratos ativos, além da prospecção de novos medicamentos à base de produtos naturais, no sentido de contribuir para a elucidação dos efeitos farmacológicos, toxicológicos e a eficácia das ervas medicinais. Pesquisas para a identificação e autenticação de extratos vegetais se mostram valiosas, visto que mais de 95% da biodiversidade mundial ainda não foi avaliada para qualquer atividade biológica (DAVID *et al.*; 2015).

Estima-se que cerca de 65-80% da população dos países em desenvolvimento utilizam ervas medicinais em seus cuidados com a saúde, seja pelo conhecimento da medicina popular, medicina indígena, quilombola ou científica (BRASIL, 2012; CRUZ; GONÇALVES, 2022), impulsionando a elaboração de políticas públicas voltadas para esta área, em consonância com a Organização Mundial de Saúde, a qual reconhece e incentiva o desenvolvimento da fitoterapia por seus países membros. Assim, em 2006, a fitoterapia como prática integrativa complementar para tratamento de pacientes no sistema público de saúde (SUS) foi aprovada no Brasil. A seguir, em 2008 o Programa Nacional de Plantas Medicinais e Fitoterápicos aprovou o uso de 12 plantas para tratamento e prevenção de doenças no SUS, além de elencar 71 plantas medicinais, com uso disseminado pela população, na Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde (RENISUS), programa federal brasileiro que objetiva a promoção de pesquisa, desenvolvimento e inovação a fim de regulamentar o uso de fitoterápicos e insumos à base de plantas medicinais. *Bauhinia* spp. consta da RENISUS, representada pelas espécies *Bauhinia forficata*, *Bauhinia variegata e Bauhinia affinis*.

A *Bauhinia forficata* desperta o interesse da comunidade científica por apresentar atividade frente a uma diversidade de patologias, com destaque para a Diabete Melito tipo II. Seus efeitos benéficos têm sido associados à fração de flavonoides glicosilados, ainda que não se tenha estabelecido na literatura, marcadores químicos fidedignos para a padronização desta fração. Além disso, o conhecimento científico sobre a fração volátil desta espécie, fração esta rica em compostos com reconhecida atividade biológica que, portanto, não deveriam ser negligenciados em estudos que relacionam composição e atividade.

Outro ponto de atenção é a falta de regulação na comercialização destas ervas, uma vez que são isentas de registro na ANVISA e estão sujeitas a regulação de alimentos, o que pode facilitar fraudes e adulterações. Assim, uma análise química abrangente, compreendendo diferentes frações, torna-se uma ferramenta indispensável, pois através dela é possível contribuir para a elucidação química do material vegetal, em sua totalidade, garantindo a qualidade e segurança na sua comercialização.

GENERAL INTRODUCTION

The use of plants for medicinal purposes, for the treatment, cure and prevention of diseases, is one of the oldest forms of medicinal practice of humanity, with a continuous and growing interest in the field of phytotherapy, mainly in relation to identity, quality and effectiveness of herbal medicines, representing an important field of study in areas such as medicine, biology, pharmacy and chemistry.

Recent advances in analytical and bioinformatics technologies, as well as areas such as genomics, proteomics, metabolomics, have boosted research in the area of natural products, allowing for more comprehensive research than those based on traditional isolation techniques. Interest in the topics of identification, quality, authentication of active substances/extracts, in addition to the prospection of new medicines based on natural products, in order to contribute to the elucidation of the pharmacological and toxicological effects and the effectiveness of medicinal herbs is highlighted. Research for the identification and authentication of plant extracts proves to be valuable, since more than 95% of the world's biodiversity has not been evaluated yet, for any biological activity (DAVID *et al.*; 2015).

It is estimated that about 65-80% of the population of developing countries use medicinal herbs in their health care, whether for knowledge of folk medicine, indigenous, quilombola or scientific medicine (BRASIL, 2012; CRUZ; GONÇALVES, 2022),

encouraging the development of public policies aimed at this area, in line with the World Health Organization, which recognizes and encourages the development of phytotherapy by its member countries. Thus, in 2006, phytotherapy as a complementary integrative practice for treating patients in the public health system (SUS) was approved in Brazil. Then, in 2008, the National Program of Medicinal Plants and Phytotherapeutics approved the use of 12 plants for the treatment and prevention of diseases in the SUS, in addition to listing 71 medicinal plants, with widespread use by the population, in the National List of Medicinal Plants of Interest to the Single Health System (RENISUS), a Brazilian federal program that aims to promote research, development and innovation in order to regulate the use of herbal medicines and supplies based on medicinal plants. Bauhinia spp. consists of RENISUS, represented by the species *Bauhinia forficata*, *Bauhinia variegata* and *Bauhinia affinis*.

Bauhinia forficata arouses the interest of the scientific community for presenting activity against a variety of pathologies, especially Diabetes Mellitus type II. Its beneficial effects have been associated with the glycosylated flavonoid fraction, although reliable chemical markers for the standardization of this fraction have not been established in the literature. In addition, the scientific knowledge about the volatile fraction of this species, a fraction that is rich in compounds with recognized biological activity that, therefore, should not be neglected in studies that relate composition and activity.

Another point of attention is the lack of regulation in the commercialization of these herbs, since they are exempt from registration with ANVISA and are subject to food regulation, which can facilitate fraud and adulteration. Thus, a comprehensive chemical analysis, comprising different fractions, becomes an indispensable tool, because through it it is possible to contribute to the chemical elucidation of plant material, in its entirety, guaranteeing quality and safety in its commercialization.

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OBJETIVOS

Objetivo geral

Avaliar a composição química da fração volátil e não volátil de amostras comerciais e botanicamente identificadas de *Bauhinia forficata,* bem como avaliar seu uso como fonte de compostos bioativos na obtenção de um extrato hidroetanólico.

Objetivos específicos

- Isolar os óleos essenciais de amostras comerciais e botanicamente identificadas de *B. forficata* por hidrodestilação em aparelho de Clevenger;
- Caracterizar os óleos essenciais de amostras comerciais e botanicamente identificadas de *B. forficata* por CG-EM e CG-DIC;
- Obter infusões de *B. forficata* a partir das amostras comerciais e botanicamente identificadas;
- Caracterizar as frações volátil e não volátil das infusões preparadas com amostras comerciais e botanicamente identificadas de *B. forficata*;
- Avaliar o potencial antioxidante, antimicrobiano e inibidor de α-amilase das infusões de *B. forficata*;
- Otimizar a recuperação de compostos antioxidantes das folhas de *B. forficata* por meio de uma extração do tipo sólido-líquido.

OBJECTIVES

General objective

- To evaluate the chemical composition of the volatile and non-volatile fractions of commercial and botanically identified samples of *Bauhinia forficata*, as well as to evaluate its use as a source of bioactive compounds to obtain an antioxidant hydroethanolic extract.

Specific objectives

- Isolate essential oils from commercial and botanically identified samples of *B. forficata* by hydrodistillation using the Clevenger apparatus;
- Characterize essential oils from commercial and botanically identified samples of *B. forficata* by GC-MS and GC-FID;
- Obtain infusions of *B. forficata* from commercial and botanically identified samples;
- Characterize the volatile and non-volatile fractions of infusions prepared with commercial and botanically identified samples of *B. forficata*;
- Evaluate the antioxidant, antimicrobial and α-amylase inhibitory potential of B. forficata infusions;
- Optimize the recovery of antioxidant compounds from *B. forficata* leaves through solid-liquid extraction.

CHAPTER 1

BAUHINIA FORFICATA EXTRACTS: AN OVERVIEW OF THE LITERATURE ON CHEMICAL AND BIOLOGICAL ASPECTS.

1. PHYTOTHERAPY AND PUBLIC POLICIES IN BRAZIL

The use of plants for medicinal purposes, in the treatment, cure, and prevention of diseases is one of the oldest forms of medicinal practice by humanity. Plants used in phytotherapy are called "medicinal plants", since they are raw materials of plant origin that contain substances that can be used for certain therapeutic purposes or that work as precursors of semi-synthetic drugs (ROSA *et al.*, 2012). Even though plants are traditionally employed for this purpose, it is estimated that more than 95% of the world's biodiversity (approximately 2 million species of plants, animals, fungi, microorganisms and the like) has not been evaluated for any biological activity. In this sense, research for the identification and authentication of plant extracts is an extremely valuable source of knowledge (DAVID *et al.*, 2015).

Ensuring access to safe, effective, quality, and affordable medicines for everybody is an apparently intangible challenge in Brazil. The medicinal plants and herbal medicines industry represents an excellent alternative to this end. On the other hand, it constitutes an important source of innovation in healthcare and can strengthen local production and innovation based on the exploitation of the rich Brazilian biodiversity, representing the possibility of expanding the therapeutic options offered to users of the Unified Health System (SUS) in order to improve healthcare and social inclusion (HASENCLEVER *et al.*, 2017).

The World Health Organization (WHO) recognizes and encourages the development of herbal medicine by its member countries, encouraging this practice in public healthcare. In Brazil, the National Policy on Medicinal Plants and Phytotherapeutics (BRASIL, 2006) was approved in 2006 through the Presidential Decree No. 5,813/2006. A few years later, in early 2009, the government established the National Program of Medicinal and Phytotherapeutic Plants, which aims to "guarantee safe access and rational use of medicinal and phytotherapic plants in the country, which contributes to the development of technologies and innovations, as well as such as the strengthening of production chains and arrangements, the sustainable use of Brazilian biodiversity, and the development of the Health Productive Complex" (BRASIL, 2009).

Among the guidelines established for the use of medicinal plants and herbal medicines is the elaboration of the National List of Medicinal Plants and Herbal Medicines of Interest to SUS (RENISUS). This list includes 71 plant species with medicinal potential, which were

selected by regions based on their popular indication and in according to the categories of the International Code of Diseases (ICD-10), through in-depth research carried out by professionals specialized in the area of medicinal plants and phytotherapics linked to the National Health Surveillance Agency (ANVISA) and the Ministry of Health of Brazil.

Medicinal plants can be used fresh (in natura), when collected at the time of use, or dried, when subjected to drying process. Medicinal plants used in traditional and folk medicine are home remedies of plant origin, also defined as homemade preparations of medicinal plants, for extemporaneous use (immediate use), and that do not require specialized techniques for handling and administration. They are consumed in the form of teas, alcoholic beverages (extract), syrups, compresses, baths, and in some cultures they are also associated with various healing rituals. The plants sold to prepare teas or infusions are regulated, under Brazilian law, as food, and therefore, therapeutic claims are not allowed on the labels of such products. ANVISA defines tea as: "a food product consisting of one or more parts of whole plant species(s), fragmented or ground, with or without fermentation, toasted or not, and aroma and/or spice may be added to impart aroma and/or flavor". These products are exempt from the mandatory registration with ANVISA (RDC278/05; BRASIL, 2005a), and its production and commercialization follow, including the quality control, the general standards set in place by legislation applied to food and also specific regulations for coffee, barley, tea, yerba mate, and soluble products (RDC277/05; BRASIL, 2005b). On the other hand, when medicinal plants or their parts are shredded, crushed, or sprayed, whether stabilized or not, they are called herbal drugs (BRASIL 2010a). The production and trade of industrialized plant drugs are regulated through RDC10/10 (BRASIL, 2010 b), which contains a list of 66 plant species that are officially made available in the form of industrialized plant drugs and must follow the Good Manufacturing Practices (RDC13/13; BRASIL, 2013). *Bauhinia forficata* L is commercialized in several forms. The dried herb can be used for the preparation of infusions, which is subject to the legislation provided for food as well as herbal or phytotherapic drugs.

2. *BAUHINIA FORFICATA* LINK – BOTANICAL ASPECTS

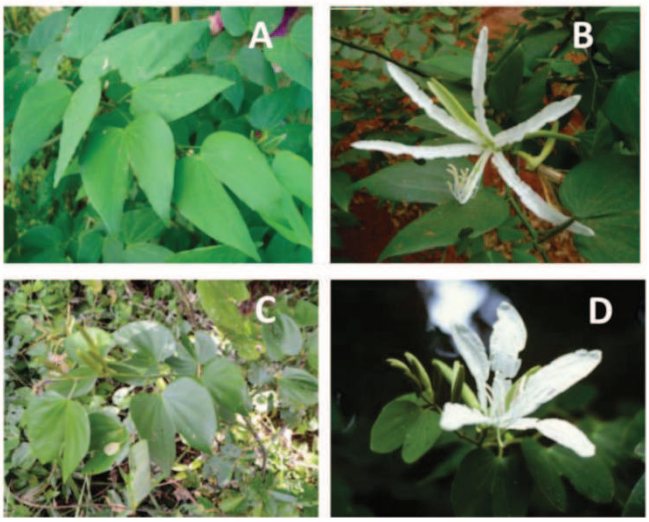
Bauhinia is a genus named by Carolus Linnaeus in 1753 in honor of the French-Swiss botanist Gaspar Bauhin. This genus comprises 300 species widely distributed in tropical and

subtropical forests, 64 of which can be found in Brazil. Such species belong to the subfamily Caesapinoideae and the family Fabaceae (VAZ; TOZZI, 2005). They are commonly known as “pata-de-vaca” or “cow's paw” due to the shape of their leaves, which are composed of two leaflets joined at the base. In recent years, interest in plants of the genus Bauhinia has increased considerably around the world, as experimental studies have confirmed ethnopharmacological observations (CECHINEL-ZANCHETT *et al*, 2018). Most species are of Asian origin, but there are species native to Brazil such as *B. longifolia* and *B. forficata* (LÓPEZ; SANTOS, 2015).

The species *B. forficata* is divided into two subspecies: *Bauhinia forficata* Link subsp. *forficata* and *Bauhinia forficata* subsp. *pruinosa* (Vogel) Fortunato & Wunderlin, which despite some small morphological differences in the flowers and leaves, do not show variations in their chemical composition (DOMINGOS; JÚNIOR, 2016).

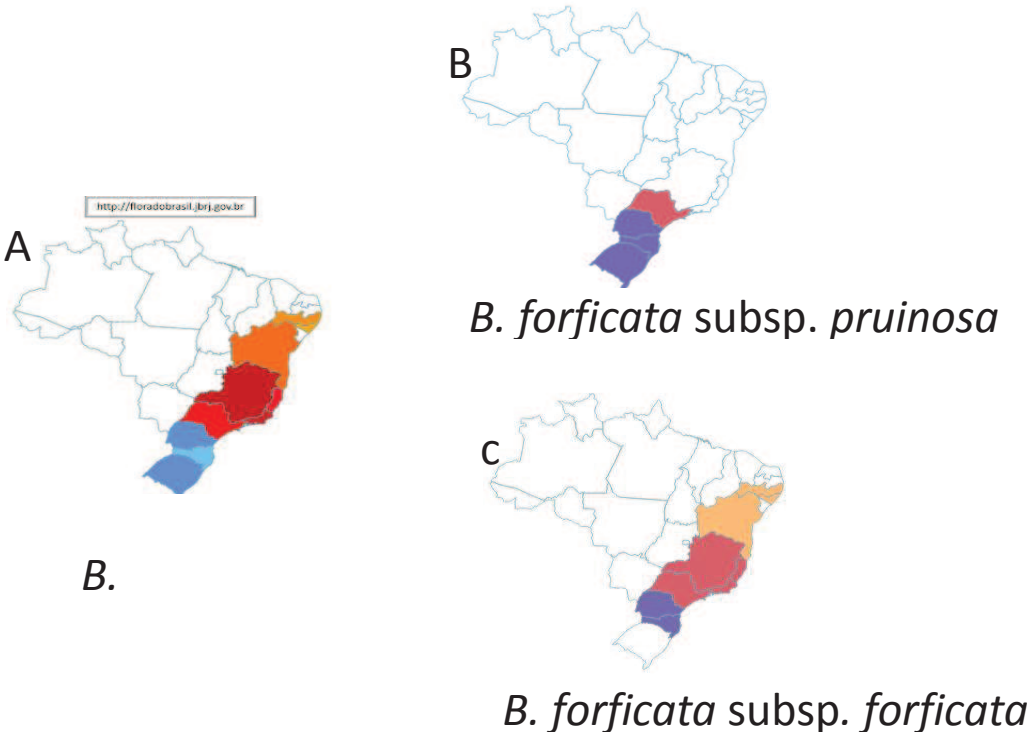
The *Bauhinia forficata* Link subsp. *forficata* belongs to the genus Bauhinia, the Fabaceae family and Caesalpinioideae subfamily (SILVA-LÓPEZ; SANTOS, 2015). Despite the morphological similarity with other species, its leaves differ by presenting paired spines at the leaf base and very pointed apices (DOMINGOS; JÚNIOR, 2016; LÓPEZ; SANTOS, 2015). The flowers are white and with narrow petals (Figure 1.1). The “true pata-de-vaca”, as it is popularly known, is a deciduous, large or arboreal, perennial shrub, which reaches up to 8 m in height. It occurs only in Brazil, spreading through the Northeast and Southeast regions, reaching the state of Santa Catarina (Figure 2.1) (VAZ, 2015).

Figure 1.1. Bilobed leaves (A) and flower (B) of Bauhinia forficata Link subsp. forficata. Bilobed leaves (C) and flower (D) of Bauhinia forficata subsp. pruinosa



Source: DOMINGOS; JÚNIOR, 2016

Figure 1.2. Distribution map of *B. forficata* in Brazil.



Source: (A) http://floradobrasil.jbrj.gov.br; (B) and (C) adapted from Vaz, 2015.

The present review will address *B. forficata* in general, since information regarding the subspecies, in most articles, was not cited. Aspects related to chemical composition and biological activity of its aqueous, alcoholic and hydro-alcoholic extracts will be addressed, based on information published in the literature in relevant scientific journals.

3. CHEMICAL CHARACTERIZATION OF *B. FORFICATA* EXTRACTS

Before approaching the chemical characterization of the extracts, it is important to emphasize that the quality of a natural extract and, consequently, of its biological activity are related to the composition, which depends on the extraction process, the solvent used, the origin of the raw material, as well as the conditions of storage and pretreatment (CRUZ *et al.*, 2019). As reported by Jung *et al.* (2021), emergent extraction techniques have been investigated to recover antioxidant compounds from plants. However, due to the high cost of operation, when compared to conventional methods, its implementation must be very well evaluated.

The determination of the phytochemical profile of crude extracts is an onerous task due to the diversity of chemical structures present in the plant and variability in composition related to extrinsic factors (PINTO *et al.*, 2002; TOSTES *et al.*, 2019). However, technological advances in analytical techniques, especially hyphenated techniques, play an important role in the elucidation of complex chemical compositions of matrices of plant origin, with sensitivity and selectivity that was unthinkable until a few years ago. This has made it easier to analyze raw extracts, without the need for the exhaustive work of isolation that, many times, leads to compounds already known. Therefore, chemical screening strategies have been developed using hyphenated techniques (PINTO *et al.*, 2002).

High Performance Liquid Chromatography (HPLC) is the most used analytical separation technique for acquiring chromatographic profiles of natural extracts and can be coupled to different analyzers and detectors. HPLC technique became especially important in the early 1990s after the development of interface systems that allowed its coupling to atmospheric pressure ionization sources (APCI — atmospheric-pressure chemical ionization, APPI — atmospheric-pressure photo ionization, and ESI — electrospray ionization). Among these mild atmospheric pressure ionization methods, ESI is the method of choice for most metabolomics studies, allowing the formation of protonated and deprotonated species, adducts and, in particular cases, molecular ions from electron removal (ZHAO *et al.*, 2008).

With the development of high-resolution equipment such as time of flight (ToF) and Orbitrap analyzers (GLAUSER *et al.*, 2013), which present an accuracy generally lower than 2mDa (millidaltons), it was possible to obtain the molecular formulas of compounds with very proximate accurate masses and which may be chromatographically coeluted. Additionally, other information is obtained in fragmentation experiments (MS/MS) in which product ions are generated in the ionization source itself or using hybrid analyzers such as QqTOF (hybrid System with Quadrupole and TOF (time of flight) analyzers), in which the quadrupole works as a collision cell by Collision-induced dissociation (CID) (SEGER *et al.*, 2013). Those data are fundamental for the emerging metabolomic science. Specifically, in the analysis of plant extracts, HPLC-MS (high-performance liquid chromatography coupled to mass spectrometry) makes possible the structural elucidation of the different classes of secondary metabolites, such as alkaloids, phenolic acids, terpenes, flavonoids, among others (PILON *et al.*, 2020).

The first studies dealing with the chemical composition of *B. forficata* extracts date back to the early 2000s. Silva *et al.* (2000) isolated two phytoconstituents present in the *B. forficata* leaves by column chromatography and identified ß-sitosterol and kaempferol-3,7-dirhamnoside (kaempferitrin) by spectroscopical data and comparison with authentic samples. Thus, the kaempferitrin was established as a chemical marker of the species, as it is not present in other species of the genus. Since then, a series of studies were carried out in order to evaluate the biological actions of kaempferitrin, since it was believed that this compound was responsible for the antihyperglycemic activity related to these extracts (DE SOUZA *et al.*, 2004a; DA SILVA *et al.*, 2000). In fact, the hypothesis was proven later, when De Souza *et al.* (2004b) observed a significant hypoglycemic effect in normal rats as well as in those alloxan-induced diabetic animals by administering different doses of kaempferitrin isolated from the hydroethanolic leaf extract of *B. forficata*.

Pinheiro et al. (2006) evaluated through liquid chromatography the content of kaempferitrin in hydroalcoholic and aqueous extracts of B. forficata leaves collected in different regions of Brazil, pointing out kaempferitrin as the major compound in only one evaluated sample. On the other hand, they were able to identify other glycosylated flavonoids derived from kaempferol and quercetin. This work drew attention to the fact that kaempferitrin could not be considered a unique marker of the species B. forficata. Many other works developed later showed that, in fact, other samples of the species did not contain

kaempferitrin (ENGEL *et al.*, 2008; FARIAS *et al.*, 2014; FERRERES *et al.*, 2012; TOSTES *et al.*, 2019; JUNG *et al.*, 2021). Table 1.1 summarizes data on the chemical composition of B. forficata extracts and Figure 1.3 displays structures of main compounds reported in B. forficata extracts.

Table 1.1. Chemical compounds identified in aqueous and/or hydroalcoholic extracts of *B. forficata*

Botanical denomination	Part of the plant	Type of extract	Analytical technique	Identified compound	Reference
B. forficata	leaves	Methanolic extract	Thin layer chromatography (TLC)	Kaempferitrin	Da Silva *et al.* (2000)
B. forficata	leaves	Ethyl acetate and n-butanolic extract	Column chromatography	β-sitosterol; kaempferol 3,7-di-*O*-α-L-ramnopiranoside; quercetin 3-*O*-[β-D-glucopiranosíde-(1→6)-α-L-ramnopiranoside]-7-*O*-α-L-ramnopiranoside; kaempferol 3-*O*-[β-D-glucopiranoside-(1→6)-α-L ramnopiranoside]-7-O-α-L-ramnopiranoside; quercetin 3-*O*-[β-D-glucopiranoside-(1→6)-α-L ramnopiranoside]-7-O-α-L-ramnopiranoside	Pizolatti *et al.* (2003)
B. forficata	leaves	Aqueous and hydro-ethanolic extract	HPLC-DAD	*O*-glycosyl flavonoid derivatives of kaempferol and quercetin	Pinheiro *et al.* (2006)

Table 1.1 – Continued

Botanical denomination	Part of the plant	Type of extract	Analytical technique	Identified compound	reference
B. forficata Link B. *forficata* subsp. *pruinosa* (Certificated and commercial samples)	leaves	Hydro-methanolic extract	HPLC-DAD-ESI/MS^n	Flavonol-3-*O*-glycoside-7-*O*-glycosides; Flavonol-3-*O*-glycosides; Flavonol-3-*O*-(galloyl)glycosides.	Ferreres *et al.* (2012)
B. forficata	leaves	Methanolic extract	TLC	Steroid triterpenes, flavonoids, proanthocyanidins, leucoanthocyanidins and reducing sugars	Marques *et al.* (2012)
B. forficata Link subsp. *pruinosa* (Vogel) Fortunato & Wunderlin	leaves	Infusions (0,01-1%)	LC-MS (íon trap)	quercetin-3-*O*-(2-rhamnosyl) rutinoside, kaempferol-3-*O*-(2-rhamnosyl) rutinoside, quercetin-3-*O*-rutinoside and kaempferol-3-*O*-rutinoside	Salgueiro *et al.* (2013)
B. forficata subsp. *pruinosa* (Vogel) Fortunato & Wunderlin	leaves	Hydro-ethanolic extracts	LC/ESI-MS	quercetin-3-*O*-(2 rhamnosyl)rutinoside; kaempferol-3-*O*-(2-rhamnosyl)rutinoside; quercetin-3-*O*-rutinoside; kaempferol-3-*O*-rutinoside	Farias *et al.* (2014)

Table 1.1 – Continued

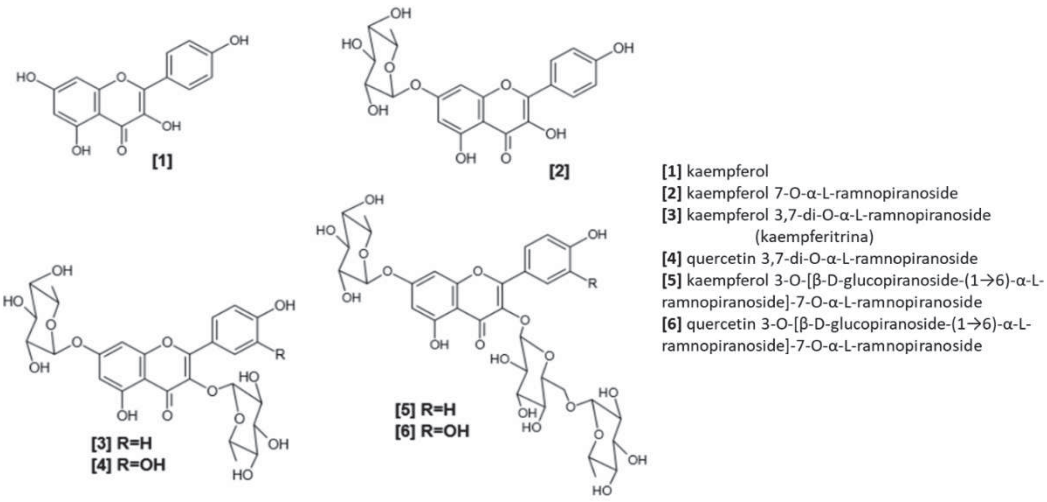
Botanical denomination	Part of the plant	Type of extract	Analytical technique	Identified compound	reference
B. forficata	leaves	70 % Methanolic extract	UHPLC–qTOF–PDA–MS	kaempferol-3-*O*-dirhamnoside, Quercetin-3-*O*-rhamnosyl rutinoside, Kaempferol-3-*O*-rhamnosyl rutinoside, Isorhamnetin-3-*O*-rhamnosyl-rutinoside, 2-benzyltartaric acid	Farag *et al.* (2015)
B. forficata	leaves	Infusion (30%)	HPLC-DAD	gallic acid, chlorogenic acid, rutin, quercetin, isoquercetin, kaempeferol, caffeic acid.	Ecker *et al.* (2017)
B. forficata	leaves	Hydro-methanolic extract	HPLC-DAD-ESI/MS	3,4-dicaffeoylquinic acid	Meinhart *et al.* (2017)
B. forficata subsp. *pruinosa*	leaves	hydro-methanolic extracts	HPLC-ESI-MS^n	Quercetin - 3 - (2-rhamnosyl) rutinoside; Kaempferol- 3- (2-rhamnosyl) rutinoside Quercetin- 3-rutinoside Kaempferol - 3,7 - dirhamnoside (kaempferitrin) Kaempferol - 3-rutinoside	Santos *et al.* (2018)

Table 1 – Conclusion

Botanical denomination	Part of the plant	Type of extract	Analytical technique	Identified compound	reference
B. forficata subsp. *forficata*	leaves	Hydro-methanolic extract	HPLC-DAD; HPLC-	Kaempferol triglycosides; Quercetin diglycoside;	Tostes *et al.* (2019)

			DAD-ESI/MS)	Qercetin glycoside; kaempferitrin; kaempferol diglycoside; isorhamnetin diglycoside.	
B. forficata Link	leaves	Hydro-ethanolic extract	UPLC-qTOF/MS	dihydroxybenzoic acid-hexoside Caffeoyltartarate (Epi)Catechin dimer (Epi)Catechin Quercetin 3-*O*-rhamnosyl rutinoside Kaempferol 3-*O*-rhamnosyl rutinoside Rutin Quercetin *O*-arabinoside Kaempferol 3-*O*-rutinoside Quercetin 3-*O*-rhamnoside Kaempferol 3-*O*-glucoside Kaempferol 3-*O*-galactoside Isorhamnetin 3-*O*-rutinoside Kaempferol *O*-pentoside Isorhamnetin	Jung *et al.* (2021)

Figure 1.3. Kaempferol and quercetin glycosides from *B. forficata*



Source: Adapted from Pizolatti *et al.*, 2003.

4. BIOLOGICAL ACTIVITY AND MEDICINAL USE OF *B. FORFICATA*

B. forficata is used in folk medicine as a hypoglycemic, purgative, diuretic, antidiarrheal, depurative, and renal tonic. It has the peculiar property of reducing urine excretion in cases of polyuria and glucosuria, regulating blood glucose, especially in patients with type 2 diabetes (ENGEL *et al.*, 2008).

4.1. HYPOGLYCEMIC ACTIVITY

The first report on the medicinal use of B. forficata concerns its hypoglycemic activity, as reported by Juliane (1929) through clinical trials with diabetic patients. However, the hypoglycemic action of B. forficata is empirically observed in its use as an antidiabetic in folk medicine, being regularly considered by rural communities as “real cow’s paw”, used in the form of infusions and other herbal preparations (SILVA; FILHO, 2002).

Pepato et al. (2002) evaluated extracts prepared by decoction of leaves of B. forficata and verified that those extracts showed hypoglycemic action only in diabetic rats, not reducing blood glucose of non-diabetic animals. In this way, they concluded that the mechanism of action of the compounds of B. forficata is different from the mechanisms of action of insulin and sulfonylureas (drugs that promote the release of insulin from the β cells of the pancreas), confirming the hypothesis that this plant contains compounds with

antiinsulinase activity, which may be useful in maintaining the levels of any residual insulin that may be present in diabetics. That is, for moderate diabetes the decoction of B. forficata increases the efficiency of insulin.

Lino *et al.* (2004) confirmed the validity of the clinical use of *B. forficata* in the treatment of type II diabetes mellitus, since their results showed that extracts from this plant can reduce the levels of glucose, triglycerides, total cholesterol and HDL (high-density lipoprotein) cholesterol. Preparations of an extract of dried leaves of *B. forficata* obtained with ethanol-water (1:2 v/v) were administered to diabetic rats and a drop in glucose levels was observed. This observation reinforced the hypoglycemic role of this plant and this effect appeared to be dependent on the presence of quercetin-3,7-di-*O*-α-L-rhamnopyranoside and Kampferol-3,7-di-*O*-α-L-rhamnopyranoside (kampferitrin), (DA CUNHA *et al.*, 2010).

Ferreres *et al.* (2012) demonstrated that the glycemic reduction promoted by *B. forficata* hydromethanolic extracts is the result of inhibition of α-glucosidase, the enzyme responsible for catalyzing the final process in carbohydrate digestion (α-glucosidase inhibitors are usually used to control blood glucose levels in patients with diabetes mellitus). According to the authors, such action may be due to the presence of quercetin and kaempferol derivatives of the species. Souza *et al.* (2018) also observed that the flavonoids present in the leaves of *B. forficata* hydroethanolic extract act as hypoglycemic agents by reducing the intestinal absorption of carbohydrates, inhibiting α-glucosidase.

As already mentioned, although B. forficata is widely used in traditional medicine for therapeutic purposes, clinical trials are rare. Heller et al, 2013 evaluated the hypoglycemic, anthropometric, and hypotensive effects of B. forficata tea in 54 people for eight months, and found that the group that consumed the tea had decreased blood pressure and reduced weight and body mass index (BMI) compared with the control group. In contrast, Vanzetto et al. 2011, analyzed anthropometric data from 37 patients with type 2 diabetes who consumed B. forficata tea for eight months, but found no significant difference.

Córdova Mariangel et al. (2019) evaluated the effects of B. forficata infusion on lipid profile when used as complementary therapy in type 2 diabetic patients and verified that its use as complementary therapy could help to reduce the levels of triglycerides and total cholesterol. However, this decrease was not clinically significant, suggesting additional studies.

Recently, Toneli *et al.* (2022) conducted a double-blind randomized trial using capsules containing granules of a standardized extract of *B. forficata* leaves to determine the adjuvants effects and possible harms of this phytopharmaceutical preparation on the glycemic control of patients with type 2 diabetes. It was demonstrated that the adjuvant treatment with a standardized capsules of *B. forficata* for four months improved metabolic control in type 2 diabetes patients as compared to placebo.

