UNIVERSIDADE ESTADUAL DE FEIRA DE SANTANA
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ANA EDILÉIA BARBOSA PEREIRA LEAL

CONTROLE DE QUALIDADE DA DROGA VEGETAL E AVALIAÇÃO DA ATIVIDADE HIPOLIPIDÊMICA *IN VIVO* DO EXTRATO AQUOSO DOS FRUTOS DE *Passiflora cincinnata* MAST. (PASSIFLORACEAE)

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Orientador: Prof. Dr. Jackson Roberto Guedes da Silva Almeida

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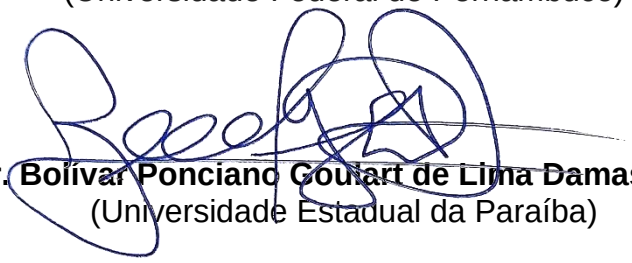

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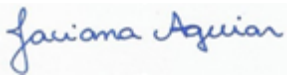

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“Nas grandes batalhas da vida, o primeiro passo para a vitória é o desejo de vencer”.

Mahatma Gandhi

RESUMO

O presente estudo foi dividido em dois capítulos. O primeiro capítulo propôs investigar patentes relacionadas a atividades biológicas do gênero *Passiflora*, onde foi observado o grande potencial de várias partes de diferentes espécies, para a obtenção de fitoprodutos. O segundo capítulo propôs obter extratos de cascas dos frutos de *P. cincinnata* e avaliar sua atividade hipolipidêmica *in vivo*, como etapa importante para o desenvolvimento de fitoterápicos. Assim, foram realizados testes físico-químicos com a droga vegetal, conforme Farmacopeia Brasileira, análise de constituintes químicos presentes nos extratos, através de cromatografia líquida de alta eficiência acoplada a detector de arranjo de diodos (CLAE-DAD), assim como avaliação da toxicidade aguda, atividade hipolipemiante do extrato (administrado por via oral: 100 e 200 mg/kg), em camundongos hiperlipidêmicos induzidos, e investigação do possível mecanismo de ação, através de simulações de dinâmica molecular. Os ensaios físico-químicos apresentaram-se dentro dos padrões farmacopeicos. A presença de vitexina, orientina e isoorientina foi confirmada por CLAE-DAD. Nenhum sinal clínico de toxicidade foi observado nos animais avaliados. A análise hipolipidêmica apresentou uma redução nos níveis de colesterol total (CT) e aumento da lipoproteína de alta densidade (HDL-c), estatisticamente significativos ($p < 0,05$), quando comparados ao controle apenas induzido, enquanto os índices de triglicerídeos (TG) não mostraram uma resposta significativa. A análise de docking molecular mostrou que os compostos de um modo geral apresentaram estabilidade em praticamente toda a simulação, principalmente a vitexina, quando associado à enzima LCAT, demostrando que o extrato avaliado possui atividade hipolipemiante, possivelmente devido à presença dos compostos bioativos identificados.

Palavras-chave: *Passiflora*. *Passiflora cincinnata*. Flavonoides *C*-glicosídeos. Hiperlipidemia. Fitoterápicos.