Although most studies show positive results with regard to confirmation of antidiabetic and hypoglycemic activities, some are controversial (FERRERES *et al.*, 2012; SALGUEIRO *et al.*, 2016). Said controversy may be related to different aspects, such as: the experimental model used by the researchers, the types and methods of preparing extracts and/or fractions, the different doses and routes of administration chosen. Also, concerning natural products, several factors may influence the content of secondary metabolites, namely genetic constitution of the species, environmental conditions (soil type), seasonal variations, and conditions of the cultivation and storage of the plant (MARQUES *et al.*, 2013; SILVA; FILHO, 2002).

4.2. ANTIOXIDANTE ACTIVITY

The antioxidant activity of B. forficata is widely described in the literature and is associated to the marked presence of flavonoids , — a representative group of plant antioxidants. This activity is extremely important in the prevention of complications of diabetes mellitus and other pathological processes associated with oxidative stress (DAMASCENO *et al.*, 2004; KHALIL, PEPATO; BRUNETTI, 2008; MARQUES *et al.*, 2013; SILVA *et al.*, 2012). Santos et al. (2018) also attribute the antioxidant effect of B. forficata infusion to the presence of flavonoids. According to Salgueiro et al. (2013), B. forficata subsp. pruinosa has important antioxidant properties and chelators that may be related to the presence of different phenolic compounds, mainly flavonoids as well as other components such as terpenes, steroids, aromatic acids, quinones, lactones, and alkaloids. Salgueiro et al. (2016) showed that B. forficata subsp. pruinosa tea is able to eliminate reactive oxygen species (ROS), decreasing liver oxidative injury in diabetic mice, but did not record a change in glycaemia. Thus the authors suggest that the protective effect of B. forficata may be attributed especially to its antioxidant capacity related to main compounds, namely quercetin and kaempferol derivatives.

4.3. ANTIMICROBIAL ACTIVITY

The antimicrobial activity of extracts and fractions of B. forficata was reported in some studies through the radial diffusion method in agar with microorganism species. Significant growth inhibition was observed with pathogenic strains of *Escherichia coli* and *Staphylococcus aureus* (DE SOUZA *et al.*, 2004*), Streptococcus mutans* and *Streptococcus sanguis* (SOUZA *et al.*, 2014), *Bacillus cereu*s and *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Proteus mirabilis* and *Salmonella typhimurium* (PEREIRA *et al.*, 2014). Although most studies show positive results regarding antimicrobial activity, Miceli *et al.* (2015) did not observe such activity against Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, Gram-negative bacteria: *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcescens*, and the fungus *Candida albicans*.

4.4. OTHER ACTIVITIES OF *B. forficata*

Oliveira *et al.* (2005) showed that the aqueous extract of aerial parts of *B. forficata* is capable of neutralizing the coagulation induced by the venom of *Bothrops jararaca* and *Crotalus durissus terrificus* snakes, probably due to the inhibition of serine proteases involved in blood clotting disorders. A similar action was observed by Vasconcelos *et al.* (2004), which worked with lyophilized *Tityus serrulatus* scorpion venom.

Arigony (2005) demonstrated an antiedematogenic activity of *B. forficata* extracts when using the method of paw edema in rats induced by carrageenan. According to the author, this activity may be related to a possible synergism between its major constituents kaempferol and glycosylated quercetin.

Substances isolated from *B. forficata* dry leaves exhibited anticancer activity: a cyclin-dependent protein kinase inhibitor that interferes with the cell cycle of HeLa cells, inhibiting their proliferation (LIM *et al.*, 2006); a lectin obtained from seeds of *B. forficata* also induced the death of human breast cancer cells by inhibiting the integrin-dependent adhesion of these cells to the extracellular matrix, thus interfering with tumor progression (SILVA *et al.*, 2014).

4.5. OVERALL VIEW

It is clear that the wide use of the species *B. forficata* in the therapeutic treatment of diabetes mellitus is based on a plurality of scientific studies with different experimental models, and the antidiabetic property is attributed to the presence of kaempferol and quercetin

glycosides present in the leaves. Many other studies on the potential action of *B. forficata* for the treatment of various pathologies have been conducted and are promising. These studies support the use of this plant in folk medicine and support the Brazilian State's initiatives to produce new phytotherapics with technological quality, safety and efficacy, aiming at expanding the number of herbal medicines that are currently financed by the Federal Government. However, there is still a lack of standardization of these extracts establishing a chemical marker preferably represented by a set of compounds that would make it possible to control the quality of this potential plant raw material, which is abundant and easily accessible to the Brazilian population.

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CHAPTER 2

BAUHINIA LINNAEUS: AN OVERVIEW OF THE CHEMISTRY AND BIOACTIVITY OF ESSENTIAL OILS

Bauhinia Linnaeus: una descripción general de la química y bioactividad de los aceites esenciales

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This chapter was accepted for publication in Boletín Latinoamericano y del Caribe de Plantas Medicinales as a review paper

ABSTRACT

Bauhinia genus comprises 300 diferent species distributed in tropical and subtropical forests. Infusions of some species have been frequently used in folk medicine to treat several ailments, especially diabetes. Studies are focused on the extracts and little is reported about their essential oils. This review aims to compile data about the chemical composition and biological activities of essential oils from diferent species of the genus Bauhinia, in order to show the potential of these oils, since they have a rich composition in terpenoids, with emphasis on sesquiterpenes and diterpenes, which have a broad spectrum of biological actions and can be explored in various application areas.

Keywords: Bauhinia, essential oils, chemical composition, terpenes, biological activity

RESUMEN

El género Bauhinia comprende 300 especies diferentes distribuidas en bosques tropicales y subtropicales. Las infusiones de algunas especies se han utilizado con frecuencia en la medicina popular para tratar varias dolencias, especialmente la diabetes. Los estudios se centran en los extractos y se informa poco sobre sus aceites esenciales. Esta revisión tiene como objetivo recopilar datos sobre la composición química y actividades biológicas de los aceites esenciales de diferentes especies del género bauhinia, con el fin de mostrar el potencial de estos aceites, ya que tienen una composición rica en terpenoides, con énfasis en sesquiterpenos y diterpenos, que tienen un amplio espectro de acciones biológicas y pueden explorarse en diversas áreas de aplicación.

Palabras clave: Bauhinia, aceites esenciales, composición química, terpenos, actividad biológica.

1. INTRODUCTION

Bauhinia is a genus named by Carolus Linnaeus, in 1753, in honor of Jean Bauhin (1541-1613) and Gaspard Bauhin (1550-1624) Swiss doctors and botanists (QUATTROCCHI, 2012). It is comprised of 300 species widely distributed in tropical and subtropical forests, 64 of which can be found in Brazil. Such species belong to the subfamily Caesapinoideae and to the Fabaceae family (VAZ & TOZZI, 2005).

Widely used by the Brazilian population for the prevention and treatment of diabetes, plants of the genus Bauhinia Linnaeus are popularly known as cow's hoof or ox-claw because of the shape of their bilobed leaves, and they may present arboreal, shrub or climbing plants. In recent years, interest in plants of the genus Bauhinia has increased considerably around the world, since experimental studies have confirmed ethnopharmacological observations. Most species are of Asian origin, but there are species native to Brazil such as Bauhinia longifolia and Bauhinia forficata (LÓPEZ & SANTOS, 2015).

Traditionally, there is widespread use of Bauhinia forficata Link, which presents white flowers with linear petals. The first pharmacological trial of this species dated back to the beginning of the 20th century, and the findings by Carmela Juliani in 1929 (JULIANI, 1941) had already pointed to its hypoglycemic properties. However, in the Cerrado and Amazon regions, folk medicine has also recorded the use of Bauhinia rufa Steud. Bauhinia variegata L., in turn, is widely employed in Brazil in urban afforestation, mainly in the south and southeast regions, owing to its ornamental potential and frost tolerance (LORENZI *et al.*, 2003), and the same occurs with Bauhinia tormentosa L. and Bauhinia purpurea L. However, recent studies have shown that some species, so far considered to be ornamental plants only, e.g., B. tomentosa and B. purpurea, can present potentially medicinal compounds with antimicrobial activity (CHANDRASHEKAR & KUMAR, 2011; GOPALAKRISHNAN & VADIVEL, 2011).

The scientific literature that addresses the chemical composition and the therapeutic potential of Bauhinia species focuses on their organic extracts, with a prevalence of alcoholic and hydroalcoholic extracts, obtained from different parts of the plant, such as leaves, stems, barks or flowers. Free and glycosylated flavonoids are characteristic of the Leguminosae family and possess large occurrence on the Bauhinia genus. Quercetin 3-O-α-rhamnoside, kaempferol 3-O-α-rhamnoside, quercetin 3-O-α-arabinoside, quercetin 3-O-α-(2″-

galloyl)rhamnoside, kaempferol 3-O-α-(2″-galloyl)rhamnoside, kaempferol 3,7-dirhamnoside (kaempferitrin), Quercetin 3—(2-rhamnosyl)rutinoside are some examples (SANTOS *et al.*, 2019). Alkaloids are rarely encountered on this genus and only few compounds have been reported so far. Some examples are trigonellin in Bauhinia candicans (IRIBARREN & POMILIO, 1983) and the carbazols mahainbine, bicycloamhanimbine and girinimbine in Bauhinia variegata (ZHAO *et al.*, 2005)

The results support, for most species, the therapeutic properties of leaf extracts, indicating that they are mainly due to the presence of flavonoids (PAULA *et al.*, 2002; PIZZOLATTI *et al.*, 2003; FERRERES *et al.*, 2012). Such flavonoids in the extracts of plants may also be important for prevention and treatment of diseases which cause an increase in oxidative activity (SALGUEIRO *et al.*, 2016). Increasing experimental and clinical evidence suggests that oxidative stress can be the pathogenesis of diabetes. Increase of free radicals and the concomitant mitigation of antioxidants lead to increase in lipid peroxidation, development of insulin resistance and damage to cellular organelles and enzymes, which can result in diabetes mellitus complications (CHATTERJEE *et al.*, 2007; LYONS & JENKIS, 1997).

More recent hypotheses about possible mechanisms of flavonoids have been postulated, including the influence of the interaction of these polyphenols and gut microbiota and the possibility that flavonoids or their metabolites could modify gene expression or act as potential modulators of intracellular signaling cascades (GONZÁLEZ-PARAMÁS *et al.*, 2019)

Among all therapeutic activities presented by the genus Bauhinia, hypoglycemic action is the most commonly reported in the literature. Lino *et al.* (2004) evaluated aqueous, ethanolic and hexane extracts of B. forficata in a model of alloxan-induced diabetes in rats and found a decrease in the levels of glucose, triglycerides, total cholesterol and LDL, validating the clinical use of this plant for the treatment of type II diabetes mellitus. Similarly, Menezes *et al.* (2007) reported that aqueous extracts obtained from B. monandra and B. forficata exhibited hypoglycemic activity when evaluated in normoglycemic rats, suggesting that this action may be related to the presence of glycosylated flavonoids.

The composition of polar fractions extracted from Bauhinia species are well described in the literature, however, when it comes to the volatile fraction of the plant, that is, their essential oils, data are scarce and controversial. It may be related to the fact that volatile terpenoids, when compared with other classes of secondary metabolites present less

taxonomic utility in angiosperms and show irregular occurrence patterns. (DUARTE-ALMEIDA, 2004)

Essential oils have been known since antiquity for their biological activity, including antibacterial, antifungal, antiviral and anti-inflammatory effects. In this way, spices and herbs have been suggested to have healing or disinfecting properties. These oils can also be active against higher organisms such as nematodes, helminths, insects, etc. (KUMAR, 2011).

Volatile terpenoids, namely monoterpenes and sesquiterpenes, are major components of volatile oils; in comparison with other classes of secondary metabolites (for example, flavonoids), terpenes have had less taxonomic utility in angiosperms, showing irregular occurrence patterns (DUARTE–ALMEIDA, 2004). However, several sesquiterpenes present pleasant and commercial characteristics, and they are recognized for their potential as an aromatic compound. This interesting class of compounds has also been studied in the last years for their biological potential (N'ÉRI-NUMA *et al.*, 2019).

A high degree of polymorphism in the genus Bauhinia determines a large number of subspecies, different varieties and forms, producing essential oils with varied chemical composition, and offering variable level of potential applications (VAZ, 2013). There are few reports in the literature about the chemical composition of essential oils from plants of the genus Bauhinia. Essential oils extracted from Bauhinia plants, despite presenting low yields, have been reported to possess interesting biological properties. The larvicidal activity of B. cheilanta, B. pulchella Benth and B. ungulata L. has been recently reported, as well as its cytotoxic potential on lung carcinoma, breast and cervical adenocarcinoma human tumor cells (SILVA *et al.*, 2020; DE SOUZA *et al.*, 2016).

This systematic review aims to provide an overview of the chemical composition and biological activity of essential oils of various species of the genus Bauhinia, focusing on available literature data at the last twenty years.

2. LITERATURE REVIEW

2.1. STRATEGY FOR STUDY SELECTION

The titles and abstracts were thoroughly studied, and the articles relevant to the research questions (1 - Which are the chemical profiles of the essential oils of the Bauhinia plants? 2 - Which are the bioactive properties associated with these essential oils?) were

collected and cross-verified by one of the coauthors. Independent reviewers resolved any disagreement about a study as per the inclusion and exclusion criteria as below.

2.2. INCLUSION CRITERIA

Literature reporting the chemical characterization and bioactive properties of the essential oils (volatile fraction) of plants of the genus Bauhinia. This bibliographic review included full texts available in English, Spanish and/ or Portuguese, with paid and/or free access.

2.3. EXCLUSION CRITERIA

Literature reporting the chemical characterization and bioactive properties of the non-volatile fraction of Bauhinia plants was excluded from the study. For instance, studies dealing with infusions and hydroalcoholic extracts containing phenolic compounds (like flavonoids) were not included. Furthermore, published theses were not used as resources to collect information.

2.4. DATABASES AND RESOURCES UTILIZED

Searches were carried out in the following databases: Google Scholar, Periódicos CAPES, Scielo, Science Direct and Pubmed. Furthermore, books were used as resources to collect information.

2.5. KEYWORDS SEARCHED

“Bauhinia”, “essential oil”, “volatile fraction”, “chemical composition”, combined in pairs using the Boolean operator “and”.

2.6. TIME PERIOD

The literature of the last twenty years was searched; the data was collected and kept updated starting on December 2000 till September 2021.

2.7. ARTICLES FOUND

Fourteen papers met the inclusion criteria, describing specifically the chemical composition of essential oils of species of the genus Bauhinia, comprising a total of seventeen

different species. Among the fourteen selected articles, six of them showed some biological activities for oils.

3. CHEMICAL COMPOSITION

EOs have been rarely described in the Leguminosae family, except for the genus Bauhinia (DUARTE-ALMEIDA, 2004; SARTORELLI & CORREA, 2007). Essential oils can be obtained by expression, fermentation, enfleurage, or extraction, although hydrodistillation is the most common technique (SPERANZA & CORBO, 2010). In fact, all articles used in the elaboration of this review applied the hydrodistillation technique to isolate the volatile fraction of plant. Concerning extraction yield, all works pointed out to very low and quite variable values. Duarte-Almeida (2004), for example, reported the chemical composition of seven Bauhina species, but only two of them (*B. brevipes* and *B. rufa*) had measurable amounts of oil, 0.25 and 0.30%, respectively. In the others, the amount of oil was negligible and immeasurable. It should be noted the studies used different parts of the plant material. While some used fresh leaves and flowers, others used dry leaves, which significantly affects the result of the extraction process yield. Moreover, although all the works used hydrodistillation, time of extraction ranged from 2 to 8 hours. These factors could explain the great variability in the values found, ranging from 0.006 to 0.3%.

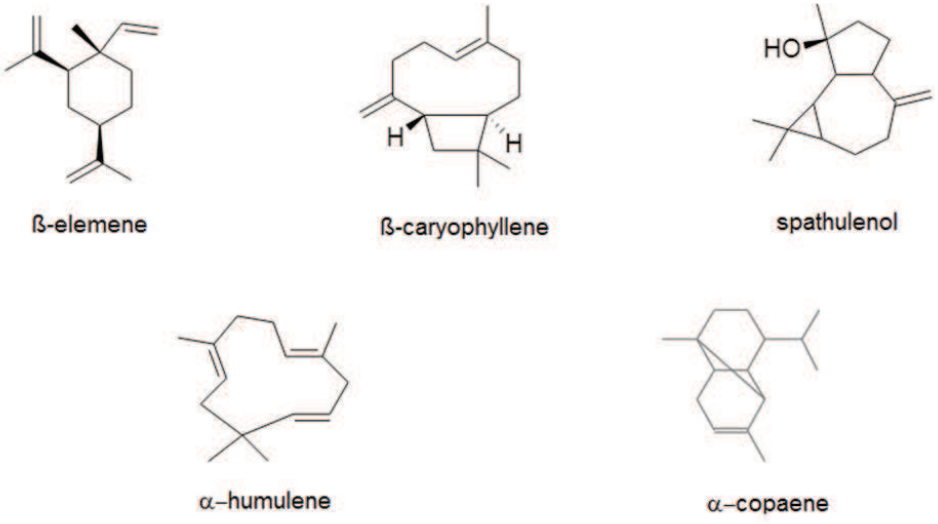
Despite showing a low yield in the extraction of the volatile fraction when compared to other species, the elucidation of the chemical composition of essential oils from Bauhinia is relevant and related to some aspects. Species of the genus Bauhinia are widely used in Brazilian folk medicine in the form of infusions. During this preparation, the volatiles are condensed, returning to the solution. In this way, part of the plant's essential oil can migrate to the infusion, which is consumed, and may be associated with the beneficial effects of this preparation on human health.

In studies conducted to stablish the chemical composition of essential oils, identification of the individual components is often carried out with the aid of gas chromatography coupled with a quadrupole mass spectrometer (GC/MS). However, someone skilled in the art can realize that the identification of these compounds in some works is carried out in a non-reliable way. In these specific studies, the identification is exclusively based on the evaluation of the mass spectra of the compounds. The use of standard compounds and retention indexes (e.g.: Kovats index) is not employed in the identification

process to endorse the spectrometric data. Thus, these compounds could not be considered definitely identified, but only tentatively identified, and mistakes must be considered as a real possibility. For instance, this kind of problem can be noted in the manuscript published by Vasudevan *et al.* (2014). They indicated the use of retention times to endorse the spectrometric data. If you don´t have standard compounds as reference to analyze in the same chromatographic conditions used to study the samples, the retention times are not a trustful mean to complete the identification process. On the other hand, although linear retention indexes (LRIs) have been calculated by Vasudevan *et al.* (2013), wrong identifications were also done in their work, probably because they forgot to compare the calculated indexes with those available in the literature. For instance, the compounds 3-hexen-1-ol and octacosane were probably not present in the leaf oil of Bauhinia acuminata that was analyzed in this study. Considering non-polar capillary columns, the LRIs attributed for these compounds (LRI3-hexen-1-ol = 1571; LRIoctacosane = 2496) didn´t match the LRIs available in the literature (LRI3-hexen-1-ol = 852; LRIoctacosane = 2800) (Miyazawa *et al.*, 2011; Kaib *et al.*, 2004). Another important point, when working with the common names of volatile compounds, is to check for synonyms. For instance, De Almeida *et al.* (2015) concluded that elixene was being reported by the first time as a component of B. pentandra. However, this compound had been previously indicated as a component of this species by Duarte-Almeida *et al.* in the year 2004, referring to it as γ-elemene (a synonym). In addition, the linear retention index calculated for elixene (1509) by De Almeida *et al.* (2015) is also far from the mean linear retention index (1439) found in the literature (MONDELO *et al.*, 2002; SHELLIE *et al.*, 2003) for this compound when using the same capillary column (RTX-5), making identification less reliable. So, data available in the literature related to the chemical composition of essential oils must be analyzed with care to avoid such mistakes.

The composition of the essential oil of genus Bauhinia investigated during the last twenty years is reported in Table 2, while Table 3 shows the three major compounds of each oil. One hundred fifty six compounds are listed as components of the essential oils of the seventeen species. Based on the information in this table, it can be seen that all oils have quite a different composition, even when the same species was analyzed by different research groups. β-elemene was identified in twelve species, β-caryophyllene in eleven species and α-humulene (α-caryophyllene), spathulenol and α-copaene were identified in ten species (Figure 2.1).

Figure 2.1. Sesquiterpenoid compounds characterized in a higher number of species of Bauhinia.



Source: acquired by the author in Chemdraw® software

β-Elemene, compared with their isomers γ, α and δ, has been shown to be a broad-spectrum anti-tumor drug that exerts effects through multiple pathways and multiple targets. Recent studies have shown that β-elemene exerts anti-tumor effects by inhibiting cell proliferation, arresting the cell cycle and inducing cell apoptosis (ZHAI *et al.*, 2019). In recent clinical studies, β-elemene has been shown to be a promising adjunctive treatment, providing a synergistic effect in improving patient outcome in the treatment of malignant disease (WANG *et al.*, 2012; ZHENG *et al.*, 2014; JUN *et al.*, 2016). This valuable compound is present in 70% of the oils reported in the literature, in concentrations ranging from traces to 56.9%. The δ-elemene isomer also seems to be an important compound of essential oils in the genus Bauhinia: it is present in nine oils at relatively high levels (1 - 38.4%) and it also has important antitumor activity, showing positive results against cancer cells of the colon/rectum (XIE *et al.*, 2009) and the lungs (XIE *et al.*, 2011).

Regarding the class of bicyclic sesquiterpenoids, which have raised attention in the bio-pharmacological field, β-caryophyllene is particularly relevant. This natural compound was approved by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), and it is used as a flavor enhancer (MACHADO *et al.*, 2018) and in cosmetics (GERTSCH, 2008). In nature, β-caryophyllene mainly occurs as trans-

caryophyllene ((E)-caryophyllene) mixed with small amounts of the isomers (Z)-β-caryophyllene and α-humulene (α-caryophyllene), and its oxidation derivative, i.e., caryophyllene oxide. Francomano *et al.* (2019) published a review of the biological properties of β-caryophyllene in which they demonstrated (with a series of pre-clinical studies) the bioactive potential of this molecule, highlighting antioxidant, anti-inflammatory, neuroprotective, sedative and muscle relaxant activities. α-caryophyllene (or α-humulene), according to the literature, shows cytotoxic activity against tumor cells A-549 (lung carcinoma), HeLa (cervical carcinoma) and HT-29 (adenocarcinoma of colon) (DA SSILVA *et al.*., 2007). In general, α and β-caryophyllene have antiedemic, phage-repellent, anti-inflammatory, anti-tumor, bactericidal, insect-resistant and anti-allergenic actions. Caryophyllene oxide, in turn, has analgesic, anti-inflammatory (CHAVAN *et al.*, 2010), antitumor (PARK *et al.*, 2011) and gastroprotective activities (SANCHEZ *et al.*, 2014). This set of compounds was identified in a large part of the analyzed oils, with, at least, one of the compounds being found in these samples. They are one of the three major compounds in fourteen of the seventeen oils reported in the literature. β-caryophyllene was characterized as a major compound in *B. pentandra* (46.6%), *B. forficata* (18.5%), *B. longifolia* (17.4%) (DUARTE-ALMEIDA, 2004), *B. ungulata* (15.9%) (MEDEIROS *et al.*, 2016), B. rufa (15.8%) (DA SILVA *et al.*, 2019), *B. pentandra* (13.64%) (DE ALMEIDA *et al.*, 2015), *B. acuminata* (13.87%) (VASUDEVAN *et al.*, 2013) and *B. tormentosa* (14.24%) (VASUDEVAN *et al.*, 2014). E-caryophyllene was a major constituent in *B. chileanta* (21.65%) (SILVA *et al.*, 2020) and *B. ungulata* (14.5%) (DE SOUZA *et al.*, 2016). Caryophyllene oxide is one of the three major compounds of *B. ungulata* analyzed by De Souza *et al.* (2016), Gramosa *et al.* (2008) and Medeiros *et al.* (2016), with percentage concentrations of 23%, 18.3% and 9.2%, respectively.

Another compound that is noteworthy owing to frequency and concentration of appearance in several species of the genus Bauhinia is sesquiterpenoid spathulenol. Duarte-Almeida (2004) reported this compound as a major constituent in four species, namely, *B. brevipes* (15.9%), *B. longifolia* (27%), *B. rufa* (14.1%) and *B. variegata* (13.3%). In the oil of *B. ungulata* studied by Gramosa *et al.* (2009), spathulenol accounts for 47.7% of the total identified constituents, while Gois *et al.* (2011) has reported a concentration of 23.4 % of sphatulenol in the oil of B. acuruana. Spathulenol presents activity against Gram-positive and Gram-negative bacteria (Bougatsos *et al.*, 2004). Recently, Dzhul-beh *et al.* (2019) isolated

spathulenol from *Azorella compacta* Phil and showed its in vitro growth inhibition and bactericidal activity against drug-resistant clinical isolates of *Mycobacterium tuberculosis*.

The works reported in the literature used fresh or dried leaves or fresh flowers for essential oil extraction. In general, the aroma of the flowers must have a different chemical composition from that of the vegetative parts, in order to serve as a reference for specific pollinators (SAHOO *et al.*, 2013; SHARMA *et al.*, 2013).

Steffanello *et al.* (2009) showed differences in the composition of essential oils from flowers and leaves of *Eugenia pyriformis* (Myrtaceae). In leaf oils, the main components were β-pinene, limonene, 1,8-cineol and caryophyllene oxide, while in flower oils, the main components were E-caryophyllene (22.8%) and germacrene D (15.3%), which are common volatile compounds of flowers pollinated by bees and wasps. In this review, this comparison can be made with the species *Bauhinia variegata*, since two studies were found on the volatile composition of its flowers and one for the leaves. In the leaf oil, the main compounds described by Duarte-Almeida (2004) were Germacrene D (24.7%), γ-elemene (18.7%) and spathulenol (13.3%). However, data found in the two studies on flower oil are controversial. While Sharma *et al.* (2013) pointed that the major compounds of oils from Indian flowers of *Bauhinia variegata* were nerolidol (20.8%), α-bisabolol (17%) and β-bisabolene (10%), Sahoo *et al.* (2013) found cis-murrol-5-em-4-α-ol (24%), γ-elemene (19%) and α-pinene (5.1%) as the major components. Although they both were collected in India, they are from different regions of the country and details of the time of collection were not specified, which certainly impacts the variation in the composition of the oils.

It was also possible to compare the essential oils extracted from the leaves and flowers of *Bauhinia rufa*, since two works reported the composition of the leaf oils (Duarte-Almeida *et al.*, 2004; Da Silva *et al.*, 2019) and one work reported the composition of essential oils extracted from flowers from four different locations (De Menezes Filho *et al.*, 2020). Viridiflorol appears as one of the major compounds of flowers (8.32-15.08%) and leafs (26%) of *B. rufa*. When it comes to chemical classes, there is a consensus that sesquiterpenes represent an overwhelming majority among the identified compounds.

However, some exceptions were found. Five species of Bauhinia presented phytol, a diterpene, as a major constituent: *B. pentandra* (DE ALMEIDA *et al.*, 2015), *B. acuminata* (VASUDEVAN *et al.*, 2013), *B. scandens, B. purpurea* and *B. malabarica* (VASUDEVAN *et al.*, 2014), which were composed by 58.78%, 65.9%, 88.32, 90.38, and 62.17% of this

compound, respectively. For those oils, sesquiterpenes and their oxygenated derivatives were the second major groups of compounds. The presence of phytol in essential oils is attributed to the degradation of chlorophyll owing to the excessive desiccation of the vegetal matrix, the high temperatures used in the extraction process and the endogenous enzymatic reaction of chlorophyllase (KRAÜTLER, 2008). Phytol presents interesting applications in cosmetics, fine fragrances, shampoos and is also used as precursor for the manufacture of vitamin E and K1 (VASUDEVAN *et al.*, 2014). Phytol seems to have anti-inflammatory activity in acute inflammation models, mainly by inhibition of neutrophil migration, owing to a reduction of IL-1β and TNF-α levels and oxidative stress (CARVALHO *et al.*, 2020).

4. BIOLOGICAL PROPERTIES OF *BAUHINIA* OILS

In recent years, several research studies with natural products, using essential oils as raw materials, were performed.

Experiments on biological activities of essential oils from different Bauhinia species were established in only five of the thirteen works that described the chemical composition of oils. Below is the reported of the relevant findings about biological properties of the different essential oils of Bauhinia obtained from different parts of the world.

4.1. LARVICIDAL AND ACARICIDAL ACTIVITY

Several authors have developed their own criteria to characterize the potential of mosquito larvicides based on natural products, since the World Health Organization (WHO) has not established a standard criterion for determining larvicidal activity of natural products. In general, the classification in use takes into account the LC50 value, which is the lethal concentration required to kill 50% of the population. Essential oils with LC50 > 100 mg/L are considered as not active; LC50 < 100 mg/L as active; and those with LC50 < 50 mg/L are considered as highly active. In addition, samples whose results are expressed as mortality percentage are considered active when they are able to kill almost 100% of larvae at 100 mg/L. (KOMALAMISRA *et al.*, 2005).

Essential oils from some species of the genus Bauhinia have shown to be promising in combating *Aedes aegypti* larvae. Gois *et al.* (2011) evaluated the larvicidal potential of the essential oil of *B. acuruana*, exhibiting an LC50 value of 56.2 mg/L, that is, it presented a

LC50 value considered to be active. This essential oil was mostly composed of spathulenol (23.4%), epi-α-cadinol (20.7%) and caryophyllene oxide (16.4%). Sesquiterpenes and their oxygenated derivatives, together, accounted for 91.4% of the composition of this oil. In turn, De Souza *et al.* (2016) evaluated the essential oils of *B. pulchella* and *B. ungulata*. These oils presented LC50 values of 105.9 mg/L and 75.1 mg/L, respectively. Importantly, *B. ungulata* presented an oil rich in sesquiterpenes and its oxygenated derivatives (84.5%), with emphasis on caryophyllene oxide (23%), (E)-caryophyllene (14.5%) and α-copaene (7.2%), while oil from B. pulchella was rich in monoterpenes (55.8%), represented, for instance, by α-pinene (23.9%), β-pinene (12.2%) and tricyclene (7,3%). Another species of Bauhinia, reported in the literature, which also showed important larvicidal activity was *B. cheilantha* (Bong.) Steud. Silva *et al.* (2019) found a LC50 value of 40.84 mg/L for this specific oil that was also rich in sesquiterpenes (78.59%), a higher percentage than the one reported for monoterpenes (18.51%). It is noteworthy that the larvicidal activity tests described in all the works on essential oils from Bauhinia were performed with the same methodology, against instar III larvae of *Aedes aegypti*, using Temephos® (O,O`-(thiodi-4,1-phenylene)bis(O,O-dimethyl phosphorothioate) as positive control.

It is not easy to determine the relationship between larvicidal activity and the chemical composition of essential oils, since the interaction between the compounds present in the oil can interfere with the activity of the mixture. However, some authors have shown that oils rich in sesquiterpenoid compounds exhibit important larvicidal activity (FEITOSA *et al.*, 2009; ARRIAGA *et al.*, 2007; MAGALHÃES *et al.*, 2010). It can explain the lowest activity of *B. pulchella* oil, since this species showed a majority composition of monoterpenes, while other oils evaluated were mostly composed of sesquiterpenoids.

Simas *et al.* (2004) demonstrated the importance of the lipophilicity of terpenes for larvicidal activity against Aedes aegypti, comparing monoterpenes and sesquiterpenes based on their structures. The sesquiterpenoid farnesol, an isomeric form of nerolidol, showed larvicidal activity with LC50 of 13 mg/L. However, the monoterpenoid geraniol, a biosynthetic precursor of farnesol, was less active, presenting a LC50 value equal to 81.6 mg/L. These results suggest that the higher lipophilicity of the tested sesquiterpenes, when compared to monoterpenes, is an important property for larvicidal activity, since this feature provides sesquiterpenes with a higher ability to disrupt and penetrate the lipoprotein matrix of the insect cell membrane (SATYAN *et al.*, 2009).

Some authors showed that the synergistic phenomenon exists and results in a higher bioactivity of the mixture when compared to purified compounds (DIAS & MORAES, 2014). From a commercial point of view, this information is important, since the economic feasibility for obtaining an extract is much higher than that for obtaining a pure substance, which requires a series of complex isolation processes (SIMAS *et al.*, 2004).

Recently, Da Silva *et al.* (2019) evaluated the acaricidal activity of EOs from two species of Bauhinia, namely *B. rufa* Steud and *B. dumosa* Benth, by determining the fumigant, residual contact and repellency properties of these oils against Tetranychus urticae. Both oils were toxic to mites, regardless of the method in use, although they were more effective by fumigation than residual contact. With respect to chemical composition, they were both almost exclusively composed of sesquiterpenes: *B. rufa* was composed of 95.3% and *B. dumosa* of 97.2% of them. The mode of action of essential oils on arthropods and fumigation effectiveness depends on ambient temperature, air intake and the volatile nature of the essential oil (LIM *et al.*, 2012). Toxicity of oils has a stronger effect when applied through the airways than through residual contact, since they need to penetrate the coat layers to have an effect on mites. One of the factors that can influence the performance of essential oils in the bioassay of residual contact is the dilution process carried out to apply the oil. The degree of hydrophobicity of the oil solution or compound solution affects its penetration into the body of mites. More hydrophobic oil solutions will have less acaricidal activity (BADWAY *et al.*, 2010). On the other hand, in the fumigation bioassay, there is no contact of mites directly with the substance tested, but it does not exclude the possibility of synergistic effects of the minority compounds in the hermetically sealed environment, quickly reaching the airways of mites. Essential oils of *B. rufa* and *B. dumosa* seem to affect mites through both mechanisms (fumigation and residual contact), offering considerable advantages for integrated management of *T. urticae* (DA SILVA *et al.*, 2019). These findings enhance the interest for this potential activity attributed to Bauhinia oils.

4.2. CYTOTOXIC AND ANTIMICROBIAL ACTIVITY

Oils from *B. pulchella* and *B. ungulata* were evaluated by de Sousa *et al.* (2016) against HL-60 (promyelocytic leukemia), MCF-7 (breast adenocarcinoma), NCI-H292 (lung carcinoma) and HEP-2 (cervical adenocarcinoma) cells, using the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-2H tetrazolium bromide) colorimetric method.

Doxorubicin was used as positive control. With the exception of the HL-60 cell line, where the two essential oils showed the same degree of cytotoxicity, the essential oil of B. ungulata was more active in all other cell lines. The same protocol was used by Silva *et al.* (2020), who evaluated the cytotoxic activity of *B. chileantha* essential oil, presenting more intense activity on MCF-7 and HL-60 cell lines. These are the major compounds present in the oils: caryophyllene oxide (23%), (E)-caryophyllene (14.5%), α-copaene (7.2%) – *B. ungulata*; caryophyllene oxide (22.4%), α-pinene (23.9%) and β-pinene (12.2%) – *B. pulchella*; (E)-caryophyllene (21.65%), α-pinene (11.75) and bicyclogermacrene (8.19%) – *B. chileantha*. The cytotoxic activity of (E)-caryophyllene and caryophyllene oxide are already well established in the literature, as mentioned above. However, it was found that, in addition to direct activity on tumor cells, these compounds act by potentiating the action of classic chemotherapy drugs, such as doxorubicin and paclitaxel, pointing to a potential use of these compounds as therapeutic adjuncts in chemotherapeutic treatments (AMBROZ *et al.*, 2015; KIM *et al.*, 2014; LEGAULT *et al.*, 2007).

α and β-pinene have aroused interest in the scientific community for having an anti-inflammatory, anti-tumor and anti-microbial potential (NAM *et al.*, 2014; SILVA *et al.*, 2002). Despite being oxygenated monoterpenes, not belonging to the major class of sesquiterpenes, they can be found in some species of Bauhinia, in relevant quantities, possibly acting synergistically in the cytotoxic activity of oils.

Particularly for Bauhinia essential oils, two works were found in this bibliographic review. Medeiros *et al.* (2016) evaluated the antimicrobial activity of *B. ungulata* against gram negative (*Salmonella typhimurium* and *Citobacter freundii*) and gram positive (*Staphylococcus aureus* and *Bacillus cereus*) bacteria. They also evaluated antifungal activity against *Candida albicans*. Concentrations assayed were 250, 125, 62.5, 31.25, 15.6, 3.9 and 1.95 µg mL-1, and ampicillin (antibacterial), miconazole and nystatin (antifungals) were used as positive controls. EOs, mostly composed of β-caryophyllene (15.9%), caryophyllene oxide (9.2%) and α-humulene (8.1%), showed satisfactory inhibition (inhibition percentage higher than 50%) on the four microorganisms evaluated. Importantly, the inhibition on *S. aureus* was interesting, since the oil showed activity in the eight concentrations tested, ranging from 1.95 to 250 µg mL-1. De Menezes Filho *et al.*, (2020) evaluated the antimicrobial activity of essential oils extracted from *B. rufa* flowers on four species of the genus Candida (*C. albicans* ATCC 2115-1, *C. guilliermondi* ATCC 2018-2, *C. krusei* ATCC 2047-3 and *C. tropicalis*

ATCC 2591-4) using the disk diffusion method and Ketoconazol® as a positive standard. The inhibition results are expressed in millimeters (mm) in a halo of antibiosis caused by the tested strains. At all evaluated concentrations (2, 4, 6 and 8%) the essential oils showed a relevant inhibitory activity, specially at 6 and 8%, presenting halos from 10 to 29 mm, while the positive control presented halos from 25-30 mm. The presence of high concentration of β-pinene in the essential oils of *B. rufa* flowers may contribute for this great antifungal activity. Andrade *et al.*, 2018 investigated effectiveness of β-pinene inhibition on *Candida spp.* growth showing that this compound has antifungal activity and most likely acts through interference with the cell wall, through molecular interaction with Delta-14-sterol reductase and, to a lesser extent, with the 1,3-β- glucan synthase. β-pinene also found to effectively reduce Candida biofilm adhesion.

5. CONCLUSIONS

Plants of the genus Bauhinia are still little explored as to the potential of their essential oils, and few studies have been found in the literature. One of the reasons may be the low yield when extracting these oils, which makes it difficult to perform many analyses. On the other hand, plants of this kind are very easily found and abundant, making them an inexpensive alternative in the search for new biological applications.

To the best of our knowledge, this work is the first review that provides an overview of the chemical composition and biological activity of essential oils of various species of the genus Bauhinia. This review showed that most essential oils from Bauhinia are mainly composed of sesquiterpenes, which have a broad spectrum of biological actions and can be explored in various application areas. However, more studies are needed for proper standardization of essential oils. This is an arduous task, since these secondary metabolites are subjected to great variations, such as metabolic stress of plants, seasonal variations and others.

Table 2. 1. Summary of descriptive characteristics of included studies

Authors	Vasudevan *et al.* (2013)	Vasudevan *et al.* (2014)	Da Silva *et al.* (2019)	Medeiros *et al.* (2016)
Species (yield (w/w%))	*B. acuminata* (0.008)	*B. tormentosa* (0.008); *B. scandens* (0.006); *B. purpurea* (0.008); *B. malabarica* (0.009)	*B. rufa* (0.01); *B. dumosa* (0.03)	*B. ungulata* L. (0.006)
Material	Fresh leaves	Fresh leaves	Fresh leaves	Fresh leaves
Technique/extraction time	HD Clevenger, 6h	HD Clevenger, 6h	HD Clevenger, 2h	HD Clevenger, time not mentioned
Number of identified compounds, method of identification	19 RI, MS	6, 2, 4, 8 RI, MS	25, 22 RI, MS, coinjetion with standards	18 RI, MS
Major class (%)	D (65)	S (49), D (88), D (90), D (62)	S (95), S (97)	S (65)
Biological activity	-	-	Acaricidal against *T. urticae*	Acetylcholinrsterase inibition; antibacterial (Gram negative)

HD-hydrodestilation, RI-retention index, MS-mass spectrometry, S-sesquiterpenes and oxygenated sesquiterpenes, D-diterpenes; (-) = not addressed.

Table 2. 1. Continued

Authors	Silva *et al.* (2020)	De Menezes Filho *et al. (*2020)	Sharma *et al.* (2013)	De almeida *et al.* (2015)	Gramosa *et al.* (2009)
Species (yield (w/w%))	*B. chileanta* S. (0.024)	*Bauhinia rufa* (0.045-0.098)*	*B. variegata* (0.3)	*B. pentandra* (not mentioned)	*B. ungulata* L. (0.007)
Material	Fresh leaves	Flowers	Fresh flowers	Fresh leaves	Air dryed leaves
Technique/ extraction time	HD Clevenger, 3h	HD Clevenger, 3h	HD Clevenger, 3h	HD Clevenger, 5h	HD Clevenger, time not mentioned
Number of identified compounds, method of identification	44 RI, MS	30-34* RI, MS	27 RI, MS	6 RI, MS	13 RI, MS
Major class (%)	S (78)	S (39-87)*	S (60)	D (59)	S (95)
Biological activity	Larvicidal and cytotoxic	Antifungal against *Candida (C.albicans, C. guilliermondi, C.krusei, C.tropicalis)*	-	-	-

HD-hydrodestilation, RI-retention index, MS-mass spectrometry, S-sesquiterpenes, D-diterpenes; im (immesurable, but sufficient amount for injection and analysis). (-) = not addressed

Table 2. 1. Conclusion

Authors	Gois *et al.* (2011)	Sahoo *et al.* (2013)	De Souza *et al.* (2016)	Duarte-Almeida (2004)	Sartorelli (2007)
Species (yield (w/w%))	*B. acuruana* (0.01)	*B. variegata* (0.25)	*B. pulchella* Benth (0.01) *B. ungulata* L. (0.02)	*B. aculeata* (im) *B. brevipes* (0.25) *B. forficata* (im) *B. longifolia* (im) *B. pentandra* (im) *B. rufa* (0.3) *B. variegata* (im)	*B. forficata* (0.02)
Material	Fresh leaves	Fresh flowers	Fresh leaves	Air dryed leaves	Fresh leaves
Technique/ extraction time	HD Clevenger, 2h	HD Clevenger, 6h	HD Clevenger, 2h	HD Clevenger, 5h	HD Clevenger, 8h
Number of identified compounds, method of identification	30 RI, MS	51 RI, MS	28, 22 RI, MS	11, 18, 11, 7, 7, 19, 13 MS	15 RI, MS
Major class (%)	S (91.4)	S (78.3)	M (56), S (84.5)	S for all samples (100, 84, 87, 89, 100, 100)	S (96)
Biological activity	Larvicidal *Aedes egipti*	-	Larvicidal *Aedes egipti* and cytotoxic	-	-

HD-hydrodestilation, RI-retention index, MS-mass spectrometry, S-sesquiterpenes, D-diterpenes; im (immesurable, but sufficient amount for injection and analysis). (-) = not addressed

Table 2.2. Relative percentage of constituents identified in essential oils of plants of the genus Bauhinia. (compounds listed in alphabetical order)

Compounds	*B. for*	*B. acur*	*B. pul*	*B. ung*	*B. bre*	*B. pen*	*B. lon*	*B. ruf*	*B. dum*	*B. var*	*B. acum*	*B. acul*	*B. torm*	*B. scan*	*B. chei*	*B. purp*	*B. mal*
Monoterpenes																	
Camphene	-	-	2.2 [c]	-	-	-	-	-	-	0.3 [b]	-	-	-	-	0.4 [m]	-	-
3-Carene	-	-	-	-	-	-	-	0.4-1.0 [n]	-	-	-	-	-	-	-	-	-
p-Cymene	-	-	0.9 [c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Fenchene	-	-	-	-	-	-	-	Tr [d]	-	-	-	-	-	-	-	-	-
Limonene	-	-	1.0 [c]	-	-	-	-	-	-	0.3 [b]	-	-	-	-	1.2 [m]	-	-
Myrcene	-	-	-	-	-	-	-	0.1 [l]	-	0.4 [b]	-	-	-	-	0.6 [m]	-	-
(E)-β-Ocimene	-	-	-	-	-	-	-	-	-	0.2 [b]	-	-	-	-	-	-	-
β-Ocimene	Tr [d]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Pinene	1.5 [d]	-	23.9 [c]	1.4 [c], 0.8 [k]	-	-	-	1.1 [d], 0.5 [l], 0.4-3.2 [n]	0.6 [l]	5.1 [b]	-	-	-	-	11.8 [m]	-	-
β-Pinene	Tr [d]	-	12.2 [c]	0.2 [k]	-	-	-	Tr [d]; 11.2-19.7 [n]	0.3 [l]	2.2 [b]	-	-	-	-	2.4 [m]	-	-
α-Phellandrene	-	-	-	-	-	-	-	0.1 [l]	-	-	-	-	-	-	-	-	-
Sabinene	Tr [d]	-	1.2 [c]	-	-	-	-	1.0-1.2 [n]	-	0.1 [b]	-	-	-	-	0.4 [m]	-	-
α-Thujene	-	-	-	-	-	-	-	-	-	0.3 [b]	-	-	-	-	-	-	-
Tricyclene (M)	-	-	7.3 [c]	-	-	-	-	-	-	-	-	-	-	-	1.2 [m]	-	-
Oxygenated Monoterpenes																	
1,8-Cineole	-	-	0.5 [c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fenchone	-	-	-	-	-	-	-	0.9 [n]	-	-	-	-	-	-	-	-	-
Linalool	-	-	-	-	-	-	-	0.9-3.1 [n]	0.2 [l]	0.1 [b]	-	-	-	-	-	-	-
Myrcenol	-	-	-	-	-	-	-	0.3 [l]	-		-	-	-	-	-	-	-
Myrtenal	-	-	-	-	-	-	-	1.2-3.1 [n]	-	-	-	-	-	-	-	-	-
E-Pinocarveol	-	-	0.9 [c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pinocarvone	-	-	0.5 [c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2.2. Continued

Oxygenated Monoterpenes																	
(E)-Sabinene hydrate	-	-	-	-	-	-	-	-	-	0.1[b]	-	-	-	-	-	-	-
Terpinen-4-ol	-	-	0.8[c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Terpineol	-	-	0.6[c]	-	-	-	-	-	-	0.1[b]	-	-	-	-	0.2[m]	-	-
E-verbenol	-	-	-	-	-	-	-	4.1-11.0[n]	-	-	-	-	-	-	-	-	-
Sesquiterpenes																	
allo-aromadendrene	-	0.7[a]	0.4[c]	3.2[c], 2.1[h]	2.7[d]	-	-	1.2[d]	0.9[l]	1.0[b], 7.4[d]	-	Tr[d]	-	-	0.8[m]	-	-
α-Amorphene	-	-	-	-	4.8[d]	-	-	6.6[d]	-	-	-	-	-	-	-	-	-
δ-Amorphene	-	-	-	-	-	-	-	2.18[n]	-	-	-	-	-	-	-	-	-
Aromadendrene	-	-	-	1.0[k]	7.0[d]	-	5.6[d]	Tr[d]; 0.2-1.0[n]	-	Tr[d], 2.0[f]		Tr[d]	-	-	-	-	-
Z-α-Bergamotene	-	0.3[a]	-	-	-	Tr[d]	-	-	-	-	-	Tr[d]	-	-	-	-	-
E-α-Bergamotene	-	0.8[a]	0.2[c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bicyclogermacrene	14.0[d]	0.7[a]	-	-	-	-	12.3[d]	7.1[d], 0.9-1.3[n]	-	-	-	-	-	-	8.2[m]	-	-
cis-α-Bisabolene	-	-	-	-	-	-	-	9.1[d]	-	1.2[f]	-	-	-	-	-	-	-
β-Bisabolene	-	-	-	-	-	-	-	-	0.2	10.1[f]	-	-	-	-	-	-	-
β-Bourbonene	-	-	0.5[c]	1.4[c]	Tr[d]	-	-	0.3-1.1[n]	-	0.1[b], Tr[d]	-	12.4[d]	-	-	-	-	-
α-Bulnesene	17.3[e]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cadalene	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	1.5[j]
α-Cadinene	-	-	-	-	-	-	-	-	-	1.6[b]	-	-	-	-	-	-	-
β-Cadinene	-	-	-	-	-	-	-	-	-		-	-	6.1[j]	-	-	-	-
γ-Cadinene	-	0.8[a]	0.9[c]	1.6[c], 2.3[h]	5.8[d]	-	6.9[d]	2.6[d]	-	-	-	-	-	-	-	-	-
δ-Cadinene	-	-	-	1.1[k]	7.5[d]		-	4.5[d]		3.6[b], 8.1[d]	-	-	-	-	-	-	-

Table 2.2. Continued

Sesquiterpenes																	
α-Calacorene	-	-	-	0.8[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
β-Caryophyllene	18.5[d]	1.5[a]	2.2[c]	4.2[h], 15.9[k], 14.5[c]	-	46.6[d], 13.6[g]	17.4[d]	15.8[l]	6.4[l]	0.7[b], 2.4[d]	13.9[i]	Tr[d]	14.2[j]		21.7[m]	-	-
9-epi-(E)-Caryophyllene	-	-	-	-	-	-	-	0.4[l]	-	-	-	-	-	-	-	-	-
Cyclosativene	-	-	-	1.2[c]	-	-	-	0.2[l]	-		-	-	-	-	-	-	-
α-Copaene	5.6[d]	0.4[a]	1.3[c]	7.2[c], 2.9[h], 3.5[l][k]	3.4[d]	-	-	2.4[l]	1.0[l]	2.9[d]	-	-	-	-	0.4[m]	-	4.7[j]
β-Copaene	-	0.2[a]	1.3[c]	4.6[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
Copaene Isomer	22.8[d]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Cubebene	-	-	-	0.6[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
β-Cubebene	-	-	-	-	1.5[d]	-	-	-	-	-	-	-	21.8[j]	-	-	-	-
α-Curcumene	-	-	-	-	-	-	-	-	-	0.8[f]	-	-	-	-	-	-	-
Cyperene	-	-	2.2[c]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Elemene	-	-	-	-	-	22.6[d]		-	-	-	-	-	-	-	-	-	-
β-Elemene	9.7[d]	2.3[a]	1.1[c]	4.9[c], 2.3[h], 1.8[k]	1.2[d]	Tr[d]		4.8[l]		2.6[b], 3.4[d]		56.9[d]	6.9[j]		4.9[m]	-	2.8[j]
γ-Elemene	Tr[d], 38.4[e]	-	-	1.3[k]	11.8[d]	17.5[d], 11.7[g]	-	1.0[l]	10.5[l]	19.0[b], 18.7[d]	-	-	-	-	-	-	-
δ-Elemene	-	0.3[a]	-	-	-	-	-	-	-	-	-	-	-	-	7.4[m]	-	-
Eremophyllene	-	-	-	-	-	-	-	-	-	-	-	8.4[d]	-	-	-	-	-
α-Farnesene	-	-	-	-	-	-	-	-	-	-	0.1[i]	-	-	-	-	-	-
(E)-β-Farnesene	-	0.4[a]	-	-	-	-	-	-	1.5[l]	-	-	-	-	-	-	-	-
(Z)-β-Farnesene	9.1[e]	-	-	-	-	-	-	-	-	3.2[f]	-	-	-	-	-	-	-
Germacrene A	-	-	-	-	-	-	-	-	-	0.2[b]	-	-	-	-	-	-	-

Table 2.2. Continued

Sesquiterpenes																	
Germacrene B	-	0.7[a]	-	-	-	-	-	7.1[d], 3.9[l]	37.6[l]	- -		-	-	-	-	-	-
Germacrene D	-	0.7[a]	-	1.5[h]	7.7[d]	8.4[g]	9.0[d]	7.2[d], 0.7[l], 3.8-7.9[n]	-	1.1[b], 24.7[d]	-	-	-	-	2.0[m]	-	-
6,9-Guaiadiene	-	-	-	0.8[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
α-Guaiene	-	-	-	0.6[c]	-	-	-	-	-	0.5[b]	-	-	-	-	-	-	
β-Guaiene	-	-	-	-	-	-	-	0.6[l]	0.7[l]		-	-	-	-	-	-	6.5[j]
α-Gurjunene	-	-	-	-	-	-	-	Tr[d]	-	-	-	-	-	-	-	-	-
β-Gurjunene	-	-	-	-	-	-	-	-	-	0.3[b]	-	-	-	-	-	-	-
γ-Gurjunene	-	-	-	-	-	-	-	-	-	0.8[b]	-	-	-	-	-	-	-
α-Humulene	3.5[e]	0.6[a]		0.6[c], 3.5[h], 8.1[k]	Tr[d]	Tr[d], 2.7[g]	-	0.4[l]; 0.4-2.0[o]	0.5[l]	0.5[b], Tr[d]	1.8[i]	-	-	-	3.6[m]	-	-
Isocaryophyllene	-	-	-	-	-	-	-	-	-	2.2[d]	-	-	-	-	-	-	-
Isocomene	-	-	-	-	-	-	-	-	-	0.1[b]	-	-	-	-	-	-	-
Ledene	-	-	-	-	Tr[d]	-	-	-	-	7.4[d]	-	Tr[d]	-	-	-	-	-
α-Muurolene	-	-	0.7[c]	2.8[c]	-	-	-	-	-	0.5[b]	-	-	-	-	-	-	-
γ-Muurolene	-	-	-	2.8[h], 1.1[k]	-	-	-	12.2[l]	3.7[l]	0.1[b]	-	-	-	-	-	-	-
γ-Patchulene	-	-	-	-	-	-	-	-	2.0[l]	-	-	-	-	-	-	-	-
Selina-3,7(11)-diene	-	-	-	-	-	-	-	-	-	1.3[b]	-	-	-	-	-	-	-
α-Selinene	-	-	-	-	-	-	-	-	-	1.9[b]	-	-	-	-	-	-	-
β-Selinene	-	0.4[a]	1.3[c]	1.4[c]	1.5[d]	-	-	-	-	0.3[b], 0.7[f]	-	-	-	-	-	-	-
7-epi-α-Selinene	-	-	-	-	-	-	-	-		0.3[b]	-	-	-	-	-	-	-
β-Sesquiphellandrene	-	1.8[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sinularene	-	-	-	-	-	-	-	Tr[d]	-	-	-	-	-	-	-	-	-
Valencene	-	-	-	3.0[c]	-	-	-	7.1[l]	-	-	-	Tr[d]	-	-	-	-	-

Table 2.2. Continued

Sesquiterpenes																	
β–ylangene	-	-	-	-	-	-	-	2.1[l]	-	-	-	-	-	-	-	-	-
Oxygenated Sesquiterpenes																	
α-Bisabolol	0.8[e]	-	-	4.7[k]	-	-	-	0.9[l], 4.1-7.2[n]	0.2[l]	17.1[f]	-	-	-	-	-	-	-
α-Bisabolol oxide	-	-	-	-	-	-	-	-	-	1.7[f]	-	-	-	-	-	-	-
α-Cadinol	3.7[e]	-	0.7[e]	1.0[e], 0.4[h]	7.4[d]	-	-	9.1[d], 10[l]	-	4.6[b]	0.4[i]	-	-	-	-	-	-
epi-α-Cadinol	-	20.7[a]	-	-	-	-	-	12.8[l]	0.8[l]	-	-	-	-	-	-	-	-
trans-Calamenen-10-ol	-	0.5[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Caryophylla-4(12),8(13)-dien-5α-ol	-	-	-	-	-	-	-	-	-	-	0.2[i]	-	-	-	-	-	-
Caryophylla-4(12),8(13)-dien-5β-ol	-	-	-	-	-	-	-	-	-	-	1.0[i]	-	-	-	-	-	-
14-Hydroxy-9-epi(E)-Caryophyllene	-	0.6[a]	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Caryophyllene oxide	9.4[e]	16.4[a]	22.4[c]	23.0[e], 18.3[h], 9.2[k]	-	13.3[d], 0.7[g]	-	2.7[l]	1.1[l]	-	3.2[i]	Tr[d]	-	-	4.6[m]	-	-
5-Cedranone	-	-	-	-	-	-	-	-	-	0.4[b]	-	-	-	-	-	-	-
α-Chenopodiol-6-acetate	-	-	-	-	-	-	-	3.0-3.1[n]	-	-	-	-	-	-	-	-	-
Cubenol	-	-	-	1.4[k]; 1,1[c]	-	-	-	0.5[l], 0.5-1.0[n]	-	0.8[b]	-		-	-	-	-	-
epi-Cubenol	-	-	-	-	-	-	-	0.3-0.9[n]	-	0.2[b]	-	-	-	-	-	-	-
1,10-di-epi-Cubenol	-	-	-	-	-	-	-	-	-	1.4[b]	-	-	-	-	-	-	-
2,3-Dihydrofarnesol	-	1.4[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8-α-11-elemenediol	-	-	-	2.7[h]	-	-	-	-	-	-	-	-	-	-	-	-	-
Elemol	-	0.1[a]	-	-	-	-	-	1.6-10.2[n]	-	-	-	-	-	-	-	1.9[j]	-
γ-Eudesmol	-	1.0[a]	-	-	-	-	-	0.8-0.9[n]	-	-	-	-	-	-	-	-	-

Table 2.2. Continued

Oxygenated Sesquiterpenes																	
Epi-γ-Eudesmol	-	-	-	7.5[k]	-	-	-	-	-	-	-	-	-	-	-	-	-
Farnesol	-	-	-	-	-	-	-	-	-	0.7[f]	0.2[i]	-	-	-	-	-	-
(Z,E)-Farnesol	1.6[e]	-	-	1.2[k]	-	-	-	-	-	-	-	-	-	-	-	-	-
(Z,Z)-Farnesol	1.0[e]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Germacrene-D-4-ol	0.7[e]	-	-	-	-	-	-	4.7-7.8[n]	-	1.5[b]	-	-	-	-	-	-	-
Globulol	1.2[e]	2.4[1a]	-	-	-	-	-	2.2[d], 3.2-9.0[n]	-	-	-	-	-	-	1.8[m]	-	-
Guaia-3,1-(14)-dien-11-ol	-	-	-	-	-	-	-	2.2-4.1[n]	-	-	-	-	-	-	-	-	-
Guaiol	-	-	-	-	-	-	-	0.3-0.7[n]	-	-	-	-	-	-	-	-	-
Hexahydrofarnesyl acetone	-	-	-	-	-	-	-	-	-	4.4[f]	-	-	-	-	-	-	-
14-Hydroxy-α-humulene	-	-	-	-	-	-	-	-	-	0.9[b]	-	-	-	-	-	-	-
Humulene epoxide II	-	-	1.7[c]	4.6[c], 5.2[h]	-	-	-	-	-	-	0.4[i]	-	-	-	-	-	-
Isoaromadendrene epoxide	-	-	-	-	-	-	-	-	-	-	0.1[i]	-	-	-	-	-	-
Isospathulenol	-	-	-	-	-	-	10.8[d]	-	-	-	-	-	-	-	-	-	-
Junenol	-	-		0.8[c]	-	-	-	-	-	-	-	-	-	-	-	-	-
Juniper Camphor	-	-	-	-	-	-	-	-	-	0.7[b]	-	-	-	-	-	-	-
Lanceol	-	-	-	-	-	-	-	0.2-3.3[n]	-	1.7[f]	-	-	-	-	-	-	-
Lepidozenol	-	-	-	-	-	-	-	3.1[d]	-	-	-	22.3[d]	-	-	-	-	-
cis-Murrol-5-en-4-α-ol	-	-	-	-	-	-	-	-	-	24.4[b]	-	-	-	-	-	-	-
α-Muurolol	-	0.4[a]	-	-	-	-	-	-	-	-	0.3[i]	-	-	-	1.4[m]	-	-
epi-α-Muurolol	1.7[e]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(E)-Nerolidol	-	2.4[a]	-	3,3[k]	-	-	-	-	29.1[l]	20.8[f]	-	-	-	-	-	-	-

Table 2.2. Continued

Oxygenated Sesquiterpenes																	
Occidentalol	-	-	-	-	-	-	-	-	-	2.3[b]	-	-	-	-	-	-	-
Oplopanone	-	-	-	-	-	-	-	3.8-10.7[n]	-	-	-	-	-	-	-	-	-
Sclareolide	-	-	-	-	-	-	-	-	-	-	0.2[i]	-	-	-	-	-	-
Spathulenol	2.1[e]	23.4[a]	2.9[c]	1.8[c], 47.7[h], 2.1[k]	15.9[d]	-	27.0[d]	14.1[d], 4.4[l]	0.2[l]	13.3[d]	-	-	-	-	4.8[m]	-	-
Valerianol	-	5.7[a]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Viridiflorol	-	1.6[a]	-	-	2.8[d]	-	-	2.0[L], 26.0[d], 8.3-15.1[n]	-	-	-	-	-	-	0.7[m]	-	-
Diterpenes																	
Isophytol	3.3[e]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phytol	-	-	-	-	-	58.8[g]	-	-	-	-	65.9[i]	-	10.1[j]	88.3[j]		90.4[j]	62.2[j]
Phytone	-	-	-	-	-	-	-	-	-	-	-	-	32.8[j]	2.5[j]	-	1.9[j]	-
Miscellaneus																	
1[H]Cycloprop[e]azulene (HC)	-	-	-	-	-	-	-	-	-	0.8[f]	-	-	-	-	-	-	-
1-Decanol (A)	-	-	-	-	-	-	-	0.7-5.3[n]	-	-	-	-	-	-	-	-	-
Dichloroacetic acid (CA)	-	-	-	-	-	-	-	-	-	6.6[f]	-	-	-	-	-	-	-
8,11,14-Docosatrienoic acid methyl ester (E)	-	-	-	-	-	-	-	-	-	2.0[f]	-	-	-	-	-	-	-
1,6,10-Dodecatrien-3-ol (A)	-	-	-	-	-	-	-	-	-	-	0.3[i]	-	-	-	-	-	-
Eicosane (HC)	0.2[e]	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-
Ethyl 9-hexadecenoate (E)	-	-	-	-	-	-	-	-	-	0.9[f]	-	-	-	-	-	-	-
Ethyl n-heptadecanoate(E)	-	-	-	-	-	-	-	-	-	1.6[f]	-	-	-	-	-	-	-
Hexadecanoic acid (CA)	-	-	-	-	-	-	-	-	-	4.8[f]	-	-	-	-	-	-	-
Hexadecanoic acid, ethyl ester (E)	-	-	-	-	-	-	-	-	-	6.1[f]	-	-	-	-	-	-	-
Hexadecanoic acid, methyl ester (E)	-	-	-	-	-	-	-	-	-	1.4[f]	-	-	-	-	-	-	-
9-Hexadecenoic acid (CA)	-	-	-	-	-	-	-	-	-	0.8[f]	-	-	-	-	-	-	-
Hexadecadienoic acid, methyl ester (E)	-	-	-	-	-	-	-	-	-	0.8[f]	-	-	-	-	-	-	-

Table 2.2. Conclusion

Miscellaneus																	
3-Hexen-1-ol (A)	-	-	-	-	-	-	-	-	-	-	0.2 i	-	-	-	-	-	-
β-Ionone (NI)	-	-	-	-	-	-	-	-	-	-	0.1 i	-	-	-	-	-	-
isomethyl-α-Ionone (NI)	-	-	-	-	-	-	-	-	-	-	0.2 i	-	-	-	-	-	-
Linoleic acid ethyl ester (E)	-	-	-	-	-	-	-	-	-	3.5 f	-	-	-	-	-	-	-
2-Methyl-decane (HC)	-	-	-	-	-	-	-	-	-	1.3 f	-	-	-	-	-	-	-
Myristic acid isopropyl ester (E)	-	-	-	-	-	-	-	-	-	1.2 f	-	-	-	-	-	-	-
Nonadecane (HC)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.8 j	-
Octacosane (HC)	-	-	-	-	-	-	-	-	-	-	0.2 i	-	-	-	-	-	-
Octadecanal (AD)	-	-	-	-	-	-	-	-	-	0.7 f	-	-	-	-	-	-	-
Octadecane (HC)	-	-	-	-	-	-	-	-	-	0.9 f	-	-	-	-	-	-	-
1-Octadecene (HC)	-	-	-	-	-	-	-	-	-	-	0.3 i	-	-	-	-	-	-

Tr=traces. B. forf, B. forficata Link; B. acur, B. acuruana Moric.; B. pul, B. pulchella Benth; B.ung, B. ungulata L.; B. bre, B. brevipes Vogel; B. pen, B. pentandra (Bong.) Steud; B. lon, B. longifolia (Bong.) Steud; B. ruf, B. rufa (Bong.) Steud; B. dum, B. dumosa; B. var, B. variegata; B. acum, B. acuminata L.; B. acul, B. aculeata L.; B. torm, B. tormentosa L.; B. scan, B. scandens L.; B. chil, B. cheilantha (Bong.) Steud; B. pur, B. purpurea L.; B. mal, B. malabarica Roxb; B. mond, B. monandra Kurz (a) Gois et al. (2011); (b) Sahooet al. (2013) (c) De Souza et al. (2016); (d) Duarte-Almeida et al. (2004); (e) Sartorilli, Correa (2007); (f) Sharma et al. (2013); (g) De Almeida et al. (2015); (h) Gramosa et al. (2009); (i) Vasudevan et al. (2013); (j) Vasudevan et al. (2014); (k) Medeiros et al. (2016); (l) Da Silva et al. (2019); (m) Silva et al. (2020); (n) de Menezes Filho et al. (2020). A = alcohol; AD = aldehyde; CA = carboxylic acid; E = ester; HC = hydrocarbones; NI = norisoprenoid.