ABSTRACT

The present study was divided into two chapters. The first chapter proposed to investigate patents related to biological activities of the genus *Passiflora*, where was observed the great potential of several parts of different species, to obtain phytoproducts. The second chapter proposed to obtain fruit peel extracts of *P. cincinnata* and to evaluate its hypolipidemic activity in vivo, as important steps for the development of herbal medicines. Thus, were performed physicochemical tests with the vegetable drug, according to Brazilian Pharmacopoeia, analysis of chemical constituents present in extracts, through high-performance liquid chromatography (HPLC-DAD), as well as, evaluation of acute toxicity, hypolipidemic activity of the extract (administered orally: 100 and 200 mg/kg), in induced hyperlipidemic mice, and investigation of the possible mechanism of action, through molecular dynamics simulations. The physicochemical assays were within the pharmacopoeial standards. The presence of the vitexin, orientin and isoorientin was confirmed by HPLC-DAD. No clinical signs of toxicity were observed in the evaluated animals. The hypolipidemic analysis presented a reduction in total cholesterol levels (TC) and an increase in high-density lipoprotein (HDL-c), statistically significant ($p < 0.05$), when compared to control only induced, while triglycerides indices (TG) did not show a significant response. The analysis of molecular docking showed that the compounds in general showed stability in practically the entire simulation, mainly the vitexin, when associated with the LCAT enzyme, demonstrating that the evaluated extract has lipid-lowering activity, possibly due to the presence of the identified bioactive compounds.

Keywords: *Passiflora*. *Passiflora cincinnata*. Flavonoids *C*-glycosides. Hyperlipidemia. Phytotherapic.

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1. INTRODUÇÃO

As dislipidemias são distúrbios metabólicos e endócrinos caracterizadas pelo metabolismo irregular no transporte de gorduras e níveis lipídicos anormais, podendo se apresentar como hiperlipidemia, através do aumento do colesterol total (CT), triglicerídeos (TG), ácidos graxos livres (AGL) e colesterol da lipoproteína de baixa densidade (LDL-c), associado a uma diminuição do colesterol da lipoproteína de alta densidade (HDL-c), de forma que esses parâmetros podem se apresentar de forma isolada ou associada, assim como, hipolipidemia, em condições de doenças hereditárias do metabolismo das lipoproteínas, apresentando hipocolesterolemia acentuada e níveis baixos ou ausentes de LDL, dependendo do gene envolvido, entre outros fatores. No entanto, por não ter expressão clínica, a hipolipidemia é raramente diagnosticada (HOOPER; BURNETT, 2013; GAO et al., 2020).

A hiperlipidemia pode ser primária, quando há uma predisposição genética, e secundária, como consequência de condições como obesidade, hipotireoidismo, síndrome nefrótica, doença hepática obstrutiva, insuficiência renal crônica, ou uso de medicamentos. Mesmo sendo diferenciadas, os fatores ambientais são sempre determinantes da expressão fenotípica. Essa condição patológica torna-se um dos grandes problemas de saúde no mundo, pois é um dos principais fatores de risco para o desenvolvimento de doenças cardiovasculares (DCVs), como doença cardíaca coronariana, derrame cerebral e aterosclerose, que é a principal causa de morte e incapacidade em muitos países desenvolvidos, e em desenvolvimento (CABEZAS et al., 2018; CHENXI et al., 2020; TENG et al., 2020).

De acordo com a World Health Organization (WHO, 2020), estima-se que 17,9 milhões de pessoas morreram por doenças cardiovasculares em 2016, representando 31% de todas as mortes em nível global. Desses óbitos, estima-se que 85% são causados por ataque cardíaco e acidente vascular cerebral (AVC). Em relação ao Brasil, as DCVs são responsáveis por 27,7% de mortes, atingindo 31,8% quando são excluídos os óbitos por causas externas (MASSA et al., 2019). Segundo a Sociedade Brasileira de Cardiologia (SBC, 2019), houve uma redução das taxas de mortalidade por doenças cardiovasculares, mas ainda há um aumento em morbidades, principalmente pelo envelhecimento e adoecimento da população, que é considerado o fator de maior impacto no custo das internações hospitalares no país.