Table 2. 3. Three major compounds identified in essential oils of Bauhinia species and local of collection

Plant species	major constituents (%)	Authors	City, Country (Region)
B. acuruana	Spathulenol (23.4); epi-α-cadinol (20.7); cariophylene oxide (16.4)	Gois *et al.* (2011)	Ceará, Brazil (Northeast)
B. variegata (flowers)	Cis-murrol-5-em-4-α-ol (24.4); γ-elemene (19); α-pinene (5.1)	Sahoo *et al.* (2013)	Lucknow, India (Northern)
B. pulchella	α-pinene (23.9); caryophyllene oxide (22.4); β-pinene (12.2)	de Souza *et al.* (2016)	Ceará, Brazil (Northeast)
B. ungulata	Caryophyllene oxide (23); (E)-Caryophyllene (14.5); α-copaene (7.2)		
B. aculeata	β-elemene (56.9); lepidonezol (22.3); β-boubonene (12.4)	Duarte-Almeida *et al.* (2004)	Minas Gerais, Brazil (Southeast)
B. brevipes	Spathulenol (15.9); γ-elemene (11.8); germacrene D (7.7)		
B. forficata	Copaene isomer (28.8); β-caryophyllene (18.5); bicyclogermacrene (14)		
B. longifolia	Spathulenol (27); β-caryophyllene (17.4); bicyclogermacrene (12.3)		
B. pentandra	β-caryophyllene (46.6); α-elemene (22.6); γ-elemene (17.5)		
B. rufa	Viridiflorol (26); spathulenol (14.1); α-cadinol (9.1)		
B. variegata	Germacrene D (24.7); γ-elemene (18.7); spathuenol (13.3)		
B. forficata	γ-elemene (38.4); α-bulnesene (17.3); caryophyllene oxide (9.4)	Sartoreli & Correa (2007)	São Paulo, Brazil (Southeast)
B. variegata (flowers)	Nerolidol (20.8); α-bisabolol (17.08); β-bisabolene (10.1)	Sharma *et al.* (2013)	Dehradun, India (North)
B. pentandra	Phytol (58.78); β-caryophyllene (13.64); elixene (11.73)	de Almeida *et al.* (2015)	Ceará, Brazil (Northeast)
B. ungulata	Spathulenol (47.7); caryophyllene oxide (18.3); humulene epoxide II (5.2)	Gramosa *et al.* (2008)	Ceará, Brazil (Northeast)
B. acuminata	Phytol (65.9); β-caryophyllene (13.87); caryophyllene oxide (3.15)	Vasudevan *et al.* (2013)	Kerala, India (Southwestern)
B. tormentosa	Phytone (32.84); β-cubebene (21.84); β-caryophyllene (14.24)	Vasudevan *et al.* (2014)	Pacha Palode, India (Southwestern)
B. scandens	Phytol (88.32); phytone (2.54)		
B. purpurea	Phytol (90.38); nonadecane (2.82); phytone (1.92)		
B. malabarica	Phytol (62.17); δ-cadinene (12.47); β-guaiene (6.53)		
B. ungulata	β-caryophyllene (15.9); caryophyllene oxide (9.2); α-humulene (8.1)	Medeiros *et al.* (2016)	Roraima, Brazil (North)
B. rufa	β-caryophyllene (15.8); epi-α-cadinol (12.8); γ-murolene (12.2)	da Silva *et al.* (2019)	Brasilia, Brazil (Midwest)
B. dumosa	Germacrene B (37.6); (E)-nerolidol (29.1); γ-elemene (10.5)		
B. chileanta	(E)-Caryophyllene (21.65); α-pinene (11.75); bicyclogermacrene (8.19)	Silva *et al.* (2020)	Ceará, Brazil (Northeast)
*B. rufa (flowers)**	β-pinene (11,23-19,74); viridiflorol (8,32-15,08); trans-verbenol (4,08-11)	De Menezes Filho *et al.* (2020)	Goiás, Brazil (Midwest)

*range of values for ois from four locations

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CHAPTER 3

CHEMICAL PROFILE OF THE VOLATILE FRACTION OF *BAUHINIA FORFICATA* LEAVES: AN EVALUATION OF COMMERCIAL AND IN NATURA SAMPLES.

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This chapter was submitted to **Food Science and Technology** as an original paper.

ABSTRACT

Bauhinia forficata L. is widely used in Brazilan folk medicine to treat a variety of pathologies. Commercial and botanically identified samples were evaluated via a gas chromatography equipped with a flame ionization detector (GC-FID) and a gas chromatography-mass spectroscopy (GC-MS). This procedure allowed the identification of 116 compounds, representing 72.45% to 96% of the total content of the investigated essential oils. The yields of the essential oils of the five samples analyzed in the present study ranged from 0.03 to 0.1 %. The classes of sesquiterpenes and oxygenated sesquiterpenes were predominant in all the essential oils. Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) were used in order to demonstrate variations in the composition of the essential oils of B. forficate L. and were able to clusterize these samples in three groups based on relationships and chemical patterns in essential oils. In natura samples showed to be different from commercial samples and CS3 group was the most distinct group of the commercial samples. Data on the chemical composition of essential oils from *B. forficata* L. are scarce and, concerning commercial samples, this is the first report. A larger universe of samples should be evaluated, commercial and in natura ones, in an attempt to establish a set of compounds that could represent a chemical marker of this species, still non-existent in literature.

Keywords: *B. forficata*, essential oils, GC-MS, sesquiterpenes

1. INTRODUCTION

The genus Bauhinia, popularly known as “pata-de-vaca”, among other denominations, belongs to the Fabaceae family, and in Brazil, 300 native species have already been cataloged. Infusions of leaves of *Bauhinia forficate Link,* also known as *“Brazilian Orchid-tree”* species are used in Brazilian folk medicine as a diuretic, hypoglycemic, tonic, depurative agent, in the fight against lymphatic filariasis (elephantiasis), and for the reduction of glycosuria. Its beneficial effects are generally associated with the presence of phenolic compounds, which are known to have antioxidant properties (SALGUEIRO *et al.*, 2016; FRANCO *et al.*, 2018).

In 2009, the Ministry of Health of Brazil published a list of Medicinal Plants of Interest to SUS (Sistema Único de Saúde) or Unified Health System, the Brazilian national healthcare system. This list known in Brazil as RENISUS aims to guide and strengthen research on the species included in the list, especially native ones. The list describes 71 species, and among them is *Bauhinia forficata Link*, highlighting the importance of advancing research that corroborates its use in folk medicine.

The main form of commercialization of this herb is dried leaves in plastic bags for preparations of homemade infusions. Thus, the product is treated as a food and under Brazilian law it is not required to indicate the content of bioactive or toxic compounds, as is done in a limited way in herbal products. In this way, the control and regulation of this type of product is practically non-existent, facilitating the possibility of fraud through the inclusion of herbs other than that determined on the label.

The literature is rich when it comes to the composition of leaf extracts (aqueous or hydroalcoholic) of *Bauhinia forficata*. Free and glycosylated flavonoids, especially canferolic and quercetinic glycosides, represent important chemical groups typical of the genus, and are the main constituents of *B. forficata* extracts (PAULA *et al.*, 2002; PIZZOLATTI *et al.*, 2003; FERRERES *et al.*, 2012).

Regarding the composition of the essential oils of this species, only two papers were reported in the literature and presented controversial results. Duarte and Almeida (2004) reported for the first time the occurrence and chemical composition of volatile oils in some species of Bauhinia, the major constituents being sesquiterpenes, namely β–caryophyllene (18.5%) and a copaene isomer (28.8%) found as major components in *Bauhinia forficata*. Controversely, Sartorelli & Correia (2007) did not find those compounds in the studied oil of

the same specimen. Instead, they identified γ-elemene (38.4%) and α-bulnesene (17.3%) as major components. These are the only records of the composition of essential oils of *Bauhinia forficata*, showing how scarce and controversial the information about the composition of this fraction of the plant actually is. When we expanded the scope of the research, considering only the genus *Bauhinia*, there are only fourteen articles that address the chemical composition of essential oils, comprising a total of seventeen species. With exception of the oils analyzed by Vasudevan *et al.*, 2013 and 2014 and De Almeida *et al.*, 2015, which presented the diterpene phytol as a major constituent of the species *Bauhinia acuminata* (65.9%), *Bauhinia scandens* (88.32%), *Bauhinia purpurea* (90.38%) and *Bauhinia malabarica* (62.17%*)*; all other samples are characterized by a major composition of sesquiterpenes.

A broader knowledge about the chemical composition of the essential oil of *Bauhinia forficata* can contribute to the elucidation of the mechanisms that involve its known pharmacological actions, since part of the volatile terpenic composition can be transferred when preparing the infusions, a form that is normally consumed. Volatile compounds also play a significant role in plant oil and infusion aroma, and they are influential in consumer choice. Furthermore, a detailed knowledge of a representative number of samples can contribute to the determination of a chemical marker of the species, not yet established, helping to standardize these oils, as a way of monitoring fraud. The lack of botanical knowledge should not be discarded, which could have led to misidentification and confusion of the raw material, a most prominent factor in this genus, as there is much morphological similarity between the species.

In this way, the association of botanical data with multivariate tools to study plants allow the comparison among samples based on chemometric methods, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), (ANDRÉS-ABELLAN, 2005).

The present work aimed to characterize essential oils extracted from leaves of *Bauhinia forficata* L, comparing a botanically identified sample and four commercial samples labeled as “pata-de-vaca” (*Bauhinia forficata* Link). A chemometric evaluation was carried out to verify the similarities between the botanically identified essential oil and the commercial ones.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Herbal parts of *Bauhinia forficata* Link, the reference sample or *in natura* sample (IN), were collected on three different dates in Petropolis city, Rio de Janeiro State (22° 30' 04.63“S, 43° 07' 58.20“W; Altitude: 958 m), and voucher specimens were deposited at the Herbarium of the Department of Botany of the Federal University of Rio de Janeiro under the registration number RFA 40.615. Four brands of commercial samples were purchased from local markets in Rio de Janeiro city. For three of them, it was possible to acquire three different lots and for one brand, it was possible to acquire only two different lots. The samples were labeled as “pata-de-vaca”, and were coded as CS1, CS2, CS3 and CS4. The reference samples (IN) were dried in an air circulation oven (50° C) for 5h (Model 12% Humidity-BRASIL, 2010) and powdered in order to be in accordance with the commercial samples.

2.2. ESSENTIAL OIL EXTRACTION FROM THE REFERENCE AND COMMERCIAL *Bauhinia forficata* L. SAMPLES

The essential oils were extracted by hydrodistillation (Clevenger apparatus) using a 2000 mL flask containing 70 g of herb and 1000 mL of distilled water. The isolation process was carried out during four hours at a temperature of 100°C. The essential oil was collected with ethyl acetate, with posterior solvent evaporation under an inert atmosphere of nitrogen gas and the final product was stored in a freezer at -18°C until the chromatographic analysis.

2.3. CHROMATOGRAPHIC ANALYSIS

2.3.1. Gas chromatography (GC) analysis

Oils obtained from aerial parts of *Bauhinia forficata* L and the commercial samples were analyzed using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with a flame ionization detector (FID). Oven temperature was maintained at 50^{o}C for 2 min initially, and then raised at the rate of 5°C/min to 240°C, staying at this temperature for 10 min. Injector and detector temperatures were set at 250°C and 260°C, respectively. Helium was used as carrier gas at a

flow rate of 1 mL/min, and 1μL of diluted samples (0.01g/mL) were injected in the splitless mode. Normalization technique was used for obtaining quantitative data.

2.3.2. Gas chromatography/mass spectrometry (GC/MS) analysis

GC/MS analysis of the oils was carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with a HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70 eV) operating under the same conditions as described above. Retention indices were calculated for all components using a homologous series of n-alkanes injected in the same conditions of the samples. Identification of components of essential oils was based on linear retention indices (LRI) relative to n-alkanes and computer matching with the Wiley275.L and Wiley7n.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature (ADAMS, 2001).

2.4. CHEMOMETRIC ANALYSIS

The chemometric methods used for data analysis were hierarchical cluster analysis (HCA) and principal component analysis (PCA). HCA comprises an unsupervised classification procedure that involves measuring either the distance or the similarity between the objects to be clustered. The samples with close similarities are sorted into the same cluster. PCA is widely used for reducing the dimensions of original data set by explaining the correlation among a large number of variables in terms of a smaller number of underlying factors (principal components, PCs) without losing much information. (HU *et al.*, 2014)

One data matrix (14 × 116) was constructed in such a way that each row corresponded to a sample and each column corresponded to a compound identified by GC-MS analysis.

The HCA and PCA analyses were performed using the Ward method and singular value decomposition (SVD) algorithm, respectively.

3. RESULTS AND DISCUSSION

3.1. ESSENTIAL OIL YIELD AND COMPOSITION

The essential oils were obtained from the dried leaves of the *in natura B. forficata* (IN) and the commercial samples (CS1, CS2, CS3 and CS4) by hydrodistillation in yelds of 0.08±0.005% (RS), 0.03±0.01% (CS1), 0.05±0.01% (CS2), 0.10±0.04% (CS3), and 0.13±0.05% (CS4), calculated from the average of three different lots. For IN, our result was four times higher than that found by Sartorelli *et al.* (2007) who obtained 0.02% yield from a botanic identified sample from São Paulo. All commercial samples showed higher yields as well. There are no records in the literature for commercial samples, but the genus is known for its low yield of essential oil (SARTORELLI *et al.*, 2007, VASUDEVAN *et al.*, 2014, DA SILVA *et al.*, 2019).

GC-FID and GC-MS analyses were performed and the identities of the compounds, their RI (calculated and literature) and their relative peak area percentages (average of three different lots) are listed in Table 1. The chemical composition of the samples proved quite different, with only 11 compouds in common between them, namely, α-copaene, β-cubebene, β-caryophyllene, α-humulene, germacrene-D, δ-cadinene, spathulenol, caryophyllene oxide, humulene epoxide II, isophytol and hexadecanoic acid (Figure 1 and Figure 2).

Figure 3.1. Percentage composition of compounds identified in all essential oils

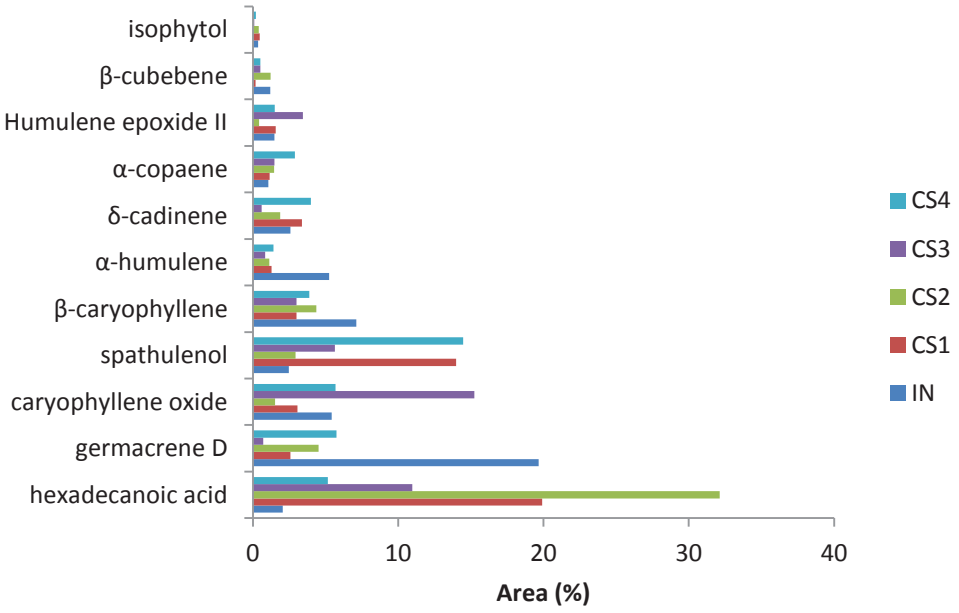
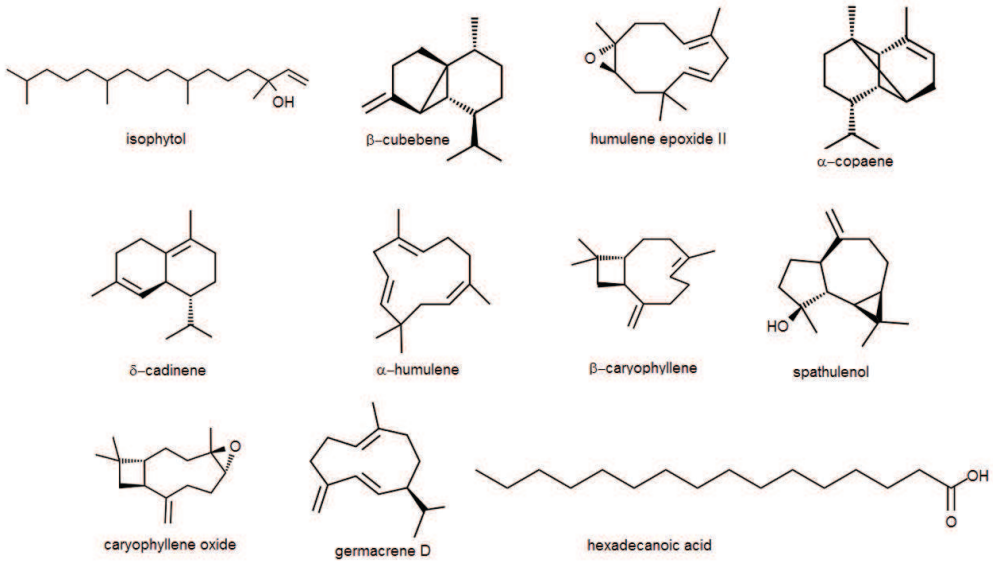
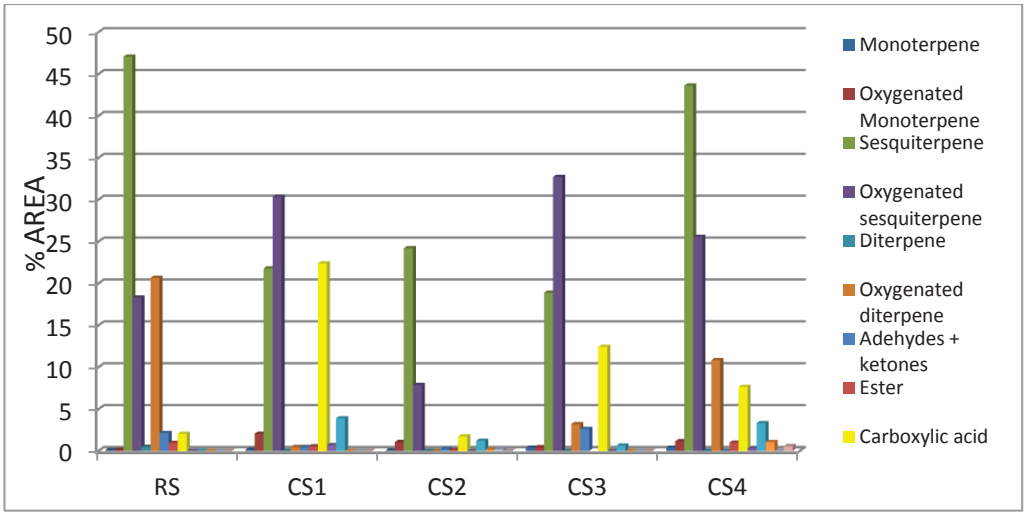


Figure 3.2. Structures of compounds identified in all essential oils



Regarding chemical classes, all samples had a terpene chemical profile. The predominant class of which was sesquiterpenes and their oxygenated derivatives. For IN (65%), CS1 (52%), CS2 (32%), CS3 (52%) and CS4 (69%).

Figure 3.3. Percentage composition based on chemical classes



For the in natura sample (IN), 38 components were identified and represent approximately 91% of the total oil content, being phytol (20%), germacrene D (19%), α-cadinol (8%) and β-caryophyllene (7%) the major compounds. 57 compounds were identified in CS1, representing 83% of the total oil. Hexadecanoic acid (19.9%), spathulenol (14%) and α-cadinol (7.6%) were the major compounds. Once again, hexadecanoic acid (32.1%) appeared as the major constituent of the essential oil of CS2 sample, followed by phytol (10.2%) and linolenic acid (8.3%). In this sample, 64 compounds were identified, representing 96% of the total oil. For the CS3 sample, 46 compounds of the oil were identified, representing 72.44% of its total content and the major compouds were caryophyllene oxide (15.2%), hexadecanoic acid (10.9%) and spathulenol (5.7%). CS4 had 94.9% of its oil composition identified, with a total amount of 65 compounds. Spathulenol (14.48%), phytol (10.64%) and germacrene D (5.74%) being the major constituents.

High sesquiterpene content in essential oils from leaves of *Bauhinia* species were previously reported by Gramosa *et al.* (2008), which showed that *B. ungulata*'s essential oil was composed exclusively of sesquiterpenes (21.6%) and its oxygenated derivatives (74.3%). Silva *et al.*, 2020 reported the composition of essential oil from *Bauhinia chileantha* and showed that the sesquiterpenoid compounds represent 78.6% of the total content of the oil. Sartoreli and Correa, 2007, also identified 15 compounds in a single sample of *Bauhinia forficata*, 14 of them being sesquiterpenes.

It is inferred that the species demonstrates a preference for the metabolic pathways of mevalonic acid, which starts from acetyl CoA and gives rise to sesquiterpenes (C_{15}) and MEP (Metileritritol 4-P) pathway, starting with the condensation of pyruvate and D-glyceraldehyde-3-phosphate to form 1-deoxy-D-xylulose 5-phosphate, producing precursors for hemiterpenes (C_5), monoterpenes (C_{10}), and diterpenes (C_{20}), since the composition of its essential oils is mostly terpenic (ARAGÜEZ and VALPUESTA, 2013).

Many sesquiterpenes, and their alcohol, aldehyde, and ketone derivatives are biologically active or precursors of metabolites with biological functions, while others have desirable fragrance and flavoring properties. Several sesquiterpenes are recognized for their potential as aroma compounds with pleasant and commercial characteristics and have also been studied in the last few years regarding their biological potentials (BUTNARIU, 2021).

Observing the 11 compounds that were characterized in all essential oils, it can be concluded that some of them are responsible for the characteristic and very similar aroma

between the samples. Germacrene D was identified in concentrations varying from 0.70 to 19.68% and for this compound an odor characteristic of woody and greasy cooked flour is attributed (AJARAYASIRI; CHAISERI 2008). Nonetheless there is no information available on the odor threshold of Germacrene D. The odor threshold is defined as the minimum concentration of a volatile compound that can allow its perception by the human olfaction. The lower the odor threshold of a substance, the greater its odorant potential (MARIANO *et al.*, 2019). Germacrenes, produced in various plant species, are known to act as insecticidal, antimicrobial, and insect pheromones (BULOW *et al.*, 2000; LI *et al.*, 2005). This volatile organic compound has been observed in bryophytes, gymnosperms, and angiosperms. Interestingly, germacrene D plays an important role as a precursor in sesquiterpenes synthesis such as selinenes and cadinenes (MALIK *et al.*, 2019). Phytol was one of the major compounds in the oils, with the exception of the CS1 sample (3.1-20.1%). An odor threshold of 0.64 ppm infers that a green, weak floral-balsamic odor can be attributed to this compound (GUO *et al.*, 2021; BUTNARIU, 2021). Phytol also presents interesting applications in cosmetics, fine fragrances, shampoos and is used as precursor for the manufacture of vitamin E and K1 (VASUDEVAN *et al.*, 2014). Carvalho *et al.* (2020) showed that phytol has an anti-inflammatory activity in acute inflammation models, mainly by inhibition of neutrophil migration, owing to a reduction of IL-1β and TNF-α levels and oxidative stress (CARVALHO *et al.*, 2020).

β-caryophyllene appears in a relevant concentration (3.00 - 7.11%) and its low odor threshold, 0.064 ppm (NIU *et al.*, 2020) signals that it is a compound that contributes to the aroma of oils. To β-caryophyllene a dry, woody-spicy and somewhat oily odor is attributed (Jirovetz *et al.*, 2006). β-caryophyllene has its use approved by the Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), as a flavor enhancer (MACHADO *et al.*, 2018) and in cosmetics (GERTSCH, 2008). Francomano *et al.* (2019) published a review of the biological properties of β-caryophyllene in which they demonstrated (with a series of pre-clinical studies) the bioactive potential of this molecule, highlighting antioxidant, anti-inflammatory, neuroprotective, sedative and muscle relaxant activities.

Caryophyllene oxide and Spathulenol, also ubiquitous in all samples at concentrations ranging from 1.52-15.25% and 2.46-14.48% respectively, are two oxygenated sesquiterpenes well-recognized as presenting several biological activities. The first has some pharmacological potentials such as anticholinesterase, analgesic, anti-inflammatory,

antifungal activities (CHAVAN *et al.*, 2010; YANG *et al.*, 2000), while the last possess several pharmacological potentials such as anti-inflammatory, antioxidant, antiproliferative, immunomodulator, and antimycobacterial (NASCIMENTO *et al.*, 2018). Regarding the contribution to the aroma of oils, there are no values in the literature for the odor thresholds of these compounds. However, an herbal aroma is attributed to spathulenol, while woody odor notes are related to the presence of caryophyllene oxide (JIROVETZ *et al.*, 2002; JIROVETZ *et al.*, 2004)). Although hexadecanoic acid was in significant quantities in the essential oils (2.05-32.13%), being a majority compound in CS1 and CS2, it is expected that its contribution to flavor would be negligible because of its high molecular weight. In fact, this compound shows a high odor threshold of 10,000 ppb (PINO: QUIJANO., 2012).

Table 3.1. Composition of essential oils from in natura (IN) and commercial samples

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$%	CS1 $Area_{(Avg)}$ %	CS2 $Area_{(Avg)}$ %	CS3 $Area_{(Avg)}$ %	CS4 $Area_{(Avg)}$ %
α-pinene$^{(M)}$	930	930*	-	-	-	0.25	0.18
o-cymene$^{(M)}$	1023	1022*	-	-	-	0.14	0.09
limonene$^{(M)}$	1028	1028*	-	0.13	-	-	0.05
eucaliptol$^{(OM)}$	1032	1032*	0.06	0.03	0.04	-	-
γ-terpinene$^{(M)}$	1059	1059*	-	-	-	-	0.02
cis linalool oxide$^{(OM)}$	1072	1072#	-	-	0.05	-	-
trans linalool oxide$^{(OM)}$	1088	1088*	-	-	0.03	-	-
linalool$^{(OM)}$	1101	1103*	-	0.13	0.15	-	-
α-tujone$^{(M)}$	1107	1106*	0.09	-	0.08	-	0.04
trans-pinocarveol$^{(OM)}$	1137	1138 *	-	0.18	0.13	-	0.01
verbenol$^{(OM)}$	1143	1143§	-	0.14	-	0.10	0.07
camphor$^{(OM)}$	1145	1145§	0.04	-	0.02	-	0.25
α-phellandren-8-ol$^{(OM)}$	1151	1159§	-	-	-	-	0.03
pinocarvone$^{(OM)}$	1164	1164§	-	0.10	-	0.07	0.09
methyl salicilate$^{(E)}$	1170	1170§		0.20	0.11	-	0.07
4-terpineol$^{(OM)}$	1176	1177*	-	-	-	0.04	0.08
α-terpineol$^{(OM)}$	1193	1194*	0.04	0.08	-	-	-
myrtenal$^{(OM)}$	1198	1198§	-	0.27	-	0.10	0.22
verbenone$^{(OM)}$	1212	1218#	-	0.11	0.09	0.10	0.25
β-cyclocitral$^{(OM)}$	1223	1223§	-	0.154	-	-	0.07

Table 3.1. continued

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$%	CS1 $Area_{(Avg)}$%	CS2 $Area_{(Avg)}$%	CS3 $Area_{(Avg)}$%	CS4 $Area_{(Avg)}$%
pulegone$^{(OM)}$	1242	1243*	-	0.1	-	-	-
carvone$^{(OM)}$	1247	1248*	-	0.106	-	-	-
geraniol$^{(OM)}$	1260	1260§	-	0.03	-	-	-
anethol$^{(OM)}$	1286	1285§	-	0.21	0.04	0.04	0.07
4-vinyl guaiacol$^{(P)}$	1317	1317§	-	-	0.05	-	-
δ- elemene$^{(S)}$	1337	1337#	0.84	0.35	0.15	-	0.21
α-cubebene$^{(S)}$	1348	1349*	-	0.93	1.10	0.45	0.90
eugenol$^{(PP)}$	1360	1360*	-	-	0.10	-	0.11
ylangene$^{(S)}$	1371	1372*	-	0.12	-	-	0.19
α-copaene$^{(S)}$	1376	1376*	1.07	1.16	1.46	1.49	2.90
β-bourbonene$^{(S)}$	1384	1384*	0.76	0.62	-	0.37	0.78
β-cubebene$^{(S)}$	1389	1389#	1.19	0.18	1.22	0.51	0.51
β-elemene$^{(S)}$	1391	1391#	-	2.1	0.79	0.44	3,92
cyperene$^{(S)}$	1398	1398§	0.13	-	-	0.42	0.77
NI	1406		-	-	-	0.60	-
methyl eugenol$^{(PP)}$	1412	1412§	-	-	-	-	0.95
β-caryophyllene$^{(S)}$	1419	1419§	7,11	3.0	4.36	3.01	3.89
α-ionone$^{(N)}$	1430	1430*	-	0,27	-	-	0.73
γ-elemene$^{(S)}$	1435	1434*	0.77	-	-	0.34	-
α-guaiene$^{(S)}$	1439	1439*	0.30	0.14	-	-	-

Table 3.1. continued

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$ %	CS1 $Area_{(Avg)}$ %	CS2 $Area_{(Avg)}$ %	CS3 $Area_{(Avg)}$ %	CS4 $Area_{(Avg)}$ %
aromadendrene$^{(S)}$	1447	1440 #	-	0.67	-	0.71	2.09
α-humulene$^{(S)}$	1452	1452 *	5.26	1.28	1.12	0.85	1.41
geranyl acetone$^{(N)}$	1456	1456 §	-	-	0.15	-	0.24
alloaromadendrene$^{(S)}$	1458	1458 *	1.41	-	0,61	1.02	0.78
aristolene$^{(S)}$	1461	1472 §	-	0.33	-	-	-
γ-muurolene$^{(S)}$	1473	1475 *	-	0.92	-	1.54	0
germacrene D$^{(S)}$	1483	1483 *	19.68	2.59	4.52	0.71	5.75
α-curcumene$^{(S)}$	1486	1487 §	-	0.12	-	-	-
β-selinene$^{(S)}$	1487	1488 §	-	0.85	0.84	1.99	2.70
β-ionone$^{(N)}$	1490	1489 §	-	0.17	0.54	-	0.54
α-selinene$^{(S)}$	1493	1494 #	-	-	-	1.53	1.23
epi-bicyclosesquifellandrene$^{(S)}$	1493	1491 §	-	0.34	-	-	-
bicyclogermacrene$^{(S)}$	1496	1498 §	2.37	2.14	2.17	-	-
α-muurolene$^{(S)}$	1501	1499 #	0.53	0.28	-	0.34	0.36
bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-$^{(S)}$	1502	1503 *	-	-	0.50	-	-
β-bisabolene$^{(S)}$	1504	1505 *	-	-	-	1.07	0.62
α-farnesene$^{(S)}$	1507	1507 §	-	-	0.54	-	-
γ-cadinene$^{(S)}$	1513	1513 #	0.56	-	0.36	0.40	
α-amorphene$^{(S)}$	1516	1514 §	-	0.39	-	0.86	1.25
δ-cadinene$^{(S)}$	1516	1516 §	2.59	3.39	1.87	0.596	3.99

Table 3.1. continued

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$ %	CS1 $Area_{(Avg)}$ %	CS2 $Area_{(Avg)}$ %	CS3 $Area_{(Avg)}$ %	CS4 $Area_{(Avg)}$ %
γ-selinene$^{(S)}$	1537	1544*	-	-	0.87	-	0.8
α-calacorene$^{(S)}$	1543	1543**	-	-	-	-	3.82
eudesma-3,7(11)-diene$^{(S)}$	1545	1545*	-	0.15	1.09	-	-
cadala-1(10) 3,8 triene$^{(S)}$	1546	1548§	-	0,40	-	-	-
NI	1555	NI	-	-	0.43	0.63	-
germacrene B$^{(S)}$	1558	1558§	2.50	-	-	0.56	-
trans nerolidol$^{(OS)}$	1568	1569*	0.51		0.41	-	1.10
dodecanoic acid$^{(CA)}$	1573	1562*	-	0.10	-	-	-
spathulenol$^{(OS)}$	1582	1581.8*	2.46	13.99	2.94	5.64	14.48
caryophyllene oxide$^{(OS)}$	1585	1583*	5.42	3.07	1.52	15.25	5.68
ledol ou viridiflorol$^{(OS)}$	1598	1590#	-	1.28	0.20	-	0.42
Globulol$^{(OS)}$	1603	1604#	-	-	0.32	-	-
Humulene epoxide II$^{(OS)}$	1607	1607§	1.48	1.56	0.42	3.43	1.51
aloaromadendrene oxide$^{(OS)}$	1613	1625*	-	-	0.36	-	-
NI	1618		-	1.50	-	-	-
NI	1629		-	-	0.86	-	-
τ-cadinol$^{(OS)}$	1643	1644*	-	-	0.61	-	-
τ-muurolol$^{(OS)}$	1647	1647§	-	1.54	-	-	-
selina-6-en-4-ol$^{(OS)}$	1656	1636*	-	-	0.95	-	-
NI	1658		-	-	-	-	4.97
α-cadinol$^{(OS)}$	1659	1658*	8.43	7.61	-	2.90	1.37
NI	1662		-	-	-	2.42	-
tumerone$^{(OS)}$	1671	1680*	-	-	-	-	0.84
eudesma 4(14)-1-diene$^{(S)}$	1673		-	-	0.23	-	-
NI	1675		-	-	-	3.95	-
cadalene$^{(S)}$	1676	1676§	-	-	0.41	3.45	4.55
NI	1679		-	-	-	2.82	-
NI	1680		-	-	0.10	-	-
NI	1687		-	-	0.33	-	-
α-bisabolol$^{(OS)}$	1688	1682*	-	-	-	0.69	-
cedren-8-13-ol$^{(OS)}$	1690	1690§	-	-	-	0.28	-

Table 3.1. continued

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$ %	CS1 $Area_{(Avg)}$ %	CS2 $Area_{(Avg)}$ %	CS3 $Area_{(Avg)}$ %	CS4 $Area_{(Avg)}$ %
NI	1691		1.08	-	-	-	-
eudesma-4,11-dien-2-ol[(OS)]	1693	1691 *	-	1.29	-	-	-
NI	1698		-	-	-	0.35	-
NI	1707		-	-	-	-	0.22
NI	1708			-	-	1.33	-
2-tetradecen-1-ol[(A)]	1714	1713 *	-	-	0.20	-	-
Pentadecanal[(AL)]	1718	1715 *	0.70	0.47	-	-	-
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol[(OS)]	1719	1714 *	-	-	0.08	0.42	0.15
NI	1722		-	-	-	0.58	-
mint sulfide[(SS)]	1739	1740 *	-	-	-	-	0.57
NI	1740		-	0.58	-	-	-
NI	1745		-	-	0.11	-	-
Aristolone[(OS)]	1751	1756 #	-	-	0.06	-	-
α-cyperone[(OS)]	1755	1755 *	-	-	-	0.66	-
Anthracene[(HC)]	1776	1770 §	-	-	-	-	0.28
myristic acid (tetradecanoic acid)[(CA)]	1777	1777 §	-	2.15	1.48	-	0.39
6,10,14-Trimethylpentadecan-2-one[(N)]	1851	1849 §	-	3.88	1.06	0.49	1.28
pentadecanoic acid [(CA)]	1871	1878 #	-	0.24	0.25	-	-
1-hexadecanol[(A)]	1879	1879 *	-	-	0.20	-	-
NI	1882		-	-	-	-	0.25
7,10 hexadecadienal[(AL)]	1891	1816 *	0.48	-	0.06	-	-
7,10,13 hexadecatrienal[(AL)]	1897	1824 *	0.70	-	0.12	-	-

Table 3.1. continued

COMPOUND	LRI_{exp}	LRI_{lit}	IN $Area_{(Avg)}$ %	CS1 $Area_{(Avg)}$ %	CS2 $Area_{(Avg)}$ %	CS3 $Area_{(Avg)}$ %	CS4 $Area_{(Avg)}$ %
2-heptadecanone$^{(K)}$	1905	1901 *	-	-	0.06	-	-
4(H)cyclopentaphenantrene$^{(HC)}$	1912	1936 §	-	0.20	-	-	-
NI	1918		-	0.08	-	-	-
farnesyl acetone$^{(N)}$	1923	1924 §	0.28	-	0.79	0.17	0.53
methyl palmitate$^{(E)}$	1930	1930 §	-	0.35	0.32	-	0.43
Isophytol$^{(DO)}$	1950	1950 *	0.36	0.47	0.40	0.09	0.20
NI	1958		-	-	0,05	-	-
Hexadecanoic (palmitic) acid $^{(CA)}$	1972	1972 *	2.05	19.90	32.13	10.96	5.15
ethyl palmitate$^{(E)}$	1998	1997 §	0.21	-	-	-	0.08
NI	2024		-	0.36	-	-	-
NI	2030		-	-	0.34	-	-
NI	2033		-	-	-	-	0.10
geranyl linalool$^{(OD)}$	2033	2034 *	0.22	-	-	-	-
Fluoranthene$^{(HC)}$	2055	2057 §	-	0.50	-	-	-
heptadecanoic acid$^{(CA)}$	2073	2071 §	-	-	0.30	-	-
9,12-Octadecadienoic acid, methyl Ester$^{(E)}$	2098	2094 *	-	-	0.27	-	0.19
9,12,15-Octadecatrienoic acid, methyl ester$^{(E)}$	2105	2105 *	-	-	0.66	-	0.23
Phytol$^{(OD)}$	2123	2122 *	20.09	-	10.16	3.11	10.64
9,12-octadienal$^{(A)}$	2146	2150 §		-	-	2.64	-

LRI_{exp}: Linear Retention index calculated for all components using a homologous series of n-alkanes analyzed in the same conditions of the sample; LRI_{lit}: Linear Retention index from literature. References for LRIlit: *Nist, 2019; #pherobase, 2019; §pubchem, 2019; ** Jordan M.J, J. Agric. Food Chem. 2002; Φ Medeiros *et al.*, Braz. J. Pharmacogn, 2012. (M)=monoterpene; (OM)=oxygenated monoterpene; (S)=sesquiterpene; (OS)= oxygenated sesquiterpene; (P)=phenol; (PP)=phenilpropanoid; (N)=norisoprenoid; (CA)=carboxylic acid; (A)=alcohol; (AL)=aldehyde; (SS)=sulfurated sesquiterpene; (HC)=hydrocarbon; (E)=ester; (K)=ketone; (D)=diterpene; (OD)=oxygenated diterpene. Area (Avg)% - percentage area of the compound in relation to the total area of the chromatogram (normalization technique) expressed as average value.

3.2. MULTIVARIATE ANALYSIS

PCA and HCA were performed from collected data to obtain an overview and understand the composition variability between essential oils from *in natura* and commercial samples.

Initially, PCA analysis was applied, and all groups among oil samples are shown by the scores in Figure 3.4. According to the results, three separation tendencies can be visualized in Figure 3.4a (2D plot) and five separation tendencies can be visualized in the Figure 3.4b (3D plot). The latter plot provides more information about oil samples because it has 87.08 % of data variance explained. Thus, 3D plot can be used to explain separation among samples. The *in natura* samples, represented by **IN** on the Figure 3, was the most distinct group, when compared to the others. The commercial samples represented by **CS1**, **CS2**, **CS3** and **CS4** can also been distinguished as different brands. The brand **CS3** is the most distinct among commercial samples.

Figure 3.4. PCA results: (a) scores plot (2D plot) and (b) scores plot (3D plot)

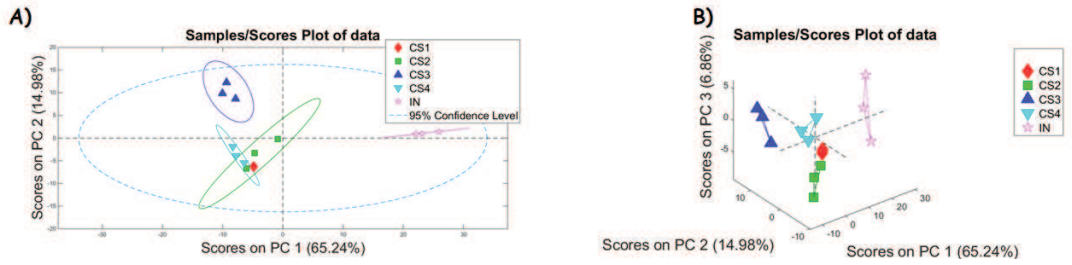
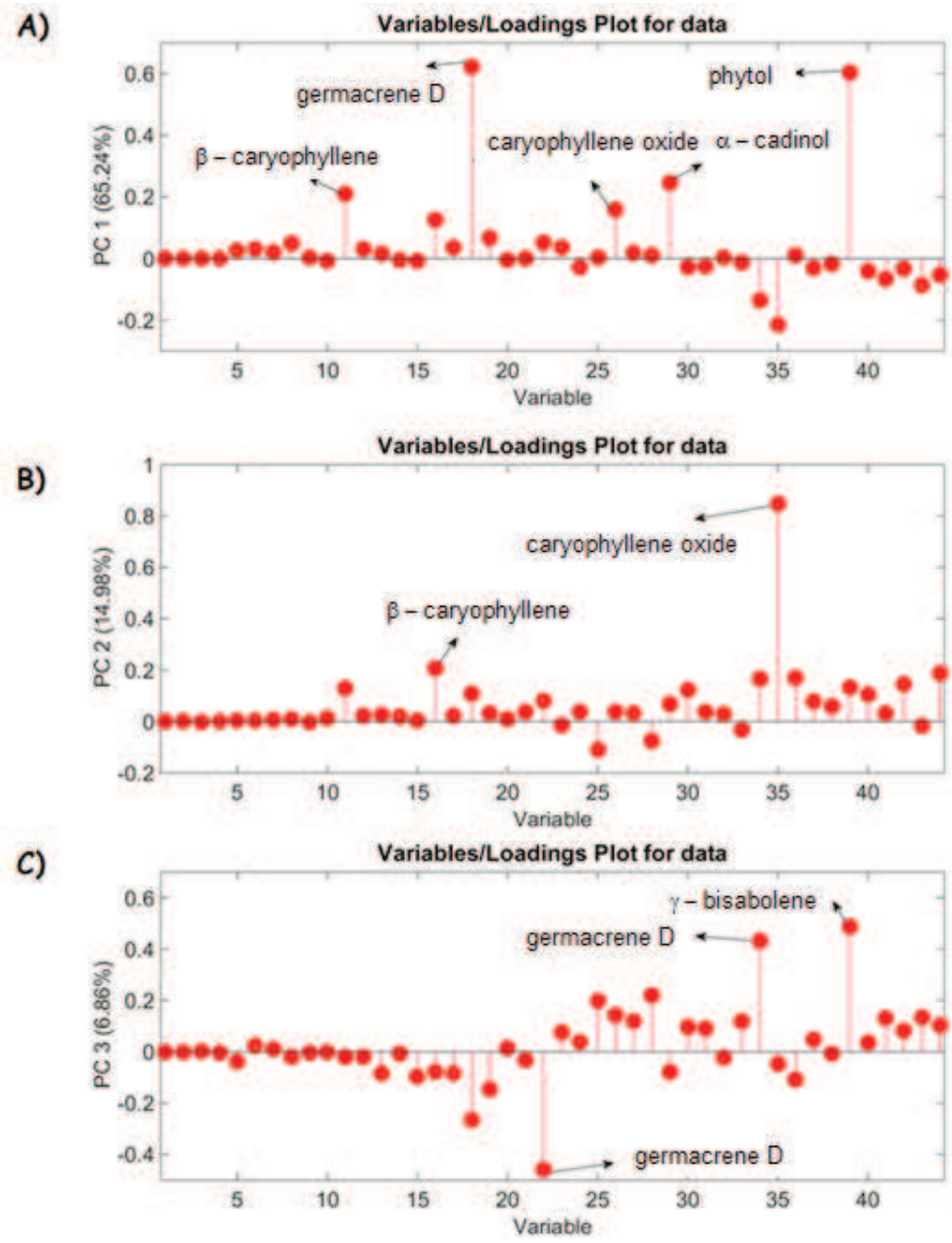


Figure 3.5 shows the loadings plot. In this plot it can be identified the composition of essential oils related to the variability described in Figure 3.4. PC1 axis presented relevant information responsible for the separation of *in natura* samples from the commercial samples. In general, the **IN** group showed higher amounts of Germacrene D, Pythol, α-cadinol, β-caryophyllene and Caryophyllene oxide compounds than commercial groups. In relation to the commercial samples, the samples with the acronym **CS3** are the most distinct. The difference is mainly due to the high concentration of the compounds caryophyllene oxide and β-caryophyllene, showed on Figure 4 b, respectively, present in the **CS3** group.

Commercial samples **CS1**, **CS2** and **CS4** are the most similar, but they are distinguished from each other, mainly due to the presence of the compounds showed in Figure

4 c, Germacrene D and γ-bisabolene, in the **CS4** samples. In addition, **CS2** samples differ from **CS1** and **CS4** samples due to the high concentration of Germacrene D.

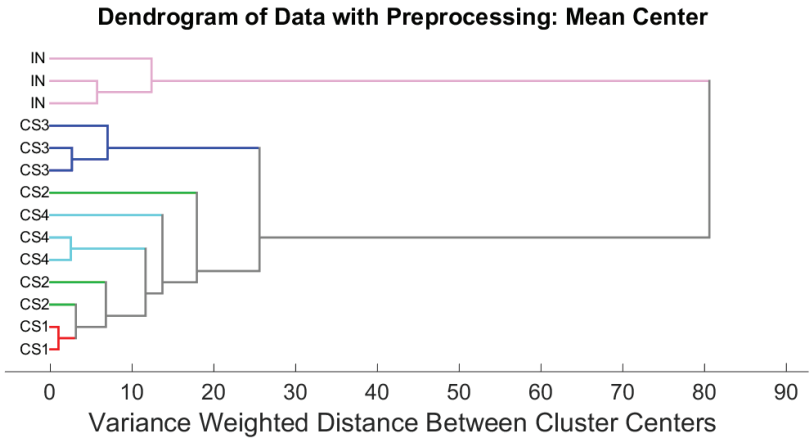
Figure 3.5. PCA results: loadings plots



To corroborate the separation showed by PCA analysis, HCA analysis was applied to this data. The HCA results in Figure 3.6 showed the same results obtained by PCA analysis,

i.e., **IN** samples are different from commercial samples and **CS3** group was the most distinct group of the commercial samples.

Figure 3.6. Dendogram representing the similarity relationship among the essential oils **IN**: in natural samples, CS1, CS2, CS3 and CS4 represents different brands



4. CONCLUSIONS

The present study compared the chemical profile of five essential oils extracted from the leaves of four commercial samples and one botanically identified considered a traceable authentic plant material of *Bauhinia forficata*. In total, 141 compounds of essential oils were detected in commercial and *in natura* samples, of which 116 were identified. The exploratory analysis of the data through PCA and HCA provided good separation of the five samples, thus indicating a distinction between them. It was also possible to identify the compounds responsible for the differences between *in natura* and commercial oils. The loadings plot by PCA indicated the compounds that are possibly responsible for the separation among the essential oils.

However, when observing the major and ubiquitous compounds, a certain similarity is perceived. Although PCA and HCA indicate greater similarity between CS1, CS2 and CS4, if we consider the major compounds for most of these oils , as they appear in practically all samples (commercial and *in natura*), namely – β-caryophyllene, spathulenol, hexadecanoic acid, phytol, caryophyllene oxide, humulene epoxide II, δ-cadinene, germacrene D, α-humulene, β-cubebene (below 1% in CS1, CS3 and CS4) and isophytol (below 1%), it could be possible to establish a set of compounds as chemical markers for the species. Therefore, it would be necessary to evaluate a much larger number of essential oils extracted from both

commercial and *in natura* samples, since there are only two records in the literature regarding the chemical identification of essential oils from *B. forficata*. The present work is the first to isolate and characterize essential oils from samples of herbs available on the market and labeled as *Bauhinia forficata*.

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CHAPTER 4

BAUHINIA FORFICATA INFUSIONS: EVALUATING THEIR VOLATILE AND NON-VOLATILE FRACTIONS.

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This chapter will be submitted to Horticulturae as an original paper.

ABSTRACT

This study aimed to evaluate Bauhinia forficata infusions prepared using samples available in Rio de Janeiro, Brazil. For such, infusions at 5% (w/v) of different brands and batches commercialized in the city (CS1, CS2, CS3, and CS4) and botanically identified (BS) were evaluated to determine their total phenolic (TPC) and flavonoid (TFC) contents, antioxidant capacity (ABTS•+, DPPH•, and FRAP assays), phytochemical profile, volatile compounds, and inhibitory effects against microorganisms and the α-amylase enzyme. The results showed that infusions prepared using botanically identified samples had lower TPC and TFC contents and antioxidant potential than the commercial samples ($p < 0.05$). The batches presented high standard deviations mainly for the commercial samples, corroborating the sample heterogeneity. Two distinct groups were obtained after the statistical analysis, with one consisting only of the commercial sample CS3. The volatile fraction of samples was mainly composed of terpenoid compounds among 41 compounds identified. In the non-volatile fraction, up to 25 compounds were identified in the sample infusions, with emphasis on the CS3 sample, which comprised most of the compounds, mainly flavonoid derivatives. This sample also stood out for presenting antimicrobial activity against Staphylococcus aureus (MBC = 0.39 RE mg/mL). All samples showed inhibitory potential against the α-amylase enzyme (IC50 value: X-Y). Despite the differences observed in this work, B. forficata presents itself as a source of bioactive compounds that can increase the intake of antioxidant compounds by the population.

Key-words: Phytochemical profile, bioactive compounds, antioxidant capacity, antimicrobial action, α-amylase inhibition, SPME.

1. INTRODUCTION

Bauhinia is a genus comprising over 300 species widely distributed in tropical and subtropical forests. In Brazil, 64 species belonging to the Fabaceae family have been identified and are commonly known as “pata-de-vaca” due to the shape of their leaves. Most species are of Asian origin; however, *Bauhinia longifolia* and *Bauhinia forficata* are native species from Brazil (VAZ & TOZZI, 2005; LOPEZ & SANTOS, 2015).

B. forficata is widely used in Brazilian folk medicine due to its beneficial effects on different diseases and human disorders such as rheumatism, local pain, uric acid, and uterine problems (CECHINEL-ZANCHETT, 2018), and it is primarily used to treat type II diabetes (TONELI *et al.*, 2022). The beneficial effects are associated with various chemicals present in *B. forficata*, such as flavonoids, alkaloids, and terpenes/terpenoids, for example (MAFFIOLETTI *et al.*, 2012; LOPEZ & SANTOS, 2015). The flavonoid compounds are highlighted since they are the major class in *B. forficata* extracts, as reported in the literature. Farag *et al.* (2015) observed the presence of quercetin and kaempferol derivatives in different species of the Bauhinia genus, including *B. forficata*.

In Brazil, *B. forficata* is mainly commercialized dried and used to make infusions. Thus, under Brazilian law, the product is treated as food, so it is not mandatory to indicate the content of bioactive or toxic compounds, as is done in a limited manner in herbal products (BRASIL, 2005). *B. forficata* infusions have been used in different in vivo studies, such as that by Salgueiro *et al.* (2016), who evaluated the effects of B. forficata infusions on oxidative stress, liver damage, and glycemia in mice. Nevertheless, data on the content of bioactive compounds, antioxidant capacity, and volatile compounds, among other parameters of this plant, to compare botanically identified and commercialized samples and their infusions are scarce in the literature. Since it is well-known that various factors such as climate, processing, and storage conditions may influence the content of bioactive compounds and the volatile fraction of medicinal plants (SOTIROPOULOU *et al.*, 2020; LAMIEN-MEDA *et al.*, 2010), there is a clear need for further studies.

Despite that, up to now, there are no data available on the volatile composition of *B. forficata* infusions. This fraction cannot be underestimated since *B. forficata* is prepared by infusion or decoction and, therefore, part of the volatile content may migrate to the beverage

and contribute to its beneficial actions besides the aroma. This approach has already been evaluated for other medicinal plants, and the migration of terpenoid compounds and other compound classes present in the essential oil of the plant for infusion was observed (TSCHIGGERL, 2010; ARSENIJEVIC *et al.*, 2016).

For such an evaluation, headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME/GC-MS) has been reported as a fast, sensitive, and solvent-free technique for analyzing the extraction and isolation of volatile and semi-volatile compounds, and it has been widely used since its invention in 1989 (MA *et al.*, 2018; LIN *et al.*, 2016). Furthermore, interference from the infusion matrix may be drastically reduced while the headspace analytes are trapped in the fiber (DU *et al.*, 2014). Thus, this technique has been successfully applied to analyze volatile compounds in infusions and tea (LV *et al.*, 2012; AUGUSTO & ZINI, 2002).

In this sense, this work aimed to determine a comprehensive chemical characterization of the volatile and non-volatile fractions of botanically identified and commercial samples of *B. forficata* used to prepare infusions at 5%. The inhibitory activity of α-amylase and the antibacterial activity of the samples were also determined.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

B. forficata leaves were collected in Petropolis, Rio de Janeiro, Brazil (22° 30’ 04.63” S, 43° 07” 58.20” W, altitude: 958 m) in different seasons (winter, spring, and summer - 2018/2019). Voucher specimens were deposited at the Herbarium of the Department of Botany of the Federal University of Rio de Janeiro, under registration number RFA 40.615. The samples were dried in an oven with forced air circulation at 45 °C, then disintegrated in a domestic blender to obtain flour, which was used to prepare the infusions. These samples were named BSB1, BSB2, and BSB3.

Four commercial samples purchased from local markets in the city of Rio de Janeiro were also evaluated. Two batches of Commercial Sample 1 (CS1) and three batches of the other samples (CS2, CS3, and CS4) were acquired, resulting in samples CS1B1, CS1B2, CS2B1, CS2B2, CS2B3, CS3B1, CS3B2, CS3B3, CS4B1, CS4B2, and CS4B3, which were used to prepare the infusions.

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2.2. LABELING ANALYSIS OF COMMERCIAL SAMPLES

The labeling of the herbal bags was evaluated following the Resolutions of the Board of Directors of the Brazilian National Health Surveillance Agency (ANVISA) RDC No. 259/2002, RDC No. 267/2005, and RDC No. 277/2005, together with the fifth edition of the Brazilian Pharmacopeia. According to these regulations, the following information must be present on tea packages: product name followed by the botanical nomenclature: species (genus + specific epithet); part used; commercial name of the product; ingredients list; name or business name and address of the producer or importer in the case of imported products; batch identification; expiration date; instructions for the preparation of the product; nutritional information. Information containing the therapeutic purpose must be absent.

An analysis of the weight corresponding to the packaging was also conducted. The samples were weighed in triplicate (three different batches of each brand), and the means and standard errors of the means were calculated. The results obtained were compared with those indicated on the packaging, checking if they were within the 9 % difference allowed by Law No. 9933 of December 20, 1999, and Inmetro Ordinance No. 96 of April 7, 2000.

2.3. PREPARING THE INFUSIONS

The infusions were prepared by adding 50 mL of boiling water to 2.5 g of the samples. After twenty minutes of rest, they were filtered and transferred to a volumetric flask, in which the volume was completed up to 50 mL using distilled water (GASTALDI *et al.*, 2018).

2.4. ANALYSIS

2.4.1. Total phenolic compounds (TPC)

The TPC analysis was performed using the Folin-Ciocalteu reagent (Imbralab, Ribeirão Preto, Brazil), following the method described by Singleton and Rossi (1965). For the reactions, 250 µL of the filtered and appropriately diluted extract was mixed with 1250 µL of 10% Folin-Ciocalteu reagent and 1000 µL of a 7.5% (w/v) sodium carbonate solution. Thereafter, the samples were heated at 50 °C for 15 min and cooled at room temperature. The

absorbance was measured at 760 nm. A calibration curve was constructed using the rutin standard with concentrations ranging from 16 mg/L to 166 mg/L (linear regression: y = 0.0034x - 0.0128; R^2 = 0.9988). The TPC content is expressed as milligrams of rutin equivalent per 100 grams (mg RE/100 g).

2.4.2. Total flavonoid compounds (TFC)

The TFC content was determined based on the method described by Zhishen *et al.* (1999) with minor modifications. Here, 0.5 mL of extract was mixed with 3.2 mL of ultrapure water and 150 µL of $NaNO_2$ (5%). After homogenization, the mixture was left to rest for five minutes. Thereafter, 150 µL of $AlCl_3$ (10 %) was added to the mixture, and 1 mL of NaOH (1 M) was added after one minute. The absorbance was recorded at 510 nm with a spectrophotometer (Metash, China) using ultrapure water as a blank. The TFC content was calculated using the calibration curve of rutin, with the concentration ranging from 99 mg/L to 595 mg/L (linear regression: y = 0.001x + 0.013; R^2 = 0.9974). The results are expressed as mg RE/100 g.

2.4.3. $ABTS^{\bullet+}$ assay

The antioxidant capacity was determined by the reduction of radical monocation, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), according to the procedure described by Gião *et al.* (2007). The radical was obtained after the addition of 7 mmol/L of ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich, Saint Louis, MO, USA) to 2.45 mmol/L of a potassium persulfate solution (1:1 (v/v)). The mixture was left to react in the dark for 16 h. To obtain an absorbance of 0.700 ± 0.020 at 734 nm, the $ABTS^{\bullet+}$ solution was diluted using ultrapure water. For the reactions, 30 µL of each filtered and diluted extract was mixed with 3000 µL of the $ABTS^{\bullet+}$ solution. After six minutes, the absorbance was measured at 734 nm with a spectrophotometer (Metash, China) using ultrapure water as a blank. The $ABTS^{\bullet+}$ antiradical activity was calculated using Trolox solutions (Sigma-Aldrich, Buchs, Switzerland) with different concentrations ranging from 240 µmol to 2000 µmol (linear regression: y = 0.0003x + 0.0094; R^2 = 0.9989). The results are expressed as micromoles of Trolox equivalents per gram (µmol TE/g).

2.4.4. $DPPH^{\bullet}$ assay

The 2,2′-diphenyl-β-picrylhydrazyl radical ($DPPH^{\bullet}$) (Sigma-Aldrich, Steinheim, Germany) scavenging activity of the extracts was determined according to the method described by Hidalgo *et al.* (2010). For the reactions, 100 μL of each diluted extract was added to 2900 μL of a $DPPH^{\bullet}$ solution (6×10^{-5} M in methanol and diluted to an absorbance of 0.700 at 517 nm). The resulting solutions were allowed to stand for thirty minutes in the dark at room temperature. Then, the absorbance was measured at 517 nm with a spectrophotometer (Metash, China) using methanol as a blank. The $DPPH^{\bullet}$ scavenging activity was calculated using Trolox solutions (Sigma-Aldrich, Buchs, Switzerland) with different concentrations ranging from 80 μmol to 680 μmol (linear regression: $y = 0.0008x + 0.017$; $R^2 = 0.9962$). The results are expressed as μmol TE/g.

2.4.5. FRAP assay

The ferric reducing/antioxidant power (FRAP) assay was performed according to the procedure reported by Benzie and Strain (1996) with minor modifications. The stock solutions included 300 mM of an acetate buffer (pH 3.6), 10 mM of 2,4,6-tri(2-pyridyl)-s-triazine (Sigma-Aldrich, Buchs, Switzerland) in 40 mM of HCl, and 20 mM of $FeCl_3{\cdot}6H_2O$. The working solution was prepared by mixing 25 mL of the acetate buffer, 2.5 mL of the TPTZ solution, and 2.5 mL of $FeCl_3{\cdot}6H_2O$. Thereafter, 100 μL of each extract was reacted with 3000 μL of the working solution at 37 °C for thirty minutes, and the absorbance was measured at 593 nm. The FRAP activity was calculated using $FeSO_4{\cdot}7H_2O$ solutions with different concentrations ranging from 150 μmol to 1200 μmol of Fe^{2+} (linear regression: $y = 0.0008x + 0.0042$; $R^2 = 0.9992$). The results are expressed as micromoles of Fe^{2+} per gram (μmol Fe^{2+}/g).

2.4.6. LC-HRMS analysis

The sample extract was dissolved in an aqueous solution containing formic acid (0.1%, v/v) and subjected to an ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry (UPLCqTOF/MS; Bruker Daltonics, MaxisImpact, QTOf Bruker) analysis. The separation was performed using a Hypersil C18 column (3 μm particle size, 2.1 mm × 150 mm). The column temperature was maintained at 40 °C. Subsequently, an aliquot of 20 μL was injected into the UPLC-ESI-qTOF system with a flow rate of 0.27 mL/min. The linear gradient elution of A (0.1% formic acid in water) and B (acetonitrile) was applied by

employing the following method: 5% of B at the beginning, 5% to 9% of B for five minutes, 9% to 16% of B for ten minutes, 16% to 36% of B for eighteen min, 36% to 95% of B for one minute, 95% of B for twelve minutes, 95% to 5% of B for one minute, and 5% of B for thirteen minutes. Data Analysis 5.1 software (Bruker Daltonics, Germany) was used to interpret the data. The MS data were acquired in the negative mode using an electrospray ionization (ESI) source. The data were scanned for each test sample at a mass-to-charge ratio (m/z) from 50 to 1200. Highly pure nitrogen was used as the nebulizing gas and ultrahigh purity helium as the collision gas, and the capillary voltage was set at 5000 V. The ESI parameters included dry gas at 200 °C at a flow rate of 8 L/min and a nebulizer pressure of two bar.

2.4.7. HS-SPME/CG-MS

The infusions that presented the best results for TPC and TFC contents and antioxidant capacity were subjected to an analysis of the volatile fraction by HS-SPME/GC-MS (Headspace Solid-Phase Microextraction followed by Gas Chromatography-Mass Spectrometry).

2.4.7.1. Volatiles isolation of infusions

The headspace volatiles analysis using SPME described by Wang *et al.* (2019) was adopted with minor modifications. Volumes of 10 mL of freshly prepared infusions were placed into 20 mL clear glass vials and immediately capped and placed on a temperature-controlled water bath at 60 °C for sixty minutes with a SPME fiber coated with 100 µm of PDMS (100% polydimethylsiloxane) (Supelco®, Bellefonte, PA, USA) pre-conditioned at 250 °C for sixty minutes and inserted into the headspace above the liquid surface. A system blank with an empty vial was run as a control assay. SPME fibers were desorbed at 250 °C for five minutes in the injection port of the chromatographic system described below.

2.4.7.2. GC-MS analysis

The GC/MS analysis of the volatile fractions was carried out using an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with an HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA, USA) equipped with an Agilent HP-5975 mass selective detector in the electron impact mode (ionization energy: 70 eV) operating according to the following

conditions. The oven temperature was initially maintained at 50 °C for two minutes, then raised at the rate of 5 °C/min to 2400 °C, staying at this temperature for ten minutes. The injector and detector temperatures were set at 250 °C and 260 °C, respectively. The samples were injected in the splitless mode. A normalization technique was used to obtain quantitative data. Linear retention indices were calculated for all components using a homologous series of n-alkanes analyzed under the same conditions as the samples. The identification of the volatile fraction components was based on retention indices (RI) relative to n-alkanes and computer matching with the Wiley275.L and Wiley7n.L libraries and comparisons of the fragmentation patterns of the mass spectra with data published in the literature (Adams, 2001).

2.4.8. Assay for α-amylase inhibition

The infusions that presented better results for TPC and TFC contents and antioxidant capacity were subjected to the inhibition assay for α-amylase, performed as reported by Meng *et al.* (2016) with minor modifications. Briefly, 100 µL of extract was mixed with an α-amylase solution (100 µL, 1.0 U/mL) (Sigma-Aldrich, St. Louis, MO, USA) in a phosphate buffer (pH 6.9) and 250 µL of a 1% starch solution. The incubation was carried out for five minutes at 37 °C. The enzyme reaction was stopped by adding dinitrosalicylic acid reagent (250 µL) (Sigma-Aldrich, Steinheim, Germany), and incubation was carried out for fifteen minutes in boiling water. For the dilution, 2 mL of distilled water was added to the final reaction mixture. The absorbance was read at 540 nm. The inhibitory effect was calculated according to Equation 1, where $Abs_{control-1}$ results from the reaction without adding the enzyme, which was replaced by the buffer solution, while the mixture of the enzyme and starch solution without extract was $Abs_{control-2}$. The results were expressed as IC_{50} (mg RE/mL). Acarbose (Supelco, Laramie, WY, USA) was used as a positive control to compare the inhibitory effects.

$$\text{Inhibition percentage (\%)} = [1 - (Abs_{sample} - Abs_{control-1})/Abs_{control-2}] \times 100 \quad (1)$$

2.4.9. Microbiology assay

Microorganisms and culture conditions: Gram-negative (*Escherichia coli* ATCC 25922) and Gram-positive (*Staphylococcus aureus* ATCC 29213) bacteria were grown in

Mueller-Hinton agar (Difco, Franklin Lakes, NJ, USA) for 24 h at 35 ± 2 ºC. The yeast *Candida albicans* ATCC 90028 was cultured in Sabouraud dextrose agar (Difco, Franklin Lakes, NJ, USA) for 24 h at 35 ± 2 ºC.