Diversos estudos têm demonstrado que os fatores de risco cardiovascular nos distúrbios metabólicos podem ser modificados, através de mudanças no comportamento, incluindo intervenções alimentares e prática de exercício físico (PRADO; DANTAS, 2002; BEZERRA et al., 2013; MUNIZ et al., 2019; RAŠKOVIC´ et al., 2019; SAKAMURI et al.,

2020), e, caso necessário, por meio de uma terapia hipolipemiante, com o uso de estatinas, resinas de troca iônica, fibratos, derivados do ácido nicotínico, inibidores da pró-proteína convertase subtilisina/cexina tipo 9 (PCSK9), entre outros (PALMER, 2019; TADA et al., 2019; WAKE et al., 2019). Esses fármacos regulam o metabolismo lipídico por diferentes mecanismos, mas apesar de bem tolerados, podem apresentar diversos efeitos colaterais, como hiperuricemia, distúrbios gastrintestinais, rubor, função hepática anormal, resistência à insulina, aumento do tempo de protrombina, queixas musculares que variam de mialgias leves a moderadas, e rabdomiólise grave, podendo alcançar grande repercussão clínica e interrupção da administração pelos pacientes, devido aos sintomas apresentados (FORTI; DIAMENT, 2008; DURAIPANDIYAN et al., 2016; HUANG et al., 2019).

O uso de plantas medicinais pela população, para o tratamento de diferentes doenças agudas e crônicas, tem sido observado há muito tempo, e isso tem chamado a atenção de pesquisadores para o estudo de plantas medicinais nativas e seus princípios ativos, discutindo também os mecanismos de ação desses constituintes. Essas ervas são utilizadas como uma terapia alternativa na busca de maior atividade farmacológica, maior biocompatibilidade, baixa toxicidade e tratamento mais acessível à população (CASTRO et al., 2014; DUTRA et al., 2016).

Por ser considerado um dos países de maior biodiversidade do planeta, com cerca de 20% do número total de espécies vegetais do mundo, o Brasil apresenta um grande potencial para o desenvolvimento de novos fármacos advindos de fontes naturais. Morfina, codeína, atropina, cafeína, digoxina, são alguns exemplos de constituintes ativos isolados de plantas medicinais, identificados pela busca contínua de medicamentos derivados de plantas. Estima-se que cerca de 25% dos medicamentos terapêuticos são desenvolvidos a partir de produtos naturais bioativos, advindos principalmente de plantas e microrganismos. Em países em desenvolvimento, e em alguns países desenvolvidos, com uma longa tradição no uso de plantas medicinais, e onde existem diretrizes apropriadas para o registro de medicamentos, normalmente esses medicamentos à base de plantas são muito populares. Mesmo que já haja uma evolução significativa, parte desta biodiversidade necessita ainda ser investigada quanto às suas potencialidades terapêuticas (CALIXTO, 2000; CRAGG; NEWMAN, 2013; CASTRO et al., 2014; RIBEIRO et al., 2014; DUTRA et al., 2016).

A grande diversidade de espécies vegetais encontradas no Brasil são distribuídas em variadas formações de vegetação, entre elas o bioma Caatinga, que se encontra na maior parte da região Nordeste, e se destaca por sua alta diversidade de espécies nativas e espécies endêmicas, com ampla utilização para fins terapêuticos e para satisfazer as necessidades da

comunidade dessa região semiárida (AGRA et al., 2007; PEREIRA-JÚNIOR et al., 2014; COSTA et al., 2015; COSTA; MARINHO, 2016; SANTOS et al., 2018).

Dentre essa grande diversidade de vegetais da Caatinga, amplamente utilizada na medicina popular, e em outros fins, destaca-se a família Passifloraceae, que apresenta cerca de 20 gêneros e 650 espécies, sendo *Passiflora* o gênero mais representativo, com aproximadamente 600 espécies, e somente no Brasil, apresentando cerca de 144 espécies nativas e cultivadas (COSTA et al., 2015; WOSCH et al., 2015). Esse gênero foi inicialmente conhecido por granadilha, pois seu fruto se parecia com a *Punica granatum*, e, posteriormente, flor da paixão, pela representação da primeira espécie descoberta, atualmente *P. incarnata* L. (CERVI, 1997). As plantas são arbustos e ervas, alpinistas, apresentando gavinhas auxiliares, com folhas, às vezes simples ou composta, inteira, palmada e lobada, frutos unicelulares, indeiscente, sementes numerosas, e belas flores ornamentais, bissexuais ou unissexuais, regulares, que podem ser de cores vivas, e formam uma coroa notável de grande diversidade (DHAWAN; SHARMA, 2004).