For antimicrobial assays, infusions that presented the better results for TPC and TFC contents and antioxidant capacity were used. The antimicrobial activity was evaluated using the broth microdilution method in 96-well polystyrene plates, standardized according to documents M07-A9 (for bacterial assays) and M27-A3 (for fungal assays). The minimum inhibitory concentration (MIC) was determined by visual inspection after the incubation at 37 ºC for 24 h of extracts at different concentrations. To determine the minimum bactericidal and fungicidal concentrations (MBC and MFC), 10 µL of the wells with no visible microbial growth were inoculated in a Mueller-Hinton culture medium and Sabouraud Dextrose Agar for 24 h at 37 ºC. The MBC and MFC were considered the lowest concentration capable of completely inhibiting microbial growth on the agar surface.

2.5. STATISTICAL ANALYSIS

The data were statistically analyzed using Statistica software version 13 (Dell Inc.), performing an analysis of variance (ANOVA) and Tukey's test to verify the differences among averages, considering the 95% confidence level. Experiments were performed in triplicate, and the results are presented as average ± standard deviation.

3. RESULTS AND DISCUSSION

3.1. LABEL ANALYSIS OF COMMERCIAL SAMPLES

The results of the evaluations are presented in Table 1. It was found that the packaging of some products did not meet the specifications for four of the evaluated criteria, implying a lack of information to consumers, who are harmed by this absence since it is their right to have the necessary information when purchasing the product. CS1 did not comply with the legislation regarding the following items: part used, ingredients list, and nutritional information parameters. Regarding the nutritional information parameter, CS4 did not comply with legislation either. As medicinal plants are classified as food by Brazilian legislation, this parameter is mandatory on labels. However, the absence of this information is of little relevance since these herbs are mainly consumed as infusions, in which the macronutrients

are found in percentages close to zero. All samples failed to comply with the list of ingredients, suggesting that the manufacturers may be unaware of the current legislation since this is an extremely simple piece of information as it merely concerns the packaged plant or plants, unlike more complex food products. CS2 and CS4 did not present the preparation instructions on their labels, thus presenting another non-conforming parameter. This is extremely relevant information since infusions of medicinal plants may harm human health when in high concentrations. Thus, information about the preparation must be shown on the labels of commercial products such as this. It should be pointed out that CS1 displayed on its label, below the preparation instructions, the following note: "consult your physician as some herbs should be used in lower proportions". Concerning the analysis of the weight corresponding to the packaging, all samples were in compliance with the specific legislation, with variations between the declared and verified weight ranging from 0.2% to 2%.

Table 4.1. Parameters evaluated for the label analysis of commercial samples of *B. forficata*

Parameter	CS1	CS2	CS3	CS4
Product name / Botanical nomenclature	IC	IC	IC	IC
Part used (bark,leaves...)	NC	IC	IC	IC
Product commercial name	IC	IC	IC	IC
Ingredients list	NC	NC	NC	NC
Nutritional information	NC	IC	IC	NC
Preparation instructions	IC	NC	IC	NC
Name and address of the producer	IC	IC	IC	IC
Batch identification	IC	IC	IC	IC
Expiration date	IC	IC	IC	IC
Absence of information containing the therapeutic purpose.	IC	IC	IC	IC

NC = noncompliant with the legislation; IC = in compliance with the legislation. CS1:commercial sample 1; CS2: commercial sample 2; CS3:commercial sample 3; CS4:commercial sample 4.

3.2. Bioactive compounds and antioxidant capacity of *B. forficata* infusions

The TPC and TFC contents and antioxidant capacity of the *B. forficata* infusions are summarized in Table 4.2. It should be pointed out that the results presented in this study for TPC and TFC contents are expressed as rutin equivalents since this compound belongs to the flavonoids class, which is the major class in this species (FARAG *et al.*, 2015). The values of

TPC varied from 1923 mg RE/100 g to 6355 mg RE/100 g. Compared to the literature, the highest value found in this study, which was for the dry basis (7222 mg RE/100 g), is superior to that reported by Port's *et al.* (2013), who evaluated different infusions of herbs from the Brazilian Amazonian region. Even though these authors did not evaluate *B. forficata*, their approach was the closest to this study, reporting results of the chemical evaluation for a *B. ungulata* infusion at 2% (g/mL) (2367 mg GAE/100 g dry basis). By calculation, at 5%, 5918 mg GAE/100 g dry basis would be found. Comparisons with data from the literature are difficult since few studies used the same species, and even when the species were the same, the results were expressed using different chemical standards, as in the example above. Also, it is easier to find data on *B. forficata* extracted with organic solvent than with hot water (infusion). Thus, our discussion will be focused on the differences observed among the brands and respective batches evaluated herein.

The values of the TPC, TFC, and antioxidant capacity by $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and FRAP assays varied from 1923 mg RE/100 g to 6355 mg RE/100 g, 482 mg RE/100 g to 3700 mg RE/100 g, 19 µmol Trolox/g to 206 µmol Trolox/g, 27 µmol Trolox/g to 204 µmol Trolox/g, and µmol Fe^{2+}/g 85 to 644 µmol Fe^{2+}/g, respectively. This corroborates that variations among samples and batches were high (Table 4.2). The botanically identified sample (BS) presented low TPC and TFC contents and antioxidant capacity compared to the commercial samples. This may be explained by differences in cultivation practices and the way the plants are processed. For example, the drying time may increase the degradation of plant bioactive compounds, whereas soil characteristics and precipitation conditions may affect the biosynthesis of secondary metabolites (SOTIROPOULOU *et al.*, 2020; LAMIEN-MEDA *et al.*, 2010).

CS4B2 presented the highest TPC content and FRAP value. For the TFC content and DPPH and ABTS assays, CS3B3 presented the highest values (Table 4.2). The literature points to a direct relationship between TPC content and antioxidant capacity; however, in this study, the sample that presented the highest TPC content did not show the highest values for antioxidant capacity by all assays employed. This corroborates that the phytochemical composition of plant extracts may interact differently with radical species, which helps explain the results found.

High standard deviations were observed in CS2 and CS4. The variation coefficient for the TFC content reached 75% in CS2, for example, confirming the heterogeneity among the

sample batches. The low standard deviation of the BS may be associated mainly with the standardization of the processing, which was followed from the harvest of leaves to drying. In addition, the harvest was from the same tree, although it took place in different seasons. This may also justify the low standard deviation of CS1 and CS3. Furthermore, conditions such as storage time, temperature, and kind of packing influence the stability of bioactive compounds.

Since the brands showed heterogeneous batches according to the statistical analysis, the CS3 sample presented significant differences for the TPC, TFC, DPPH, ABTS, and FRAP assays ($p > 0.05$), when compared whith all others.

These data provide important information about the production chain of B. forficata, rendering evident the need to standardize the steps that involve from harvest to distribution to deliver to consumers a product that guarantees its bioactive properties. B. forficata is widely used in Brazilian folk medicine due to its beneficial effects for treating rheumatism, local pain, uric acid, uterine problems (Cechinel-Zanchett, 2018), and, especially, type II diabetes (Toneli *et al.*, 2022). This is possible due to the phytochemical profile of B. forficata, which is mainly composed of flavonoids, recognized for their antioxidant capacity (Ferreres *et al.*, 2012).

Saura-Calixto and Goñi (2006) evaluated the antioxidant capacity of the Spanish diet, traditionally known for being a healthy diet as it includes olive oil, fruits, legumes, vegetables, teas and/or infusions, wines, coffee, and nuts, among others. According to the authors, the total antioxidant capacity per capita of the daily intake of the Spanish diet is 3549 µmol of Trolox equivalents, measured by an ABTS assay. The lowest and highest values of B. forficata infusions at 5% found here and expressed in µmol of Trolox equivalents using the same assay were 1368 (CS4B3) and 10212 (CS3B3), respectively. However, it should be noted that the differences between the values presented here and those found by Saura-Calixto and Goñi (2006) may be attributed to the evaluated portion sizes of the foods and beverages and the concentration at which the infusions, teas, or coffee were prepared, for example. Even so, this inference shows the contribution of B. forficata infusions to the intake of antioxidant compounds by the population.

Table 4.2. Total phenolic compounds (TPC), and total flavonoid compounds (TFC) and antioxidant capacity of *B. forficata* infusions

Samples	Assays				
	TPC[1]	**TFC[1]**	**DPPH[•2]**	**ABTS[•+2]**	**FRAP[3]**
BSB1	$2126 \pm 15^{g,h}$	648 ± 19^{e}	$20 \pm 2^{e,f}$	27 ± 4^{f}	89 ± 3^{h}
BSB2	$2126 \pm 29^{g,h}$	630 ± 9^{e}	19 ± 0^{f}	$30 \pm 2^{e,f}$	85 ± 6^{h}
BSB3	2772 ± 49^{e}	$832 \pm 11^{d,e}$	$21 \pm 1^{e,f}$	$30 \pm 2^{e,f}$	136 ± 3^{g}
Overall average	2342 ± 324^{B}	703 ± 97^{B}	20 ± 1^{C}	29 ± 3^{B}	103 ± 25^{B}
CS1B1	$2364 \pm 164^{f,g}$	1026 ± 4^{d}	$34 \pm 1^{d,e,f}$	$41 \pm 0^{d,e,f}$	127 ± 2^{g}
CS1B2	$2733 \pm 55^{e,f}$	1042 ± 24^{d}	$39 \pm 2^{d,e}$	46 ± 2^{d}	133 ± 4^{g}
Overall average	2549 ± 230^{B}	1034 ± 18^{B}	$36 \pm 3^{B,C}$	43 ± 3^{B}	130 ± 4^{B}
CS2B1	4740 ± 69^{c}	3122 ± 114^{b}	108 ± 1^{c}	99 ± 1^{c}	242 ± 5^{e}
CS2B2	$2245 \pm 79^{g,h}$	944 ± 40^{d}	$37 \pm 3^{d,e,f}$	$45 \pm 2^{d,e}$	120 ± 2^{g}

Table 4.2. conclusion

Samples	Assays				
	TPC[1]	TPC[1]	DPPH[•2]	ABTS[•+2]	FRAP[3]
CS2B3	3203 ± 215[d]	626 ± 30[e]	45 ± 2[d]	39 ± 1[d,e,f]	176 ± 1[f]
Overall Average	3396 ± 1097[B]	1564 ± 1178[B]	63 ± 34[B,C]	61 ± 29[B]	179 ± 53[B]
CS3B1	4681 ± 251[c]	2006 ± 64[d]	114 ± 2[c]	109 ± 10[c]	330 ± 11[d]
CS3B2	5448 ± 144[b]	2422 ± 147[c]	173 ± 3[b]	135 ± 12[b]	385 ±11[c]
CS3B3	4833 ± 166[c]	3700 ± 161[a]	206 ± 2[a]	204 ± 7[a]	571 ± 4[b]
Overall average	4987 ± 389[A]	2710 ± 773[A]	164 ± 42[A]	149 ± 44[A]	429 ± 109[A]
CS4B1	2169 ± 89[g,h]	1026 ± 18[d]	45 ± 2[d]	47 ± 3[d]	129 ± 1[g]
CS4B2	6355 ± 137[a]	2628 ± 90[c]	185 ± 8[b]	149 ± 6[b]	644 ± 19[a]
CS4B3	1923 ± 4[h]	482 ± 15[f]	25 ± 1[e,f]	27 ± 1[f]	86 ± 5[h]
Overall average	3483 ± 2158[A,B]	1378 ± 967[B]	85 ± 76[B]	74 ± 57[B]	286 ± 269[A,B]

Abbreviations in the "Samples" column represent the different batches of each one of the brands evaluated. Different lowercase letters in the same column indicate that the results are statistically different ($p < 0.05$). Different uppercase letters in the same column indicate a statistically significant difference among groups (BS, CS1, CS2, CS3 and CS4) ($p < 0.05$). [1]Results expressed as mg RE/100 g. [2]Results expressed as µmol Trolox/g. [3]Results expressed as µmol Fe^{2+}/g. BSB1=botanical sample batch 1; BSB2= botanical sample batch 2; BSB3= botanical sample batch 3; CS1B1=commercial sample 1 batch1; CS1B2=commercial sample 1 batch 2; CS2B1=commercial sample 2 batch 1; CS2B2=commercial sample 2 batch 2; CS2B3=commercial sample 2 batch 3; CS3B1=commercial sample 3 batch 1; CS3B2=commercial sample 3 batch 2; CS3B3=commercial sample 3 batch 3; CS4B1=commercial sample 4 batch 1; CS4B2=commercial sample 4 batch 2; CS4B3=commercial sample 4 batch 3.

3.3. LC-HRMS ANALYSIS

A total of 21 phenolic compounds (Table 4.3), among flavonoids, phenolic acids, and other phenolic compounds, were tentatively identified in the samples. The majority are kaempferol and quercetin derivatives. The samples comprised flavonoid O-glycosides, thus verifying previously reported results, which prove its pharmacological action (HWANG *et al.*, 2019, JIANG *et al.*, 2019).

It was also verified that the relative abundance of the presented ions shows significant differences among samples but there was no significant difference in the phenolic acids class. Most phenolic compounds identified in this study were free phenolic compounds, esterified with sugars or other compounds with low molecular masses, such as quercetin 3-O-rhamnoside, Kaempferol 3-O-glucoside, and Isorhamnetin.

Table 4.3. Tentatively identified compounds of *B. forficata* infusions

Compounds		λmax (nm)	m/z [M-H]- exp.	MS^2	Fórmula Molecular [M-H]-	Samples				
						BS	CS1	CS2	CS3	CS4
1	Caffeoyltartarate	240, 326	311.0401	179; 135	$C_{13}H_{11}O_9$	+				
2	(Epi)Catechin	232, 327	289.0718	245; 203	$C_{15}H_{13}O_6$				+	
3	Galloyl hexose	272	331.0670	169; 125	$C_{13}H_{15}O_{10}$	+	+	+		+
4	hydroxibenzoic Acid	305	137.0244	—	C_7H5O_3	+	+	+		+
5	dihydroxibenzoic Acid hexoside	nd	315.0719	108, 152	$C_{13}H_{15}O_9$			+	+	
6	3-Caffeoyl quinic acid (Neochlorogenic acid)	236; 325	353.0875	191	$C_{16}H_{17}O_9$	+	+		+	+
7	Kaempferol 3-*O*-rhamnosyl rutinoside	nd	739.2136	284	$C_{33}H_{39}O_{19}$				+	
8	Rutin	nd	609.1468	300	$C_{27}H_{29}O_{16}$	+	+	+	+	+
9	B4 Myricitrin	254	463.0880	316	$C_{21}H_{29}O_{12}$			+		
10	Quercetin 3-*O* glucopyranoside (Isoquercetin)	254; 351	463.0917	301; 300	$C_{21}H_{29}O_{12}$	+	+	+	+	+
11	Quercetin-*O*-pentoside (Quercetin-*O*-arabinoside)	250;351	433.0780	300; 301	$C_{20}H_{17}O_{11}$	+	+	+	+	+
12	Quercetin 3-*O*-rhamnoside	248; 342	447.0933	284; 285	$C_{21}H_{29}O_{11}$	+	+	+	+	+
13	Kaempferol 3-*O*-glucoside	248; 342	447.0975	–	$C_{21}H_{29}O_{11}$	+	+	+	+	+
14	Kaempferol 3-*O*-rutinoside	265	593.1533	327; 284; 285	$C_{27}H_{29}O_{15}$	+	+		+	+
15	Isorhamnetin	254	315.0502	300	$C_{16}H_{11}O_7$	+	+	+	+	+
16	Isorhamnetin 3-*O*-rutinoside	254; 354	623.1638	300; 315	$C_{28}H_{31}O_{16}$	+				
17	Palmitic acid	254	255.2328	_	$C_{16}H_{31}O_2$	+	+	+	+	+
18	Quercetin 3-*O*-Rhamnosyl Rutinoside	255	755.2087	300; 489	$C_{33}H_{39}O_{20}$	+			+	
19	Isorhamnetin 3-*O*-rhamnosyl	254; 350	769.2201	605; 315	$C_{34}H_{41}O_{20}$	+			+	

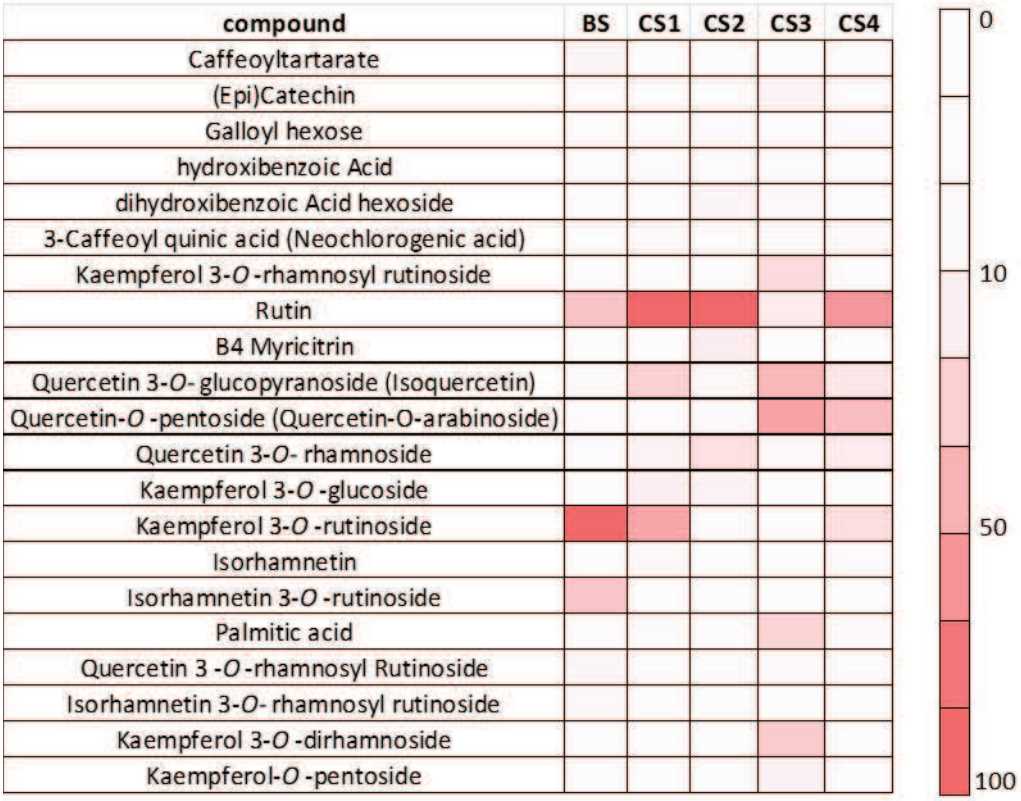
	rutinoside									
20	Kaempferol 3-*O*-dirhamnoside	264, 342	577.1595	431, 285, 284	$C_{27}H_{29}O_{14}$				+	
21	Kaempferol-*O*-pentoside	238, 272, 350	417.0833	285, 284, 255, 227	$C_{20}H_{17}O_{10}$				+	

BS: botanic sample; CS1: commercial sample 1; CS2: commercial sample 2; CS3:commercial sample 3; CS4:commercial sample 4.

Rutin, isoquercetin, Quercetin-O-pentoside, Quercetin 3-O Rhamnoside, Kaempferol 3-O-glucoside, Kaempferol 3-O-rutinoside, Isorhamnetin, and palmitic acid were the compounds detected in all samples. CS3 is the infusion with the largest number of compounds that vary according to the batch (data not shown). In CS1, CS2, and CS4, the same 11 flavonoids were identified with differences in the relative abundance of the ions. Compound 3 showed a precursor ion [M-H]- at 331.0670 m/z and a typical loss of a hexose in MS2 resulting in a [M-H]- m/z 169 fragment. It was assigned as galloyl hexose. Compound 4 was assigned as hydroxibenzoic acid based on precursor ion [M-H]- at 137.0244 m/z and an error between experimental mass and theoretical mass of 0.1 ppm (AQUINO *et al.*, 2019). Compound 6 showed a precursor ion [M-H]- at 353.0875 m/z and the quinic acid fragment in MS2 at a m/z 191. It was identified as 3-Caffeoyl quinic acid. Compound 10 was assigned as Quercetin 3-O glucopyranoside because it shows a precursor ion with 1 ppm error ([M-H]- m/z 463.0878) and by comparison with previously literature (ENGELS *et al.*, 2012). Compound 19 was assigned as Isorhamnetin 3 O rhamnosyl rutinoside based on precursor ion [M-H]- m/z 769.2190 and the presence of Isorhamnetin fragment at m/z 315. Kaempferol radical ion at m/z 284 was used to identify compound 20 as Kaempferol 3-O-dirhamnoside along with the precursor ion [M-H]- m/z 577.1595 (FARAG *et al.*, 2015). Identification of the other listed compounds by fragmentation data and exact mass were previously described by the authors (RIBEIRO *et al.*, 2020; JUNG *et al.*, 2021).

The UPLC-ESI-Q-TOF MS/MS chromatographic technique was an efficient tool to characterize and identify the phenolic compounds in *B. forficata* infusions. It is important to highlight that the advantage of this technique is that, although it is not quantitative, one may relatively quantify the compounds, even the isomeric forms (e.g., catechin, epicatechin, and Quercetin-O-pentoside), and, in case of a lack of standards, the compound assignments may be made by comparison of UV spectra and MS data (accurate mass and fragmentation) with previous literature reports (AQUINO *et al.*, 2019; ENGELS *et al.*, 2012; FARAG *et al.*, 2015).

Figure 4.1. Heatmap for the tentatively identified phenolic compound in *B. forficata* infusions, showing the relative percentage abundance (average of batches within each brand)



3.4. HS-SPME/CG-MS

The identification and relative concentrations of the volatile compounds in the five herbal infusions of *B. forficata* are shown in Table 4.3 in order of retention time and increasing linear retention index (LRI). Forty volatile compounds were tentatively identified, of which only seven were detected in all samples: 2-propyl-heptanol (7.69% to 19.42%), geranyl acetone (4.38% to 7.31%), dodecanol (3.11% to 11.37%), β-ionone (0.71% to 5.84%), spathulenol (11.78% to 30.87%), caryophyllene oxide (2.76% to 17.46%), and benzoic acid 2-ethylhexyl ester (1.12% to 20.14%). The volatile compounds included terpenoids represented by C13-norisoprenoids, sesquiterpenes, and monoterpenes, as well as hydrocarbons, alcohols, esters, aldehydes, ketones, and acids. Among all chemical groups found in the volatile content of the *B. forficata* infusions, sesquiterpenes (hydrocarbon and oxygenated) were present in a higher number (17) and represented most of the composition of the BS (63%), CS1 (61%), CS3 (50%), and CS4 (53%). Esters (31%) and alcohols (29%) accounted for most of the composition of CS2.

There are no data in the literature on the volatile composition of *B. forficata* infusions or any species of the *Bauhinia* genus; however, there are two studies that identified constituents of essential oils of this species and demonstrated that they are essentially composed of sesquiterpenoids. Duarte-Almeida (2004) and Sartoreli (2007) evaluated the composition of essential oils in *B. forficata* and reported that the content of sesquiterpenoids was 87% and 96%, respectively, providing evidence that a mostly sesquiterpenic volatile fraction composition may be characteristic of this species.

It is well established that many sesquiterpenes and their alcohol, aldehyde, and ketone derivatives are biologically active or precursors of metabolites with biological functions, while others have desirable fragrance and flavoring properties (BUTNARIU, 2021). Spathulenol (8.53% to 25.86%) and caryophyllene oxide (2.76% to 17.46%) were two of the major compounds in all samples. Both compounds are known to present several biological activities. Nascimento *et al.* (2018) demonstrated antioxidant, anti-inflammatory, antiproliferative, and antimycobacterial activities of spathulenol, and a moldy and herbaceous odor is attributed to this compound (ZELNER *et al.*, 2009). In turn, caryophyllene oxide has a floral and woody odor (EIRES & DUFOUR, 2009; HE *et al.*, 2019), and biological activities such as anticholinesterase, analgesic, anti-inflammatory, and antifungal activities were also reported (CHAVAN *et al.*, 2010; YANG *et al.*, 2000). Regarding the class of norisoprenoids, they were present in all samples at concentrations ranging from 7.56% to 14.71%, highlighting geranyl acetone and β-ionone. It is reported that they present a significant aromatic impact in fruits such as grapes, apples, lychee, and mango (KOTSERIDIS *et al.*, 2000; ONG *et al.*, 1998), with a floral odor being attributed to them (MAHATTANATAWEE *et al.*, 2005).

Attention is drawn to the identification of bisphenol A (BPA) and dibutyl phthalate (DBP) in some samples evaluated here, especially CS2, which showed important concentrations of these contaminants in its volatile fraction (9.24% and 3.82%, respectively). As any agricultural product, these herbs may be subjected to chemical contaminations due to agricultural practices, especially in stages when a plastic material is used as packaging or support or due to soil treatment, cultivation in contaminated soil, and other factors (ROZENTALE *et al.*, 2018; DI BELLA *et al.*, 2014). Furthermore, the migration of these plasticizers that constitute the packaging cannot be ruled out since it is known that this is the main source of exposure to this type of contaminant (DI BELLA *et al.*, 2014). Di Bella *et al.*

(2014) and Lo Turco *et al.* (2019) evaluated the BPA contamination of spices and herbs from different origins and found it to be present in several samples. Despite concluding that the ingestion of these contaminants does not imply a risk to human health, one cannot disregard their existence, and mechanisms to mitigate them must be evaluated, such as proposing other packaging materials free from them.

In general, the observed differences among the volatile fraction patterns of the infusions indicate the different origins of the samples with their unique ecological settings as well as features intrinsic to the medicinal herbs also observed by Arsenijević *et al.* (2016). Moreover, these authors stressed that compounds present in the volatile fraction of infusions play an important role in the antioxidant capacity of these products, thus rendering this evaluation relevant, although it was still not possible to measure it in this work. Once again, we highlight that the results obtained herein are the first step towards revealing the beneficial health effects of *B. forficata* infusions through chemical diversity after evaluating their non-volatile and volatile fractions.

Table 4.4 – Tentatively identified compounds of B. forficata infusions with their respective concentrations in %

Rt (min)	LRI[(a)]	compound	chemical class	BSB3	CS1B2	CS2B1	CS3B2	CS4B2
14.0	1185	1-decanal	aldehyde	0,095± 0,035	-	0.57±0. 22	-	-
14.3	1193	2-propyl-1-heptanol	alcohol	3.35±0 .35	7.69±0.6 2	19.42±2 .52	9.66±4. 93	8.96±3.07
16.4	1195	estragole	phenylpropanoid	-	0.3±0.00	-	-	0.44±0.21
18.44	1357	eugenol	phenylpropanoid	0.24±0 .00	-	-	-	-
20.10	1428	β-caryophyllene	sesquiterpene	0.85±0 ,10	-	-	-	-
20.30	1429	α-ionone	norisoprenoid	3.59±0 .47	1.55±0.0 8	1.64±0. 04	-	-
20.90	1448	geranyl acetone	norisoprenoid	6.88±1 .08	7.31±0.0 0	5.18±0. 76	5.02±1. 08	4.38±1.25
20.92	1452	α-humulene	sesquiterpene	1.22±0 .45	-	-	-	-
21.00	1461	alloaromadendrene	sesquiterpene	0.7±0. 03	-	-	-	-
21.20	1472	p-benzoquinone	ketone	-	0.66±0.0 4	1.50±0. 22	0.99±0. 03	-

Table 4.4 – Continued

Rt (min)	LRI[(a)]	compound	chemical class	BSB3	CS1B2	CS2B1	CS3B2	CS4B2
21.40	1480	dodecanol	alcohol	4.00±3.75	3.11±0.51	7.14±1.39	3.94±1.27	8.37±0.01
21.70	1485	deydro-β-ionone	norisoprenoid	-	-	5.30±0.50	-	1.17±0.36
21.80	1486	β-ionone	norisoprenoid	4.24±0.05	3.08±0.11	0.71±0.23	2.54±0.38	5.84±1.19
21.99	1499	germacrene D	sesquiterpene	-	0.99±0.02	-	-	-
22.70	1530	δ-cadinene	sesquiterpene	2.03±0.24	2.72±0.22	-	2.25±0.28	-
22.80	1538	dihydroactinidiolide	oxygenated monoterpene	0.7±0.1	-	-	-	-
22.90	1545	eudesma-3,7(11-diene)	sesquiterpene	0.38±0.07	-	-	-	-
23.20	1554	nerolidol oxygenated	sesquiterpene	-	-	-	3.55±0.76	-
23.20	1554	nerolidol oxygenated	sesquiterpene	-	-	-	3.55±0.76	-
24.00	1582	spathulenol	oxygenated sesquiterpene	11.78±1.02	30.87±0.15	8.53±2.34	13.98±1.39	25.86±1.76

Table 4.4 – Continued

Rt (min)	LRI[(a)]	compound	chemical class	BSB 3	CS1B2	CS2 B1	CS3B 2	CS4B2
24.10	1585	caryophyllene oxide	oxygenated sesquiterpene	15.80 ±0.42	14.32± 0.66	2.76 ±2.58	17.46± 1.48	14.11±0 .28
24.40	1598	ledol	oxygenated sesquiterpene	4.05± 0.21	-	-	-	-
24.50	1603	globulol	oxygenated sesquiterpene	1.47± 0.04	-	-	-	-
24.70	1607	humulene epoxide II	oxygenated sesquiterpene	14.15 ±0.78	-	1.58 ±0.14	5.71±0 .14	7.08±00 6
25.20	1631	1,7,7-trimethyl-2-vinylbicyclo[2.2.1]hept-2-ene (vinylbornene)	-	5.21± 0.27	-	-	-	-
25.40	1634	longipinocarveol	oxygenated sesquiterpene	1.68± 0.03	-	-	2.57±0 .26	-
25.50	1647	τ-muurolol	oxygenated sesquiterpene	1.75± 0.5	-	-	-	-
25.70	1659	α-cadinol	oxygenated sesquiterpene	5.04± 0.50	11.99± 0.39	-	4.36±0 .01	6.32±0. 44
27.70	1745	octanal 2-phenylmethylene	aldehyde	-	-	-	0.85±0 .27	0.31±0. 16

Table 4.4 – Conclusion

Rt (min)	LRI[(a)]	Compound	chemical class	BSB 3	CS1B2	CS2B1	CS3B2	CS4B2
27.90	1768	tetradecanoic acid	acid		0.36±0.25	1.87±0.93	1.58±1.17	0.54±0.63
28.30	1785	Anthracene	hydrocarbon					0.68±0.05
28.70	1800	Octadecane	hydrocarbon	-	-	1.04±0.38	-	-
29.60	1850	4,8,12 tetradecatrienal 5,9,13 trimethyl	aldehyde	-	-	1.91±0.59	-	1.08±0.05
30.40	1880	1-hexadecanol	alcohol	-	0.63±0.01	3.25±1.85	1.49±1.57	1.87±1.02
34.20	1881	Cyclohexadecane	hydrocarbon	-	1.39±0.00	-	0.99±0.54	-
34.50	1900	Nonadecane	hydrocarbon	-	-	0.81±0.24	-	-
34.80	1909	methyl hexadecanoate	ester	-	-	1.89±0.47	-	-
35.00	1922	dibutyl phtalate	ester	-	-	9.24±3.89	-	-
35.90	2108	bisphenol A	phenol	-	0.1±0.06	3.82±0.48	2.16±0.30	-
39.60	2360	2 methyltricosane	hydrocarbon	-	-	1.23±1.15	-	-

[(a)]Linear Retention index (LRI) calculated for all components using a homologous series of n-alkanes analyzed under the same conditions as the samples; (-) not detected. BSB3 = botanically identified sample, batch 3; CS1B2 = commercial sample brand 1, batch 2; CS2B1 = commercial sample brand 2, batch 1; CS3B2 = commercial sample brand 3, batch 2; CS4B2 = commercial sample brand 4, batch 2.

3.2. ASSAY FOR α-AMYLASE INHIBITION

In this set of experiments about the inhibition of α-amylase activity, the effect of B. forficata infusions that presented better results for TPC and TFC contents and antioxidant capacity was investigated. The results revealed that all infusions inhibited the α-amylase activity. Based on the IC50 values, the CS2B1 required the lowest phenolic compounds content to inhibit this enzyme. The IC50 values were 0.235 mg RE/mL, 0.245 mg RE/mL, 0.287 mg RE/mL, 0.489 mg RE/mL, and 0.801 mg RE/mL for CS2B1, CS4B2, CS1B2, BSB3, and CS3B2, respectively. Even though CS4B2 presented the highest TPC content, this sample exhibited a higher IC50 value. It is suggested that the inhibition of α-amylase activity may be due to other phytochemicals also present in the infusions. However, it is well-known that phenolic compounds, mainly flavonoids, are excellent inhibitors of digestive enzymes. Flavonoids and their derivatives have the ability to reduce the potency of α-amylase and α-glucosidase by either interacting with or inhibiting specific positions of the enzyme (Rohn *et al.*, 2002). However, other classes of compounds should not be neglected as reported by Papoutsis *et al.* (2021), which reported in their review positive effects of terpenoids, carotenoids, among others compounds on inhibition of α-amylase activity.

Acarbose is widely used in medicine as an inhibitor of digestive enzymes related to the breakout of polysaccharides. As these enzymes are inhibited, there is a reduction in glucose absorption and, consequently, a decrease in the postprandial blood glucose level elevation, which helps reduce the risk of Diabetes Mellitus, for example (PAPOUTSIS *et al.*, 2021). Its IC50 value was found to be 0.034 mg/mL. Thus, a lower concentration of this substance than B. forficata infusions is required to inhibit 50% of the α-amylase activity. However, it should be noted that this medicinal plant is widely used in folk medicine as an adjuvant in treating the population, especially those in vulnerable conditions who do not have access to the health system.

It is important to demonstrate that infusions prepared from commercially available herbs showed an important inhibitory action on the enzyme despite being less potent than acarbose. Furthermore, cytotoxicity was not observed when different fractions from B. forficata were evaluated by Franco *et al.* (2020). These facts reinforce the biological and pharmacological potential of B. forficata, which has an important role in Brazilian folk medicine, primarily because it is abundant and easily accessible.

3.3. ANTIMICROBIAL ASSAY

The antimicrobial action of the *B. forficata* infusions (BSB3, CS1B2, CS2B1, CS3B2, and CS4B2) was tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and yeast (*Candida albicans*). Their infusions presented initial concentrations of 1.39 mg RE/mL, 1.37 mg RE/mL, 2.37 mg RE/mL, 2.42 mg RE/mL and 3.18 mg RE/mL, respectively. No inhibitory effects were observed when the infusions were tested against *Escherichia coli* and *Candida albicans*. This may likely be related to morphological differences between microorganisms, given that Gram-negative bacteria present an extra outer membrane together with a periplasmic space that serves as a selective permeation barrier and reduces the chemical interaction and inhibition effects of antioxidant extracts such as the *B. forficata* infusions, which are mainly composed of phenolic compounds; thus, they are less susceptible to inhibition than Gram-positive bacteria (LIMA *et al.*, 2019).

Only CS3B2 showed antimicrobial action against *Staphylococcus aureus*. The value noted for MBC was 0.39 RE mg/mL. No data were found in the literature expressed as RE mg/mL for this assay, so comparisons with the literature cannot be performed. However, some works showed the antimicrobial potential of different *B. forficata* extracts. Miceli *et al.* (2015) evaluated the effects of different plant leaf extracts against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcescens*, and *Candida albicans*. These authors reported that the extracts did not display any antimicrobial effects against any of the tested strains (MICs > 625 µg/mL). According to them, the obtained results do not appear to support the ethnopharmacological use of the species as antiseptics. It should be pointed out that the tested fractions were rich in flavonoids. In turn, results reported by Farias *et al.* (2018) showed antimicrobial activity of the alcoholic extract of *B. forficata* leaves at 100 µg/mL (MIC) against *Acinetobacter baumannii* 203, *Staphylococcus aureus* 19, *Enterococcus faecalis*, and *Staphylococcus aureus* 213. Given the findings, the authors concluded that the plant extract might contribute to developing new drugs from a substance of natural origin.

Despite having a higher concentration of phenolic compounds, CS4B2 showed no antimicrobial effect. Together with CS1B2, microbiological contaminations were observed in these infusions during the microdilution method in 96-well polystyrene plates, which had a cloudy appearance. To confirm this, the infusions were seeded in plates containing a culture

medium for either yeasts (Sabouraud Agar) or bacteria (Mueller Hinton Agar) and incubated. After that, it was observed that CS1B2 and CS4B2 showed the presence of microorganisms. The respective microorganisms were cultured and isolated and subsequently subjected to identification by laser desorption/ionization time-of-flight mass spectrometry matrix-assisted (MALDI-TOF MS), with four microorganisms being identified: *Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis*, and *Bacillus amyloliquefaciens*. This contamination likely influenced the findings of the antimicrobial assays since the antimicrobial potential of phenolic compounds and other phytochemicals such as alkaloids and terpenoids is well-known in the literature. Also, it is important to highlight that the microorganisms identified are related to the soil microbiota (YADAV *et al.*, 2021), which reinforces that the contamination is inherent to agricultural practices and the processing of the products. New assays will be performed to verify the antimicrobial potential of the infusions using samples free from microbial contamination.

4. CONCLUSION

It is concluded that the samples (from different brands and batches) used to prepare B. forficata infusions presented different TPC and TFC contents and antioxidant potentials. The botanically identified sample (BS) showed lower values for bioactive evaluation than the commercial samples (CS). However, both were mostly composed of flavonoid derivatives. After the statistical analysis, it was possible to observe that the CS3 sample differed from the others. Regarding the volatile fraction isolated from *B. forficata* infusions, to the best of our knowledge, this is the first time that this evaluation has been carried out, and it is clear that it is an important fraction with regard to the aroma of these products and the possible contribution to the biological properties related to them. An inhibitory effect of all *B. forficata* infusions on the α-amylase enzyme was observed. Regarding the antimicrobial activity, only one commercial sample (CS3B2) had this potential against *Staphylococcus aureus.* Despite the differences reported in this work, *B. forficata* presents itself as a source of bioactive compounds that can increase the intake of antioxidant compounds by the population. However, it should be noted that sample standardization is required to ensure all the benefits associated to the plant be delivered to the consumer.

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CHAPTER 5

THERMAL-ASSISTED RECOVERY OF ANTIOXIDANT COMPOUNDS FROM BAUHINIA FORFICATA LEAVES: EFFECT OF OPERATIONAL CONDITIONS

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This chapter was published on **Journal of Applied Research on Medicinal and Aromatic Plants**., (2021), 100303. doi.org/10.1016/j.jarmap.2021.100303

ABSTRACT

Herein, the extraction of antioxidant compounds from Brazilian orchid tree leaves (*Bauhinia forficata* Link), also known as "pata-de-vaca" or "pezuña-de-vaca," is optimized using thermal-assisted solid-liquid extraction. The combination of operational conditions including the temperature (32–74 °C), ethanol percentage (13–97%), and solid/liquid ratio of leaves and ethanol (1:10–1:60; w/v) was employed using a rotational central composite design. The extracts were evaluated for total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant capacities by the radical monocation reduction of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), free radical scavenging activity of 2,2'-diphenyl-β-picrylhydrazyl radical ($DPPH^{\bullet}$), and ferric reducing/antioxidant power (FRAP). The phenolic profile of the optimized extract was also obtained using ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry. The statistically evaluated results revealed that the optimal operational conditions for the recovery of antioxidant compounds from plant leaves included 73.5 °C, 51% ethanol, and a solid/liquid ratio of 1:40. Under these conditions, the obtained values were 74 milligrams of rutin equivalent per gram of dried material (mg RE/g dm), 45 mg RE/g dm, 128 micromoles of Trolox equivalent per gram of dried material (μmol TE/g dm), 121 μmol TE/g dm, and 380 micromoles of Fe^{2+} per gram of dried material (μmol Fe^{2+}/g dm) for TPC, TFC, and $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP assays, respectively. The optimized extract mainly comprised kaempferol and quercetin derivatives, indicating that *Bauhinia forficata* Link leaves are an important source of antioxidant compounds.

Keywords: pata-de-vaca, Brazilian orchid tree, phenolic compound, flavonoid, antioxidant capacity, optimization.

INTRODUCTION

Bauhinia is a genus comprising more than 300 species, which is widely distributed in tropical and subtropical forests. In Brazil, 64 species have been identified, which belong to the Fabaceae family. These are commonly known as "pata-de-vaca" (cow hoof) because of the leaf shape. In recent years, the interest in the plants of the Bauhinia genus has increased considerably owing to its ethnopharmacological significance. Most species are of Asian origin, but native Brazilian species such as *Bauhinia longifolia* and *Bauhinia forficata* also exist (VAZ and TOZZI, 2005; LOPEZ and SANTOS, 2015).

"Pata-de-vaca" can be found in the Northeast (Pernambuco, Bahia, and Alagoas), Southeast (Minas Gerais, Espírito Santo, São Paulo, and Rio de Janeiro), and South (Paraná, Santa Catarina and Rio Grande do Sul) Brazil. It is an important Brazilian medicinal plant, and all parts of this plant are used for different therapeutic applications (TROJAN-RODRIGUES *et al.*, 2012, LOPEZ and SANTOS, 2015).

Varying effects on humans are observed because of a variety of chemical constituents obtained from "pata-de-vaca". These compounds are located in different parts of the plant, and range from secondary metabolites to complex molecules such as peptides and proteins. The "pata-de-vaca" leaves contain alkaloids, alcohols, sterols, flavonoids, polyalcohols, proteins, and terpenes/terpenoids, the flowers contain polyalcohols, flavonoids, and alkaloids, while terpenes/terpenoids are present in the stem and bark (MAFFIOLETTI *et al.*, 2012; LOPEZ and SANTOS, 2015).

Lino *et al.* (2004) evaluated the aqueous, ethanol, and hexane extracts of *Bauhinia forficata* for treating alloxan-induced diabetes in rats. Decreased levels of glucose, triglycerides, and total cholesterol were observed, indicating its potential in clinical usage for the treatment of diabetes. Similarly, Menezes *et al.* (2007) reported that the aqueous extracts obtained from *Bauhinia monandra* and *Bauhinia forficata* were rich in flavonoids and exhibited hypoglycemic activity when evaluated in normoglycemic rats.

The flavonoids in the "pata-de-vaca" extracts may also be useful for the prevention and treatment of diseases that are accompanied by an increase in oxidative stress such as type II diabetes (CURCIO *et al.*, 2012; SALGUEIRO *et al.*, 2016). Chronic hyperglycemia has been associated with a higher production of oxygen reactive species and oxidative damage in different tissues including liver. An increase in the oxygen reactive species has been identified as an inducer of changes in the expression and antioxidant activities of enzymes such as

superoxide dismutase and catalase as well as thiol oxidase and lipid peroxidase (PALMA *et al.*, 2014).

Comprehensive methods have been developed in the last decades to identify compounds in complex mixtures and support the development of new research fields such as plant metabolomics. Liquid chromatography coupled with mass spectrometry (LC-MS) and/or high-resolution mass spectrometry (LC-HRMS) has been demonstrated as an excellent choice for identifying bioactive compounds such as flavonoids and their derivatives from *Bauhinia* extracts (FERRERES *et al.*, 2012; FARAG *et al.*, 2015; AQUINO *et al.*, 2019). These studies show that the hydroalcoholic extracts of *Bauhinia forficata* mainly comprise kaempferol and quercetin derivatives. Ferreres *et al.* (2012) verified the authenticity of the commercial samples of *Bauhinia forficata* Link by obtaining the flavonoid profile using LC-MS.

The potential of "pata-de-vaca" is highlighted by the rich composition of phenolic compounds, mainly flavonoids. Additionally, as "pata-de-vaca" is an abundant species in Brazil, it can potentially be beneficial in the treatment of various diseases, mainly for populations that do not have access to conventional treatments. Although many studies have reported the therapeutic potential of "pata-de-vaca" extracts and infusions (ARAÚJO *et al.*, 2020), an optimized method for the thermal-assisted recovery of bioactive compounds from "pata-de-vaca" leaves based on responses such as total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant capacities in different assays is lacking in the existing literature.

Palsikowski *et al.* (2019) reported the use of supercritical CO_2 and a Soxhlet extraction method to recover antioxidant compounds from *Bauhinia forficata* Link subsp. *pruinosa*. In their study, the effects of pressure, temperature, and solvent type on the chemical composition, yield, TPC, TFC, and antioxidant capacity of the extracts were evaluated. The authors reported that the highest yield and antioxidant responses were observed in for the extract obtained after Soxhlet extraction using 95% ethanol, but an optimization of this method was not performed.

Although emergent extraction techniques have been investigated to recover antioxidant compounds from plants, these have higher operational costs than the conventional extraction methods (OREOPOULOU *et al.*, 2019). However, conventional methods have disadvantages such as long extraction times and use of relatively large quantities of organic solvents, because of which the operational conditions should be optimized. Among the conventional extraction methods, Soxhlet, hydrodistillation, maceration, and agitated solvent

extraction are used to extract bioactive compounds from plants. Agitated solvent extraction employs high temperature and agitation, which increase the efficiency of antioxidant compound recovery and decrease the processing time. This technique allows large dissemination of scientific knowledge and bioactive potential of medicinal plants, mainly in developing countries owing to the low operational costs.

Therefore, this study aimed to optimize the thermal-assisted solid–liquid extraction of antioxidant compounds from *Bauhinia forficata* Link leaves by identifying the best combination of temperature, solid/liquid ratio, and ethanol percentage.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

B. forficata leaves were collected in Petrópolis, state of Rio de Janeiro (22°30'04,63" S, 43º07'58,20" W, altitude: 958 m). Voucher specimens were deposited at the Herbarium of the Department of Botany, Federal University of Rio de Janeiro, under registration number RFA 40.615. The sample material was dried in an oven with forced air circulation at 45 °C. A residual moisture level of 12% (w/w) was attained, which was gravimetrically determined at 105 °C. It was then disintegrated in a domestic blender to obtain flour, which was used for extraction studies.

2.2. SOLID–LIQUID EXTRACTION

The extractions were performed using 125 mL flasks, which were covered to prevent solvent loss, heated in a water bath for one hour, and agitated at 130 rpm. The time and stirring variables were fixed according to the report by Ribeiro *et al.* (2018). The temperature (32–74 °C), solid/liquid ratio (1:10–1:60; w/v), and ethanol percentage in the extractive solution (13–97%) were evaluated as independent variables based on the experimental design (details are included below). The range of the independent variables was selected by considering the data from the literature. The maximum temperature was less than the boiling point of ethanol to prevent solvent loss. The obtained extracts were filtered using a quantitative filter paper and stored at −20 °C until further analysis. The samples were evaluated for TPC, TFC, and antioxidant capacity determined by the radical monocation reduction of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), free radical

scavenging capacity activity of 2,2'-diphenyl-β-picrylhydrazyl radical ($DPPH^{\bullet}$), and ferric reducing/antioxidant power (FRAP) assays.

2.3. EXPERIMENTAL DESING

The effect of the independent variables (extraction temperature, ethanol percentage in the extractive solution, and solid/liquid ratio) on the TPC, TFC and antioxidant capacity, measured using $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP assays were evaluated using the response surface methodology (RSM). The RSM was based on a rotating central composite design comprising eight factorial points (level ±1), three central points (level 0), and six axial points (level ±1.68), resulting in 17 trials. Table 1 shows the combination of independent variables (coded and actual values). Experimental data were analyzed by the RSM using the second-order polynomial equation. Analysis of variance (ANOVA), a test for determining the lack of fit and coefficient of determination (R^2), was used to verify model significance considering a significance level of 5%.

To identify the optimal conditions for the extraction of antioxidant compounds from plant leaves, the technique of the simultaneous optimization of independent variables (desirability function) was used. The desirability function is based on the conversion of each response into individual desirability (*d*). Thereafter, these are combined into an overall desirability (*D*) using the geometric mean. The *D* value ranges from zero to one, where zero corresponds to an undesirable response and one corresponds to a desirable response, in accordance with the report by Derringer and Suich (1980). Under optimal operational conditions, additional assays were performed, and the observed results were compared to those predicted by the model.

The optimized extract was compared to the macerated extract. Maceration was performed at room temperature without stirring for 48 h, employing the same percentage of ethanol in the extraction solution and identical solid–liquid ratio as in the optimized extract. In this step, statistical analysis using ANOVA and Tukey test were performed to identify significant differences in the results considering a significance level of 5%.

2.4. ANALYSIS

2.4.1. Total phenolic compounds (TPC)

TPC analysis was performed using the Folin–Ciocalteu reagent (Sigma-Aldrich, Brazil) according to the method described by Singleton and Rossi (1965). For the reactions, 250 μL of filtered and appropriately diluted extract was mixed with 1250 μL of 10% Folin–Ciocalteu reagent and 1000 μL of 7.5 % (w/v) sodium carbonate solution. Thereafter, the samples were heated at 50 °C for 15 min and cooled at room temperature. The absorbance was measured at 760 nm. A calibration curve was constructed using the rutin standard (Sigma-Aldrich, Brazil) with concentrations ranging from 16 to 166 mg L^{-1} (linear regression: $y = 0.0034x - 0.0128$; $R^2 = 0.9988$). The TPC content is expressed as milligrams of rutin equivalent per gram of dried material (mg RE/g dm).

2.4.2. Total flavonoid compounds (TFC)

The TFC content was determined based on the method described by Zhishen *et al.* (1999) with minor modifications. Here, 0.5 mL of extract was mixed with 3.2 mL of ultrapure water and 150 μL of $NaNO_2$ (5%). After homogenization, the mixture was left to stand for five minutes. Thereafter, 150 μL of $AlCl_3$ (10%) was added to the mixture, and 1 mL of NaOH (1 M) was added after one minute. The absorbance was recorded at 510 nm with a spectrophotometer (Metash, China) using ultrapure water as a blank. The TFC content was calculated using the calibration curve of rutin, with concentration ranging from 99 to 595 mg L^{-1} (linear regression: $y = 0.001x + 0.013$; $R^2 = 0.9974$). The results are expressed as mg RE/g dm.

2.4.3. $ABTS^{\bullet+}$ assay

Antioxidant capacity was determined by the reduction of radical monocation, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), according to the procedure described by Gião *et al.* (2007). The radical was obtained after the addition of 7 mmol/L ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich, Brazil)) to 2.45 mmol/L potassium persulfate (Sigma-Aldrich, Brazil) solution (1:1 (v/v)). The mixture was left to react in the dark for 16 h. To obtain an absorbance of 0.700 ± 0.020 at 734 nm, the $ABTS^{\bullet+}$ solution was diluted using ultrapure water. For the reactions, 30 μL of each filtered and diluted extract was mixed with 3000 μL of $ABTS^{\bullet+}$ solution. After six minutes, the absorbance was measured at 734 nm with a spectrophotometer (Metash, China) using ultra-pure water as a blank. The $ABTS^{\bullet+}$ antiradical activity was calculated using Trolox solutions (Sigma-Aldrich, Brazil) with different concentrations of 240–2000 μmol (linear

regression: y = 0.0003x + 0.0094; R^2 = 0.9989). The results are expressed as micromoles of Trolox equivalents per gram of dried material (μmol TE/g dm).

2.4.4. DPPH$^{\bullet}$ assay

The 2,2'-diphenyl-β-picrylhydrazyl radical (DPPH$^{\bullet}$) (Sigma-Aldrich, Brazil) scavenging activity of the extracts was determined according to the method described by Hidalgo *et al.* (2010). For the reactions, 100 μL of each diluted extract was added to 2900 μL of DPPH$^{\bullet}$ solution (6×10^{-5} M in methanol and diluted to an absorbance of 0.700 at 517 nm). The resulting solutions were allowed to stand for 30 min in the dark at room temperature. Then, the absorbance was measured at 517 nm with a spectrophotometer (Metash, China) using methanol as a blank. The DPPH$^{\bullet}$ scavenging activity was calculated using Trolox solutions (Sigma-Aldrich, Brazil) with different concentrations of 80–680 μmol (linear regression: y = 0.0008x + 0.017; R^2 = 0.9962). The results are expressed as μmol TE/g dm.

2.4.5. FRAP assay

The ferric reducing/antioxidant power (FRAP) assay was performed according to the procedure reported by Benzie and Strain (1996) with minor modifications. The stock solutions included 300 mM of acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ; Sigma-Aldrich, Brazil) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The working solution was prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$. Thereafter, 100 μL of each extract was reacted with 3000 μL of FRAP at 37 °C for 30 min, and the absorbance was measured at 593 nm. The FRAP activity was calculated using $Fe_2SO_4 \cdot 7H_2O$ solutions with different concentrations of 150–1200 μmol Fe^{2+} (linear regression: y = 0.0008x + 0.0042; R^2 = 0.9992). The results are expressed as micromoles of Fe^{2+} per gram of dried material (μmol Fe^{2+}/g dm).

2.4.6. LC-HRMS analysis

The sample extract was dissolved in an aqueous solution containing formic acid (0.1%, v/v) and subjected to ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry (UPLC-qTOF/MS; Bruker Daltonics, MaxisImpact, QTOf Bruker) analysis. Separation was performed using a Hypersil C18 column (3 μm particle size, 2.1 mm × 150 mm). The column temperature was maintained at 40 °C. Subsequently, an aliquot of 20

μL was injected into the UPLC-ESI-qTOF system with a flow rate of 0.27 mL/min. Linear gradient elution of A (0.1% formic acid in water) and B (acetonitrile) was applied employing the following method: 5% B at the beginning, 5–9% B in 5 min, 9–16% B in 10 min, 16–36% B in 18 min, 36–95% B in 1 min, 95% B for 12 min, 95–5% B in one minute, and 5% B for 13 min. Data Analysis 4.4 software (Bruker Daltonics, Germany) was used to interpret the data. The MS data were acquired in the negative mode using an electrospray ionization (ESI) source. The data were scanned for each test sample from *m/z* 50 to 1200 (mass to charge ratio). Highly pure nitrogen was used as the nebulizing gas and ultrahigh purity helium as the collision gas. The ESI data were collected in the negative mode, and the capillary voltage was set at 5000 V. The ESI parameters included dry gas at 200 °C at a flow rate of 8 L/min, and nebulizer pressure of two bar.

2.4.7. Statistical analysis

All measurements were performed in triplicate, and the results were analyzed using Statistic 13 software (Dell Inc.). The desirability function was applied to determine the operational parameters of extraction that could improve the recovery of antioxidant compounds from *B. forficata* leaves at 5% level of significance. Correlations between the antioxidant capacity, TPC, and TFC were determined using Pearson's correlation coefficient. This last statistical test was performed using Graph Pad Prism 6.0 software.

3.RESULTS AND DISCUSSION

3.1. Effect ofindependent variables

Temperature, solvent type and concentration, and solid/liquid ratio of the extraction processes are the variables that can affect the recovery efficiencies of bioactive compounds from plant tissues. In this study, the effects of the operational conditions on their recoveries are evaluated, and the obtained results are listed in Table 5.1.

The operational conditions significantly affected the recoveries of bioactive compounds from *B. forficata* leaves with 12% residual moisture. The TPC value ranged from 20 mg RE/g dm at 52.5 °C using 97% ethanol and a solid/liquid ratio of 1:35 (assay 12) to 74 mg RE/g dm when the extraction was performed at 74 °C using 55% ethanol at the same solid/liquid ratio (assay 10). The TFC value increased from 18 mg RE/g dm, when the leaves were extracted at 52.5 °C using 13% ethanol and a solid/liquid ratio of 1:35 (assay 11) to 41

mg RE/g dm, when the extract was obtained at 74 °C using 55% ethanol and a solid/liquid ratio of 1:35 (assay 10).

The antioxidant capacities of the extracts of *B. forficata* leaves were obtained by employing the $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP assays, which showed that their capacities increased 3.7, 5.1, and 3.8 times, respectively, when the plant leaves were extracted at 74 °C using 55% ethanol and a solid/liquid ratio of 1:35 (assay 10) compared to lower values observed in the experimental design (Table 1). These data confirm that operational conditions have significant effects on these responses, similar to that observed for the TPC and TFC contents. Furthermore, the results show that the compound–radical interaction is different, therefore justifying the use of different assays to evaluate the antioxidant capacity of the plant samples.

Table 5.1 - Actual and coded values of the independent variables employed to recover antioxidant compounds from B. forficata leaves, total phenolic compound (TPC) and total flavonoid compound (TFC) contents, and antioxidant capacities of the extracts.

Assay	Temperature (°C)	Ethanol (%)	Solid/liquid Ratio (g/mL)	TPC[1]	TFC[1]	$ABTS^{\bullet+}$[2]	$DPPH^{\bullet}$[2]	FRAP[3]
1	40 (-1)	30 (-1)	1:20 (-1)	49	20	59	71	213
2	40 (-1)	30 (-1)	1:50 (+1)	58	25	81	92	230
3	40 (-1)	80 (+1)	1:20 (-1)	32	20	51	58	153
4	40 (-1)	80 (+1)	1:50 (+1)	48	27	65	88	215
5	65(+1)	30 (-1)	1:20 (-1)	60	26	88	94	291
6	65 (+1)	30 (-1)	1:50 (+1)	69	29	93	103	306
7	65 (+1)	80 (+1)	1:20 (-1)	45	29	68	78	210
8	65 (+1)	80 (+1)	1:50 (+1)	60	33	66	91	258
9	32 (-1.68)	55 (0)	1:35 (0)	56	28	79	94	246
10	74 (+1.68)	55 (0)	1:35 (0)	74	41	107	143	366
11	52.5 (0)	13 (-1.68)	1:35 (0)	46	18	59	42	206
12	52.5 (0)	97 (+1.68)	1:35 (0)	20	19	29	28	97
13	52.5 (0)	55 (0)	1:10 (-1.68)	55	28	89	98	270
14	52.5 (0)	55 (0)	1:60 (+1.68)	71	35	99	113	310

15*	52.5 (0)	55 (0)	1:35 (0)	69	33	94	111	298
16*	52.5 (0)	55 (0)	1:35 (0)	65	33	101	120	284
17*	52.5 (0)	55 (0)	1:35 (0)	67	33	99	115	276

Results obtained for *B. forficata* leaves with 12% residual moisture. *Central point; $ATBS^{\bullet+}$ – antioxidant capacity by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical; $DPPH^{\bullet}$ – antioxidant capacity by 2,2'-diphenyl-β-picrylhydrazyl radical; FRAP – ferric reducing/antioxidant power. [1]Results are expressed as milligrams of rutin equivalent per gram of dried material (mg RE/g dm). [2]Results are expressed as micromoles of Trolox equivalent per gram of dried material (μmol TE/g dm). [3]Results are expressed as micromoles of Fe^{2+} per gram of dried material (μmol Fe^{2+}/g dm).

As assays 10, 11, and 12 involve the use of identical solid/liquid ratio (1:35 (w/v)), the obtained results were mainly affected by ethanol percentage and extraction temperature, which varied from 13% to 97% and 52.5 °C to 74 °C, respectively. It is well-known that the polarity of the solvent significantly affects the recovery of antioxidant compounds. The use of a more apolar solution than water favors the extraction of phenolic compounds from plant tissues as it facilitates cell wall rupture (Ignat *et al.*, 2011). In addition, it has been reported that binary solvent systems (e.g., ethanol/water) are more efficient than mono-solvent systems for the extraction of these compounds. According to the experimental design (Table 1), 55% ethanol provided an extract that was richer in the TPC content (> 70 mg RE/g dm). Dirar *et al.* (2019) extracted phenolic compounds from six medicinal plants from Sudan, employing ethanol, water, and their mixture as solvents. Better results were achieved using ethanol/water combinations.

Temperature also plays an important role in the extraction of bioactive compounds because it increases both the solute solubility and diffusion coefficients of the phenolic compounds. In addition, as reported by Markom *et al.* (2007), the surface tension and viscosity of the solvent are significantly decreased at the boiling point in compared to those at a lower temperature; therefore, the solvent can easily reach the cell wall of the plant. This behavior explains the results obtained in the present study, and the best values for the TPC and antioxidant capacity are achieved with the highest extraction temperature (74 °C). The extracts obtained at 74 °C presented TPC and TFC contents superior to 70 mg RE/g dm and 40 mg RE/g dm, respectively. Tušek *et al.* (2016) also reported the positive effect of temperature on the extraction of phenolic compounds from medicinal plants of the Asteraceae family. The plants for which the extractions were performed at 80 °C provided extracts richer in phenolic compounds than those extracted at 40 and 60 °C.

Compared to the literature reports, the extract obtained at 74 °C using 55% ethanol and a solid/liquid ratio of 1:35 (assay 10) from *B. forficata* leaves with 12% residual moisture showed a higher TFC value (41 mg RE/g dm) than that reported by Sayago *et al.* (2013) for *B. forficata* subsp. *pruinosa* leaves (9.5 mg RE/g dm) and *B. variegata* leaves (12 mg RE/g dm). These authors did not report the residual moisture contents of the plants after drying at 35 °C.

Although many factors can affect the antioxidant potential and phenolic content of plants, our results show that *B. forficata* leaves are a source of these compounds, which can provide beneficial effects to humans, mainly because of their antidiabetic and antioxidant properties (Curico *et al.*, 2012).

A strong correlation between TPC, TFC, and antioxidant capacity of the *B. forficata* extracts is observed in the present study (Table 5.2). The positive correlation coefficients (r) shown in Table 2 are high, indicating that the extracts with higher amounts of phenolic or flavonoid compounds show better antioxidant capacities. This suggests that phenolic and flavonoid compounds are important for the antioxidant capacity of *B. forficata* extracts, confirming the positive correlation of these parameters, as previously reported by Jacobo-Velázquez and Cisneros-Zevallos (2009).

All models were significant for predicting the behavior of the responses in relation to the independent variables, as the calculated F-*values* were higher than the listed F-*value* ($F_{9,7}$ = 3.68) at p = 0.05, except for TFC. The calculated F-*values* for TPC, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP responses were 126, 56, 33, and 47, respectively. Notably, the lack of fit was non-significant as it showed a p-*value* of >0.05, and the calculated F-*values* were lower than the listed F-*value* for these responses. The R^2 values of the fitted models were 0.994, 0.986, 0.977, and 0.984 for TPC, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP responses, respectively, showing that the models accounted for at least 97% of data variability obtained by this experimental design.

Table 5.2 - Pearson's correlation analysis for the experimental design results

	TPC	TFC	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	FRAP
TPC	---	r = 0.817	r = 0.940	r = 0.914	r = 0.967
TFC	r = 0.817	---	r = 0.810	r = 0.915	r = 0.831
$ABTS^{\bullet+}$	r = 0.940	r = 0.810	---	r = 0.941	r = 0.942
$DPPH^{\bullet}$	r = 0.914	r = 0.915	r = 0.941	---	r = 0.920
FRAP	r = 0.967	r = 0.831	r = 0.942	r = 0.914	---

TPC – total phenolic compounds; TFC – total flavonoid compounds; $ABTS^{\bullet+}$ – antioxidant capacity by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical; $DPPH^{\bullet}$ – antioxidant capacity by 2,2'-diphenyl-β-picrylhydrazyl radical; FRAP – ferric reducing/antioxidant power; r = Pearson's correlation coefficient; All r values are significant ($p < 0.05$).

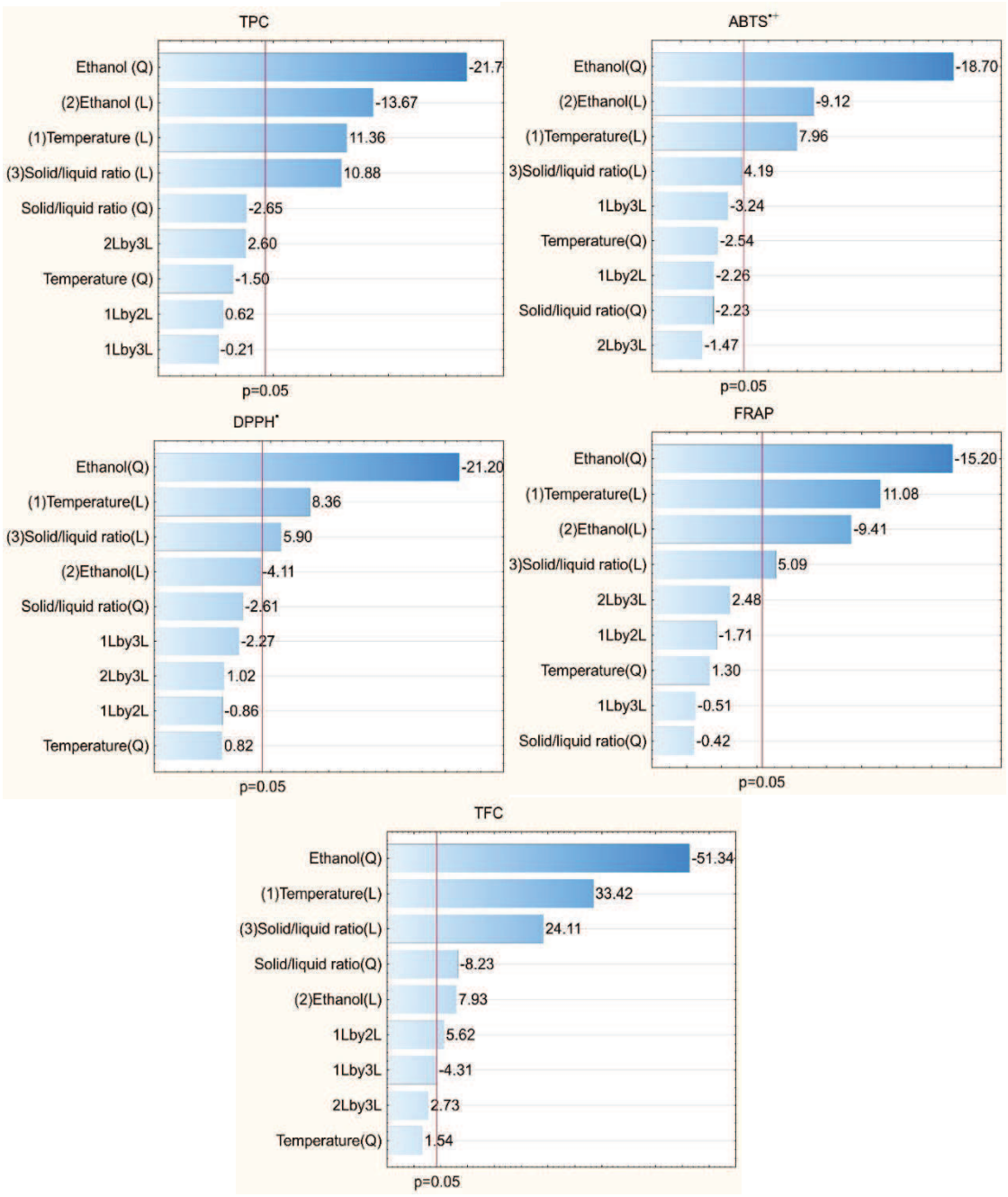
For TFC, the model afforded higher calculated F-*values* (34.9) than the listed F-*value* ($F_{9,7}$ = 3.68); therefore, it was significant. The lack of fit was also significant at p = 0.05; however, this response showed a high R^2 value (0.978), which allowed the validation of its results by the experimental design.