Dentre as espécies, destaca-se *Passiflora cincinnata*, popularmente conhecida como “maracujá-do-mato” e “maracujá-da-Caatinga” (Figura 1), que é uma espécie nativa, bastante resistente à seca, e que apresenta potencial para uso pelas indústrias alimentícia e farmacêutica. É uma trepadeira, de distribuição ampla no Brasil, que apresenta frutos oblongos ou ovoides, mas com bastante diversidade quanto ao seu tamanho, formato e maturação, e que liberam aroma agradável. A coloração da casca do fruto é verde-palha, sem brilho, embora, algumas vezes, ela possa apresentar-se de cor amarelada, e consistência deformável. Suas flores destacam-se por apresentar uma coloração cor-de-rosa pálido à violeta, e a parte mais baixa, uma cor púrpura carregada, banda média azul-rosado e azul pálido (OLIVEIRA; RUGGIERO, 2005).

A espécie apresenta uma maior resistência ao transporte e manuseio, e maior durabilidade, em comparação com *P. edulis* f. *flavicarpa* (CERQUEIRA-SILVA et al., 2010; COSTA et al., 2020). No entanto ainda existem poucos estudos farmacológicos relativos à espécie, destacando-se atividades antibacteriana (SIEBRA et al., 2018), antioxidante (DAVID et al., 2007), antinociceptiva e anti-inflamatória (LAVOR et al., 2018).

Figura 1: Representação da espécie *Passiflora cincinnata*: flor (A) e fruto (B). Fonte: Autoria própria.

Diversos estudos com espécies de *Passiflora* identificaram a presença de constituintes químicos como flavonoides, incluindo apigenina, kaempferol, vitexina, isovitexina, orientina, quercetina, rutina, sendo os flavonoides *C*-glicosilados os mais frequentemente citados para as espécies (DHAWAN; SHARMA, 2004; CHAPARRO-ROJAS et al., 2014; WOSCH et al., 2017; LEAL et al., 2018), além de saponinas (REGINATTO et al., 2001), alcaloides β-carbolínicos (OGA et al., 1984), óleo essencial (BUCHBAUER; JIROVETZ, 1992) e glicosídeo cianogênico (SPENCER; SEIGLER, 1984). Esses constituintes lhes conferem importantes atividades farmacológicas, como antimicrobiana, antidiabética (SARAVANAN; PARIMELAZHAGAN, 2014), antinociceptiva, anti-inflamatória (SASIKALA et al., 2011), ansiolítica (AKHONDZADEH et al., 2001; GAZOLA et al., 2018), cicatrizante (GONÇALVES-FILHO et al., 2006), antitussígena (DHAWAN et al., 2002), anti-hipertensiva (ICHIMURA et al., 2014), citotóxica (SILVA et al., 2020), hipolipemiante (CHAU; HUANG, 2005) e atividade sobre o sistema nervoso central (SNC) (GOSMANN et al., 2011).

A presença de flavonoides em diversas plantas tem atribuído a elas um potencial antioxidante, com inibição da peroxidação lipídica e maior atividade de enzimas antioxidantes, como catalase e superóxido dismutase, e redução do risco de doenças cardiovasculares. Estes compostos bioativos possuem capacidade de sequestrar radicais livres em organismos vivos, regular a dislipidemia e metabolismo de lipídio hepático, além de outras atividades (ANILA; VIJAYALAKSHMI, 2002; TALCOTT et al., 2003; ASSINI et al., 2013; MAHMOUD et al., 2019).

Alguns estudos mostram que vitexina e orientina reduzem o acúmulo de gotículas de gordura, triglicérides e os níveis de expressão da proteína PPARy (receptor ativador por

proliferador de peroxissoma), que modula os genes envolvidos no metabolismo dos lipídeos, relacionados com a hidrólise dos TGs (lipase lipoproteica e apolipoproteína CIII), degradação e síntese de ácidos graxos, e HDL (KIM et al., 2010; KOPF, et al., 2014). Isso foi sugerido também em ensaio com extrato de *Ficus carica*, enriquecido com vitexina, em que houve efeito hipocolesterolêmico em ratos hiperlipidêmicos induzidos (BELGUITH-HADRICHE et al., 2016).

Existem diversas pesquisas com maracujazeiros, relacionando suas espécies, principalmente *P. edulis*, com a redução nos níveis lipídicos (RAMOS et al., 2007; JANEBRO et al., 2008; TEIXEIRA et al., 2014). No entanto, a espécie *P. cincinnata*, que apresenta potencial agronômico e de ocorrência espontânea na região semiárida do Nordeste brasileiro, não tem recebido atenção da pesquisa nessa área (ARAÚJO et al., 2008).

O emprego de plantas medicinais já faz parte da cultura popular, embora, nas últimas décadas, o interesse pela fitoterapia aumentou consideravelmente pelos usuários, pesquisadores e serviços de saúde. A Organização Mundial da Saúde (OMS) reconhece que 80% da população dos países em desenvolvimento já utilizam práticas tradicionais nos seus cuidados básicos de saúde, e 85% usam plantas ou preparações destas, expressando a sua posição quanto à necessidade da valorização e utilização de plantas medicinais no âmbito sanitário, e na atenção básica à saúde (ROSA; CÂMARA, 2011).

Muitas preparações que utilizam plantas medicinais ainda necessitam de estudos científicos mais detalhados, incluindo padronização química, testes biológicos *in vitro* e em modelos animais, e avaliação clínica que, para esta etapa, o controle de qualidade já validado passa a ser uma prática totalmente indispensável (SOUZA-MOREIRA, 2010). E para a obtenção do registro como fitomedicamento, ainda há a necessidade de comprovação da eficácia terapêutica, da qualidade, tanto da matéria-prima utilizada quanto do produto final, e estudos de toxicidade que definam o grau de risco do produto (KLEIN et al., 2009).

Os fitoterápicos são definidos pela Agência Nacional de Vigilância Sanitária (ANVISA) como medicamentos produzidos exclusivamente com matérias-primas ativas vegetais. Estes devem apresentar segurança, eficácia, qualidade constante e ser reprodutível, sendo necessário para o seu registro a realização de testes que visam garantir a integridade e pureza da droga vegetal, como análise de solventes e excipientes utilizados na extração do derivado, relação aproximada droga vegetal:derivado vegetal para extratos secos, a determinação de água, solubilidade e densidade aparente, além da análise quantitativa de marcadores ou controles biológicos, e perfil cromatográfico (BRASIL, 2014).

Fatores como sazonalidade, temperatura, disponibilidade hídrica, condições extrativas, propriedades dos diversos solventes, variadas técnicas de extração, têm influência na produção e obtenção de princípios ativos pelo vegetal. Assim, para a produção de medicamentos fitoterápicos, a preparação do insumo farmacêutico ativo constitui uma etapa crítica, de forma que o controle desses fatores na produção das drogas e derivados vegetais, torna-se, então, imprescindível para a qualidade e valor terapêutico do produto fitoterápico (GOBBO-NETO; LOPES, 2007; OLIVEIRA, 2015; LI et al., 2020).

Considerando o alto índice de mortalidade por aterosclerose e doenças cardiovasculares, fármacos hipolipidêmicos que podem proporcionar o surgimento de novas patologias, através de seus efeitos tóxicos e adversos, a importância das etapas de padronização sobre o controle da droga vegetal e o desenvolvimento de fitoterápicos, este estudo torna-se relevante por caracterizar e obter extratos de cascas de *P. cincinnata* Mast., e avaliar sua atividade farmacológica, no que concerne a melhora na hiperlipidemia, através de estudos pré-clínicos. Busca-se também agregar valor ao produto, até então considerado como resíduo industrial, através da obtenção de um protótipo de fitoterápico.