According to the Pareto plot (Figure 5.1), the quadratic term of ethanol percentage and linear terms of ethanol percentage, temperature, and solid/liquid ratio affected the TPC recovery ($p < 0.05$). However, ethanol percentage was an independent variable with a higher impact on this response. Negative values indicated that when the ethanol percentage was increased in the extractive solution, a lower recovery of phenolic compounds was obtained. Simultaneously, the linear terms of temperature and solid/liquid ratio showed positive values, which indicated that a direct relationship existed between these independent variables and the amount of recovered phenolic compounds. This trend was observed for the results of antioxidant capacity measured by the FRAP and $ABTS^{\bullet+}$ assays, but for the $ABTS^{\bullet+}$ assay, the linear effect of the solid/liquid ratio was not significant ($p > 0.05$) (Figure 1). For the $DPPH^{\bullet}$ and TFC responses, the quadratic effect of ethanol percentage and the linear terms of temperature and solid/liquid ratio showed significant effects on the responses ($p < 0.05$; Figure 1). The negative quadratic effect of ethanol percentage confirmed that there was a limit for this behavior; therefore, apolar solvents promoted the lower recovery of antioxidant compounds. In addition, as with other independent variables, temperature afforded a positive value, showing that an increase in temperature favored extracts with higher antioxidant capacities.

In general, the negative and quadratic effects of ethanol percentage led to a higher impact on the evaluated responses, followed by a positive linear effect of temperature. According to Oreopoulou *et al.* (2019), the efficiency of a solvent depends mainly on its ability to dissolve antioxidant compounds such as phenolics. For example, ethanol is a suitable solvent to dissolve flavonoid glucosides, while water is better to dissolve phenolic acid glycosides. These authors have also shown that ethanol may influence plant cell permeability by affecting the phospholipid bilayer of the membrane. Their work corroborates that the use of an extractive solution composed of ethanol and water to afford an extract rich in antioxidant compounds from *B. forficata* leaves. Notably, ethanol is a green solvent with low toxicity, which can allow further application of the extract.

Oreopoulou *et al.* (2019) reported that an increase in the extraction temperature led to higher permeability of cell walls, higher solubility of phenolic compounds, and higher heat and mass transfer phenomena, favoring the solvent action. However, high temperatures can also result in the degradation of some compounds, which highlights the importance of this optimization study.

Figure 5.1 - Effect of the independent variables on the total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant capacity by $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP assays. $ATBS^{\bullet+}$ - antioxidant capacity by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical; $DPPH^{\bullet}$ - antioxidant capacity by 2,2'-diphenyl-β-picrylhydrazyl radical; FRAP - ferric reducing/antioxidant power



3.2. Selection of best operational conditions

According to the contour surfaces (Figure 2), which correlate the independent variables and desirability values, the areas in dark red correspond to those where the extracts rich in antioxidant compounds can be obtained from *B. forficata* leaves. When temperature and ethanol percentage are correlated, better results are obtained by employing temperatures higher than 70 °C and an ethanol percentage of 40–60%. This shows that there is a direct relationship between temperature and recovered antioxidant compounds as well as more apolar extractive solutions, which decrease the amount of antioxidant compounds in the extract. Upon correlating the ethanol percentage and solid/liquid ratio, better results are obtained with an ethanol percentage of 40–60% and a wide range of solid/liquid ratios (1:20–1:60), indicating that solid/liquid ratio has a less significant effect on the results. This trend is maintained when the temperature and solid/liquid ratio are correlated. As observed in Figure 5.2, temperatures above 70 °C promote higher recovery of antioxidant compounds from the plant for a wide range of solid/liquid ratios (1:20–1:60).

Therefore, to determine the exact operational conditions that can afford the highest recovery of antioxidant compounds, simultaneous optimization method was used (desirability function), which assessed the individual and overall desirability. Figure 5.3 shows the individual and overall desirability profiles for the extraction conditions. The overall desirability value is 0.962 (close to the maximum value of 1.0), corresponding to the optimal operational conditions for the extraction of antioxidant compounds from *B. forficata*.

The desirability value obtained (0.962) after fitting the data corresponded to the range recognized as excellent (0.8–1.0) by Akhanazarova and Kafarov (1982). Thus, the most suitable parameters for extraction included a temperature of 73.5 °C, 51% ethanol, and a solid/liquid ratio of 1:40.

Extraction under optimal operational conditions afforded TPC of 74 mg RE/g dm, TFC of 45 mg RE/g dm, and antioxidant capacities of 128 μmol TE/g dm, 121 μmol TE/g dm, and 380 μmol Fe^{2+}/g dm in $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP assays, respectively, which were close to the values predicted by the experimental design, i.e., TPC (77 mg RE/g dm); TFC (39 mg RE/g dm); antioxidant capacities in $ABTS^{\bullet+}$ (104 μmol TE/g dm), $DPPH^{\bullet}$ (136 μmol TE/g dm), and FRAP (364 μmol Fe^{2+}/g dm) assays with coefficients of variation of <15%.

Therefore, the results indicated that the experimental design was suitable for determining the optimal operational conditions for the extraction of antioxidant compounds from *B. forficata* leaves.

Figure 5.2 - Contour surfaces of the independent variables and desirability values

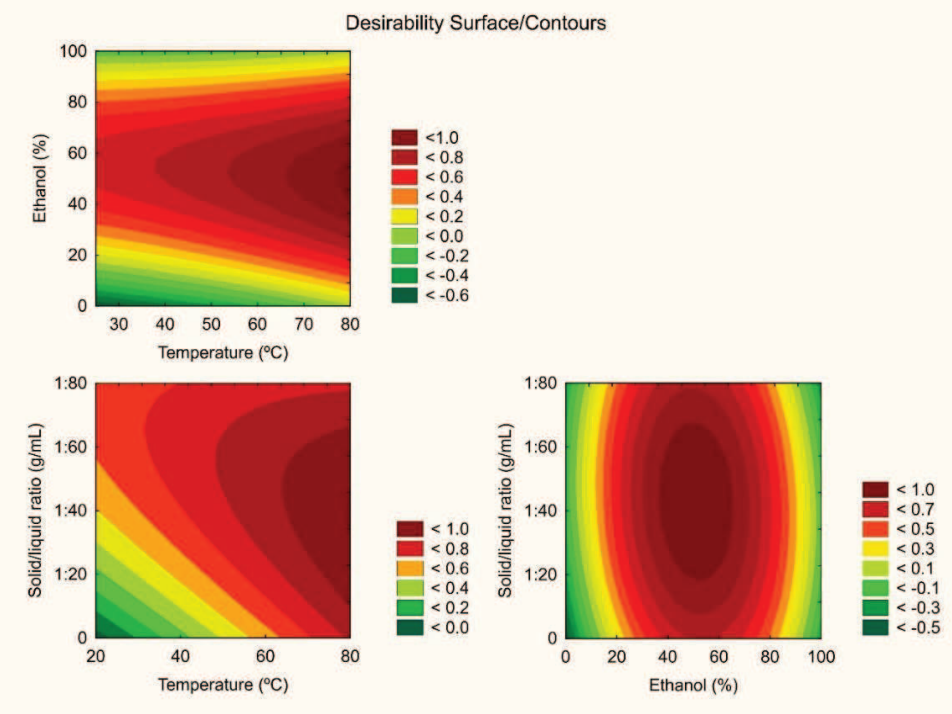
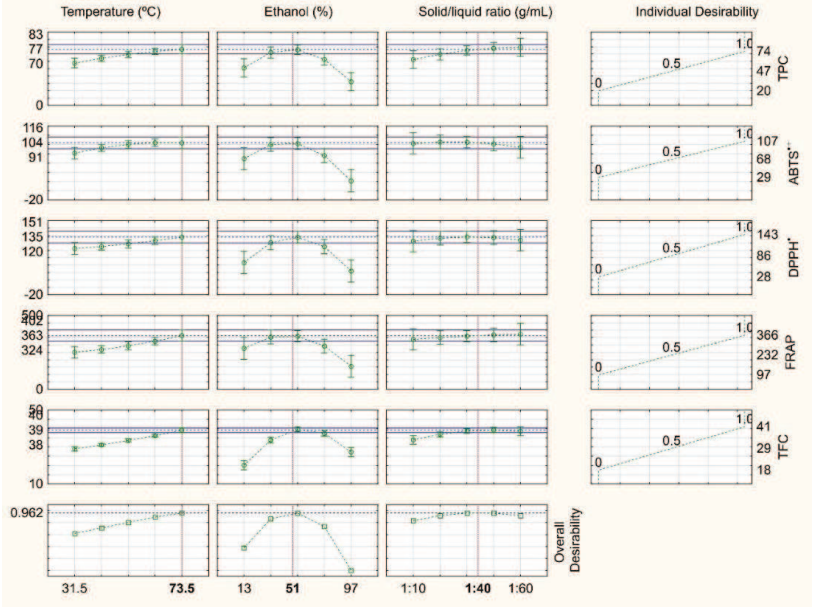


Figure 5.3 - Profiles of predicted values for individual and overall desirability for the extraction optimization.



TPC – total phenolic compounds (mg RE/g dm); TFC – total flavonoid compounds (mg RE/g dm); ATBS•+ - antioxidant capacity by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical (µmol TE/g dm); DPPH• - antioxidant capacity by 2,2'-diphenyl-β-picrylhydrazyl radical (µmol TE/g dm); FRAP - ferric reducing/antioxidant power (µmol Fe2+/g dm)

3.3.Optimized extract x Macerated extract

The results for the optimized and macerated extract obtained from *B. forficata* leaves with 12% residual moisture are listed in Table 3. The optimized extract differs from the macerated extract, showing the highest values of TPC, TFC, and antioxidant capacity ($p < 0.05$). In this case, temperature and stirring favored antioxidant compounds recovery, as maceration was performed at room temperature without stirring for 48 h, while thermal-assisted solid–liquid extraction was performed at 73.5 °C and 130 rpm for one hour.

Table 5.3 - Total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant capacities of the optimized and macerated extract from *B. forficata* leaves

Sample	TPC[1]	TFC[1]	**ABTS$^{\bullet+}$[2]**	**DPPH$^{\bullet}$[2]**	**FRAP[3]**
OE	74 ± 2[a]	45 ± 2[a]	128 ± 1[a]	121 ± 5[a]	380 ± 28[a]
ME	51 ± 1[b]	25 ± 0[b]	118 ± 2[b]	56 ± 5[b]	231 ± 5[b]

Results obtained for *B. forficata* leaves with 12% residual moisture. OE – optimized extract; ME – macerated extract; ATBS$^{\bullet+}$ – antioxidant capacity by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical; DPPH$^{\bullet}$ – antioxidant capacity by 2,2'-diphenyl-β-picrylhydrazyl radical; FRAP – ferric reducing/antioxidant power. [1]Results are expressed as milligrams of rutin equivalent per gram of dried material (mg RE/g dm). [2]Results are expressed as micromoles of Trolox equivalent per gram of dried material (μmol TE/g dm). [3]Results are expressed as micromoles of Fe^{2+} per gram of dried material (μmol Fe^{2+}/g dm). Different letters in the same column indicate statistical differences among the results ($p < 0.05$).

According to Oreopoulou *et al.* (2019), maceration is a simple, inexpensive, and popular extraction technique to recover phenolic compounds from medicinal plants. Briefly, the maceration system is allowed to stand at room temperature for several hours, even days, with occasional agitation. In comparison to the thermal-assisted solid–liquid extraction, maceration afforded a lower recovery of antioxidant compounds from *B. forficata* leaves (Table 3). The thermal-assisted solid–liquid extraction performed at medium temperature (73 °C), low constant stirring (130 rpm) for a short period of time (one hour) favored the extraction of antioxidant compounds, presented TPC, TFC, and antioxidant capacities in ABTS$^{\bullet+}$, DPPH$^{\bullet}$, and FRAP assays 1.46, 1.76, 1.08, 2.16, and 1.64 times higher, respectively, when compared to maceration.

These results were in agreement with data reported by Pudziuvelyte *et al.* (2018), who employed several extraction methods to recover phenolic compounds from *E. ciliata.* These results showed that stirring during maceration favored phenolic compound extraction,

particularly the apigenin and chlorogenic acid contents, which are important phenolic compounds.

3.4. Chromatography and Mass Spectrometry Analysis

The previously reported phytoconstituents from *Bauhinia* species predominantly include flavonoid *O*-glycosides, majority of which are derivatives of kaempferol and quercetin (FILHO *et al.*, 2009; FERRERES *et al.*, 2012; AQUINO *et al.*, 2019).

The identification of compounds by UPLC-qTOF/MS was based on a comparison of the relative retention time, UV spectra, and HRMS data (exact mass measurement, fragmentation pattern, and derived chemical sum formula) with those reported previously in the literature. Reliable identifications were considered for mass errors less than 6.5 ppm. The retention times, experimental deprotonated molecular ions, UV characteristics (λ_{max}: maximum wavelength), and fragment ions for the 15 tentatively identified compounds are shown in Table 4.

The phenolic acid derivative, dihydroxybenzoic acid-hexoside (**1**; *m/z* 315.0704), was tentatively identified by the fragment with *m/z* 152, corresponding to the loss of hexose moiety $[M-H-162]^-$, from which HCO_2 was lost, resulting in a fragment with *m/z* 108 (AQUINO *et al.*, 2019). For caffeoyltartarate (**2**), the main fragment ions corresponded to tartaric acid (*m/z* 149) and loss of tartaric acid $[M-H-132]^-$, represented by *m/z* 179. The data for (epi)catechin dimer (**3**) showed a fragment corresponding to the loss of an epicatechin unit (*m/z* 289) and an early elution time (Farag *et al.*, 2015). The data for epicatechin (**4**) with *m/z* 289 ($[M-H]^-$) showed fragments with *m/z* 245, corresponding to the loss of CO_2, and *m/z* 203, resulting from the Retro-Diels-Alder fragmentation (Sun and Miller, 2003; Aquino *et al.*, 2019). The glycosylated derivatives of flavonoids (**5–14**) were identified by the presence of the respective aglycone ions with *m/z* 284 and/or 285 (**6, 9, 11, 12, 14**) for kaempferol derivatives and *m/z* 300 and/or 301(**5, 7, 8, 10**) for quercetin derivatives. Fragmentation was previously proposed by Demarque *et al.* (2016). The data for methoxylated flavonoid isorhamnetin (**15**) showed a main fragment ion with *m/z* 300 $(M-H-15)^-$, resulting from the loss of CH_3 from the deprotonated molecular ion, which was followed by the loss of $^{\cdot}CO$, leading to a fragment with *m/z* 271. Notably, the signal-to-noise ratios, particularly for peaks **6**–**13**, were higher during the HRMS detection than UV detection, implying that this technique was highly sensitive.

The extract mainly comprised flavonoid *O*-glycosides, verifying the results reported in the literature. Some of these have been extensively investigated, and their pharmacological actions have been proven. Hwang *et al.* (2019) demonstrated that kaempferol-3-*O*-β-rutinoside suppressed inflammation-related gene expression through NF-κB (nuclear factor kappa beta) and MAPK (mitogen-activated protein kinase) pathways and suggested its usefulness in pharmacological research. Jiang *et al.* (2019) reported the protective effects of quercetin and isorhamnetin on glucose uptake in muscle cells at physiological concentrations, revealing the molecular mechanisms that supported the use of quercetin and isorhamnetin as novel therapeutic entities for the prevention and treatment of hyperglycemia and associated disorders.

Table 5.4 Phenolic compounds of the optimized B. forficata leaves extract

	Retention time (min)	λ_{max} (nm)	*m/z* [M-H]$^-$ experimental	MS^2	Molecular formula [M-H]$^-$	Error (ppm)	Compound
1	3.2	Nd	315.0704	108, 152	$C_{13}H_{15}O_9$	5.6	dihydroxybenzoic acid-hexoside
2	7.4	240, 326	311.0388	179, 149, 135	$C_{13}H_{11}O_9$	6.5	Caffeoyltartarate
3	10.7	230, 275	577.1351	407, 289	$C_{30}H_{25}O_{12}$	0.2	(Epi)Catechin dimer
4	11.9	232, 327	289.0718	245, 203	$C_{15}H_{13}O_6$	3.9	(Epi)Catechin
5	16.9	234, 345	755.2065	300	$C_{33}H_{39}O_{20}$	3.4	Quercetin 3-*O*-rhamnosyl rutinoside
6	18.5	Nd	739.2136	284	$C_{33}H_{39}O_{19}$	6.1	Kaempferol 3-*O*-rhamnosyl rutinoside
7	18.8	254, 353	609.1493	300	$C_{27}H_{29}O_{16}$	5.3	Rutin
8	20.7	254, 350	433.0787	300, 271, 255	$C_{20}H_{17}O_{11}$	2.5	Quercetin *O*-arabinoside
9	20.8	265, 345	593.1544	285, 284	$C_{27}H_{29}O_{15}$	5.4	Kaempferol 3-*O*-rutinoside
10	21.3	254, 350	447.0940	300; 301	$C_{21}H_{19}O_{11}$	1.5	Quercetin 3-*O*-rhamnoside

Table 5.4- Conclusion

	Retention time (min)	λ_{max} (nm)	*m/z* $[M-H]^-$ experimental	MS^2	Molecular formula $[M-H]^-$	Error (ppm)	Compound
11	21.3	254, 350	447.0940	284; 285; 255	$C_{21}H_{19}O_{11}$	1.5	Kaempferol 3-*O*-glucoside
12	21.3	254, 350	447.0940	284; 285	$C_{21}H_{19}O_{11}$	1.5	Kaempferol 3-*O*-galactoside
13	21.5	253, 353	623.1642	315	$C_{28}H_{31}O_{16}$	3.9	Isorhamnetin 3-*O*-rutinoside
14	22.9	238, 272, 350	417.0833	285, 284, 255, 227	$C_{20}H_{17}O_{10}$	1.3	Kaempferol *O*-pentoside
15	27.2	250, 330	315.0492	300, 271, 255	$C_{16}H_{11}O_7$	5.8	Isorhamnetin

Nd – Not detected; λ_{max} – maximum wavelength; *m/z* – mass to charge ratio; MS^2 – fragments of the second stage of mass spectrometry

4. CONCLUSION

The recovery of antioxidant compounds from *B. forficata* leaves was mainly affected by the ethanol percentage of the extractive solution and extraction temperature. Less apolar binary solvent systems and high temperature provided extracts rich in antioxidant compounds. The optimal operational conditions to recover the antioxidant compounds from the plant were 73.5 °C, 51% ethanol as solvent, and a solid/liquid ratio of 1:40. Under these conditions, the TPC, TFC, and antioxidant capacities in $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP assays were 74 mg RE/g dm, 45 mg RE/g dm, 128 μmol TE/g dm, 121 μmol TE/g dm, and 380 μmol Fe^{2+}/g dm, respectively. The thermal-assisted solid–liquid extraction afforded higher recovery efficiency of antioxidants from the plant in comparison to maceration, probably because of the

extraction temperature and agitation. Fifteen phenolic compounds were identified in the optimized extract, which mainly comprised quercetin and kaempferol derivatives. This study allowed the identification of optimal operational conditions to obtain an antioxidant-rich extract from *B. forficata* leaves, a native plant of Brazil.

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CONCLUSÕES GERAIS

Nesse trabalho foram avaliadas amostras comerciais de B. forficata, comercializadas sob a forma de ervas secas para o preparo de infusões domésticas, bem como amostras botanicamente identificadas, coletadas no município de Petrópolis, RJ, em três períodos distintos.

Por meio do isolamento dos óleos essenciais, foi possível identificar 116 compostos das mais variadas classes químicas como sesquiterpenos, sesquiterpenos oxigenados, diterpenos, aldeídos e outros. Com o uso de ferramentas quimiométricas, foi estabelecido os compostos responsáveis pela diferença entre amostras. Dentre as amostras comerciais, a amostra CS3 foi a mais distinta, pelo alto teor de β-cariofileno e óxido de cariofileno na sua composição. Aqui é importante destacar que este é o primeiro trabalho que traz informações a respeito da composição de óleos essenciais de amostras comerciais de *B. forficata*, sendo, portanto, um primeiro passo no sentido de estabelecer uma padronização de óleos essenciais desta espécie.

No que diz respeito às infusões das amostras de B. forficata comerciais e aquelas botanicamente identificadas, em consonância com trabalhos reportados na literatura, elas apresentaram composição majoritária de derivados glicosilados de flavonoides, principalmente de quercetina e kaempferol. Quanto ao conteúdo de compostos fenólicos totais e flavonoides totais e capacidade antioxidante, determinada por diferentes métodos, as amostras e seus lotes se mostraram diferentes entre si, principalmente aquelas comerciais que apresentaram alto desvio padrão. Após análise estatística, foi observado que a amostra CS3 se diferenciou das demais, amostra esta, única a apresentar ação antimicrobiana sobre Staphylococcus aureus. É importante ressaltar que apesar das diferenças entre as amostras e seus lotes, todas foram capazes de inibir a atividade da α-amilase.

Neste trabalho, foi avaliada pela primeira vez a fração volátil das infusões de B. forficata. Por meio desse estudo, foi possível identificar 41 compostos, majoritariamente representados pela classe dos sesquiterpenos, muitos deles reconhecidos por suas atividades biológicas, além de outros compostos associados ao aroma como ésteres, alcoóis e aldeídos. Assim, observa-se que esta fração não deve ser negligenciada ao tentar se estabelecer relações entre composição e atividade biológica, uma vez que ao consumir as infusões sob a forma de

chás caseiros, além da fração não volátil, composta principalmente pelos compostos fenólicos, também há a ingestão desta fração volátil.

Em vista das propriedades observadas e da abundância e facilidade de acesso à *B. forficata*, foi realizado um estudo de otimização da recuperação dos compostos antioxidantes das folhas da planta por meio de um delineamento experimental, uma vez que o extrato obtido pode ser usado como matéria-prima na formulação de fitoterápicos. As condições operacionais ideais para recuperar os compostos antioxidantes da planta foram 73,5 °C, etanol 51% como solvente e uma razão sólido-líquido de 1:40 (g/mL). No extrato otimizado foram identificados quinze compostos fenólicos, composto principalmente de derivados glicosilados de quercetina e kaempferol.

Portanto, apesar de terem sido verificadas diferenças entre as amostras aqui avaliadas, considerando todos os parâmetros estudados, a B. forficata apresenta-se como fonte de compostos bioativos, que podem aumentar a ingestão de compostos antioxidantes pela população que faz uso das infusões domésticas. No entanto, deve-se notar que uma padronização das amostras é necessária para garantir que todos os benefícios associados à planta sejam entregues ao consumidor. Mais uma vez destaca-se que ainda não há marcadores químicos validados para a espécie, permanecendo aberto esse campo da pesquisa.

GENERAL CONCLUSIONS

In this work, commercial samples of *B. forficata*, marketed in the form of dried herbs for the preparation of domestic infusions, were evaluated, as well as botanically identified samples, collected in Petrópolis county, RJ, in three different periods. Through the isolation of essential oils, it was possible to identify 116 compounds of the most varied chemical classes such as sesquiterpenes, oxygenated sesquiterpenes, diterpenes, aldehydes and others. With the use of chemometric tools, the compounds responsible for the difference between samples were established. Among the commercial samples, the CS3 sample was the most distinguished, due to the high content of β-caryophyllene and caryophyllene oxide in its composition. Here it is important to highlight that this is the first work that brings information about the composition of essential oils from commercial samples of *B. forficata*, being, therefore, a first step towards establishing a standardization of essential oils of this species.

With regard to the infusions of commercial *B. forficata* samples and those botanically identified, in line with studies reported in the literature, they showed a majority composition of glycosylated derivatives of flavonoids, mainly quercetin and kaempferol. As for the content of total phenolic compounds and total flavonoids and antioxidant capacity, determined by different methods, the samples and their lots were different from each other, especially those commercials that showed high standard deviation.

After statistical analysis, two distinct groups were observed, one of them composed only by the CS3 sample, which is the only sample to present antimicrobial action against Staphylococcus aureus. It is important to emphasize that despite the differences between the samples and their lots, all were able to inhibit α-amylase activity.

In this work, the volatile fraction of B. forficata infusions was evaluated for the first time. Through this study, it was possible to identify 41 compounds, mostly represented by the class of sesquiterpenes, many of them recognized for their biological activities, in addition to other compounds associated with aroma such as esters, alcohols and aldehydes.

Thus, it is observed that this fraction should not be neglected when trying to establish relationships between composition and biological activity, since when consuming the infusions in the form of homemade teas, in addition to the non-volatile fraction, composed mainly of phenolic compounds, it is also this volatile fraction is ingested.

In view of the observed properties and the abundance and ease of access to B. forficata, a study was carried out to optimize the recovery of antioxidant compounds from the leaves of the plant through an experimental design, since the extract obtained can be used as raw material. raw material in the formulation of herbal medicines. The ideal operating conditions to recover the antioxidant compounds from the plant were 73.5 °C, 51% ethanol as solvent and a solid-liquid ratio of 1:40 (g/mL). In the optimized extract, fifteen phenolic compounds were identified, mainly composed of glycosylated derivatives of quercetin and kaempferol.

Therefore, despite differences between the samples evaluated here, considering all the parameters studied, B. forficata presents itself as a source of bioactive compounds, which can increase the intake of antioxidant compounds by the population that uses domestic infusions. However, it should be noted that standardization of samples is necessary to ensure that all benefits associated with the plant are delivered to the consumer. Once again, it is noteworthy that there are still no validated chemical markers for the species, and this field of research remains open.

PERSPECTIVAS PARA TRABALHOS FUTUROS

O presente trabalho contribuiu para uma maior elucidação dos compostos químicos da Bauhinia forficata Link pela avaliação das suas frações volátil e não volátil, as quais têm relação direta com as propriedades biológicas da planta, aqui determinadas in vitro. Foi possível avaliar amostras comerciais disponibilizadas no mercado local, bem como amostras botanicamente identificadas, coletadas em períodos distintos. Entretanto, percebe-se que ainda existem algumas lacunas a serem preenchidas e, desta forma, sugere-se como trabalhos futuros os seguintes tópicos:

- Avaliar o potencial antioxidante e a citotoxicidade do óleo essencial das folhas de *B. forficata*.
- Avaliar o perfil fitoquímico do extrato otimizado e das infusões de B. forficata por espectrometria de massas no modo positivo de ionização.
- Avaliar o potencial inibitório do extrato otimizado e das infusões *de B. forficata* frente a outras enzimas digestivas.
- Avaliar a bioacessibilidade dos compostos bioativos do extrato otimizado e das infusões de B. forficata por meio de modelo gastrointestinal *in vitro*.
- Avaliar o efeito da ingestão das infusões de *B. forficata* nos parâmetros bioquímicos de animais induzidos à diabetes e/ou à dislipidemia.

PERSPECTIVES FOR FUTURE WORK

The present work contributed to a better elucidation of the chemical compounds of *Bauhinia forficata* Link by evaluating its volatile and non-volatile fractions, which are directly related to the biological properties of the plant, here determined in vitro. It was possible to evaluate commercial samples available in the local market, as well as botanically identified samples, collected in different periods. However, it is clear that there are still some gaps to be filled and, therefore, the following topics are suggested as future works:

- Evaluate the antioxidant potential and cytotoxicity of the essential oil from the leaves of B. forficata.
- Evaluate the phytochemical profile of the optimized extract and infusions of *B. forficata* by mass spectrometry in the positive ionization mode.
- Evaluate the inhibitory potential of the optimized extract and infusions of *B. forficata* against other digestive enzymes.
- Evaluate the bioaccessibility of the bioactive compounds of the optimized extract and of the infusions of *B. forficata* through an in vitro gastrointestinal model.
- Evaluate the effect of ingestion of *B. forficata* infusions on the biochemical parameters of animals induced to diabetes and/or dyslipidemia.

OTHER PUBLICATIONS DURING THE DEVELOPMENT OF THIS THESIS

https://doi.org/10.3390/molecules27020410. Leilson de Oliveira Ribeiro, Beatriz Pereira de Freitas, Caroline Margot Albanez Lorentino, Heloisa Freire Frota, André Luis Souza dos Santos, Davyson de Lima Moreira, Bruno Sérgio do Amaral, **Eliane Przytyk Jung**, Claudete Norie Kunigami. Umbu Fruit Peel as Source of Antioxidant, Antimicrobial and α-Amylase Inhibitor Compounds. **Molecules**, 2022.

https://doi.org/10.1007/s12649-020-01122-1. Leilson de Oliveira Ribeiro, Suely Pereira Freitas, Virginia Martins da Matta, **Eliane Przytyk Jung**, Claudete Norie Kunigami. Microencapsulation of the Extract from Euterpe edulis Co-product: An Alternative to Add Value to Fruit Agro-Chain. **Waste and Biomass Valorization**, 2020.

https://doi.org/10.1111/jfpp.14719. Leilson de Oliveira Ribeiro, Gabriele Fernanda Conrado Thomaz, Matheus Oliveira de Brito, Natália Guimarães de Figueiredo, **Eliane Przytyk Jung**, Claudete Norie Kunigami. Siriguela peels provide antioxidant compounds-rich extract by solid-liquid extraction. **Journal of Food Processing and Preservation**, 2020.

http://dx.doi.org/10.21577/1984-6835.20190120. **Eliane Przytyk Jung**, Leilson de Oliveira Ribeiro, Claudete Norie Kunigami, Fernanda de Souza Nascimento, Elizabeth da Silva Figueiredo. Ripe Banana Peel Flour: A Raw Material for the Food Industry. **Revista Virtual de Química**, 2019.

APPENDIX

Published article.

Journal of Applied Research on Medicinal and Aromatic Plants 22 (2021) 100303

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Short communication

Thermal-assisted recovery of antioxidant compounds from *Bauhinia forficata* leaves: Effect of operational conditions

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ARTICLE INFO

Keywords:
Pata-de-vaca
Brazilian orchid tree
Phenolic compound
Flavonoid
Antioxidant capacity
Optimization

ABSTRACT

Herein, the extraction of antioxidant compounds from Brazilian orchid tree leaves (*Bauhinia forficata* Link), also known as "pata-de-vaca" or "pezuña-de-vaca," is optimized using thermal-assisted solid-liquid extraction. The combination of operational conditions including the temperature (32–74 °C), ethanol percentage (13–97 %), and solid/liquid ratio of leaves and ethanol (1:10–1:60; w/v) was employed using a rotational central composite design. The extracts were evaluated for total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant capacities by the radical monocation reduction of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), free radical scavenging activity of 2,2′-diphenyl-β-picrylhydrazyl radical ($DPPH^{\bullet}$), and ferric reducing/antioxidant power (FRAP). The phenolic profile of the optimized extract was also obtained using ultra-performance liquid chromatography-quadrupole/time-of-flight mass spectrometry. The statistically evaluated results revealed that the optimal operational conditions for the recovery of antioxidant compounds from plant

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April 14, 2022

Dr.
Eliane Przytyk Jung
Instituto Nacional de Tecnologia
Rio de Janeiro
Brazil

Dear Dr. Jung:

His article entitled: **"Bauhinia Linnaeus genus: an overview of the chemistry and bioactivity of essential oils"** (BLACPMA number 2087) by the authors **Eliane Przytyk Jung, Raphael Cruz, Leilson de Oliveira Ribeiro & Ricardo Felipe Alves Moreira,** received on September 24, 2021. It has been **accepted** on April 14, 2022 for publication in BLACPMA as a Review.

Submitted article

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Assunto: Food Science and Technology - Manuscript ID CTA-2022-0341

11-Apr-2022

Dear Mr. Ribeiro:

Your manuscript entitled "Chemical profile of the volatile fraction of Bauhinia forficata leaves: an evaluation of commercial and in natura samples" has been successfully submitted online and is presently being given full consideration for publication in the Food Science and Technology.

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