Diante do que foi exposto, o capítulo 1 refere-se ao artigo de revisão, que teve a finalidade de mapear o desenvolvimento tecnológico, identificando inovações e produtos relevantes relacionados às espécies de *Passiflora*, especialmente as que demonstram atividades farmacológicas, pré-clínicas e clínicas, observando principalmente atividade hipolipidêmica e o potencial tecnológico de *P. cincinnata*.

No capítulo 2 foram realizadas as etapas de análise físico-química da droga vegetal, identificação e quantificação de compostos ativos presentes nas cascas de frutas de *P. cincinnata*, que podem servir como parâmetro para o controle de qualidade de futuros fitoterápicos, além da análise de toxicidade e avaliação da atividade hipolipidêmica da espécie, e possível mecanismo de ação envolvido, analisado por dinâmicas de docking molecular.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Mapear o desenvolvimento tecnológico relacionado às atividades biológicas com o gênero *Passiflora*, analisando, especialmente, o potencial tecnológico de *P. cincinnata*, e obter matéria-prima vegetal, relacionada a casca do fruto dessa espécie, avaliando seu potencial hipolipidêmico, em camundongos hiperlipidêmicos induzidos, e possível mecanismo de ação envolvido.

2.2 OBJETIVOS ESPECÍFICOS

- ✓ Realizar busca de patentes relacionadas às atividades biológicas de espécies de *Passiflora*, principalmente as que demonstrem ensaios *in vivo*, pré-clínicos ou clínicos.
- ✓ Realizar caracterização físico-química da droga vegetal, segundo parâmetros da Farmacopeia Brasileira;
- ✓ Identificar e quantificar marcadores químicos nos extratos;
- ✓ Analisar o perfil toxicológico, mediante possíveis efeitos adversos observáveis, nos animais tratados com o extrato das cascas dos frutos de *P. cincinnata*;
- ✓ Avaliar a atividade hipolipemiante do extrato da casca do fruto de *P. cincinnata*, em animais hiperlipidêmicos induzidos;
- ✓ Investigar o possível mecanismo de ação envolvido na atividade hipolipidêmica, através de simulações de dinâmica molecular.

CAPÍTULO 1

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Pharmacological activities of the genus *Passiflora* (Passifloraceae): a patent review

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Abstract

Background: *Passiflora* L. is a genus belonging to the Passifloraceae family, with many species widely used in folk medicine and various pharmacological activities described in the scientific literature, being a major target for the development of new therapeutic products. Studies several have identified several bioactive compounds in their composition as responsible for these activities, mainly *C*-glycosides flavonoids.

Objective: The aim of this study was carrying out a review of patents related to the genus and its application in several pharmacological activities, important for the development of new drugs and formulations.

Method: The search was carried out in 5 specialized databases, INPI, EPO, WIPO, Latipat and Derwent, using the term '*Passiflora*' combined with 'A61K and A61P', subclasses of the section A, of the International Patent Classification (IPC), that are destined at medical, dental or hygienic purposes, and therapeutic activity of chemical compounds or medicinal preparation, respectively.

Results: 1198 patents, citing the genus in the title or abstract, have been found, being 508 duplicates. After exclusion and inclusion criteria, 23 patents, written in english, portuguese and spanish, were selected, which demonstrated biological assays *in vivo*, with species of *Passiflora* as the only active constituent or incorporated in formulations with other compounds.

Conclusion: The findings of this search show growing interest in research areas and industrial, in pharmaceutical development with species of *Passiflora*, and that the different bioactive compounds present in the genus can be considered an important tool for the development of new effective and safe products with pharmacological potential.

Keywords: Bioactive compounds, Flavonoids *C*-glycosides, Medicinal plants, *Passiflora*, Patents, Pharmacological activity.

1. INTRODUCTION

Medicinal plants are able to synthesize various bioactive compounds, acting as a large stock for pharmaceutical products. Drugs derived from these plants are widely used in traditional medicines for the treatment of various diseases [1, 2].

According to World Health Organization (WHO) [3], traditional medicines include herbal medicines composed of herbs, herbal materials, herbal preparations and finished herbal products, which contain plant parts as active ingredients, or other plant materials, or combinations thereof. Most of the population living in developing countries, 70-95%, depend mainly on medicinal plants for their basic health needs.

The interest in medicinal products originating from higher plants has increased considerably worldwide, especially in developed countries such as some European countries and the United States. It is assumed that about 30% of the available therapeutic drugs are derived from natural sources, mainly of plants and microorganisms. In some therapeutic areas, such as oncology the amount of drugs derived from plants reaches 60% [4].

It stands out among medicinal plants, the genus *Passiflora* L., popularly known as passion fruit, than comprises about 20 genus and 650 species, including vines, trees and shrubs, being the largest and most diversified of the family Passifloraceae. Species of this genus are distributed in tropical and subtropical regions, being Brazil an important center of diversity, with more than 150 native species found mainly in the North and South. Important to reinforce that the large number of species and the same common name may increase the probability of errors in species identification, or even adulteration of herbal medicines [5,6,7].

Passiflora incarnata L. is the main species of North America and Europe, while in South America several other *Passiflora* species are widely distributed. A lot of these species, such as *P. edulis* Sims var. *edulis*, *P. edulis* Sims var. *flavicarpa*, *Passiflora tripartita* var. *mollissima* (Kunth) Holm-Niels. & P.M.Jørg., among others, are cultivated for having edible fruits, used, for example, in preparing juices [8], being *Passiflora edulis* the most widely cultivated in the tropics [9].

The use of *Passiflora* species as medicinal plants began in Europe, in century XVII, due to its soothing property [6]. Most of the pharmacological properties have been related to the depressant effects of the central nervous system (CNS) [9]. Vitexin, luteolin, apigenin, among others flavones, are the main chemical constituents responsible for the most reported activity for these species, the "benzodiazepine-like" anxiolytic activity. Some species have

also demonstrated results related to anti-inflammatory and antinociceptive activity [10], anxiogenic and anticonvulsants [11], hypoglycemic [12], sedative [13], antimicrobial [14], photoprotective [15], cytotoxic [16], for obesity [17], gastrointestinal anti-inflammatory activity [18], gastroprotective [19], antioxidant [20], hypolipidemic [21] among others.

The chemical composition of the species shows the presence of several substances, such as alkaloids, phenols, cyanogenic acid, saponin, and *C*-glycosides flavonoids, which are mentioned as their main constituents [10], and, because of that, several species of *Passiflora* have been widely used in the traditional system of therapeutics in many countries.

In addition to other areas of the industry, *Passiflora* has a long history as a medicine, mainly due to its sedative and anxiolytic properties [22]. Several pharmacological research with different species and parts of the genus already been carried out and demonstrated in original research articles [23-26] and review [27-30], becoming relevant the search for data related to biotechnological applications, with the purpose of analyze the tendencies of patenting the genus in therapeutic areas, and strategies for the various pharmacological activities, correlating also advances in technological development and obtaining products from these patents, to this large number of scientific researches.

In this context, this work had the objective of presenting several pharmacological activities related to *Passiflora* species, which have been patented in recent years, and that have been mainly demonstrated in *in vivo* trials, clinical or preclinical.

2. METHODS

In this review, the specialized databases, such the Instituto Nacional de Propriedade Industrial (INPI, Brazil), European Patent Office (EPO), World Intellectual Property Organization (WIPO), Latin American Patent Bank (Latipat) and Derwent Innovations Index (Derwent), were used for patent research, from July to September 2021. In the face of several studies with *Passiflora* species, patent screening took into account the International Patent Classification (IPC), section A, which is related to human needs, using in the title or abstract the term "*Passiflora*, A61K and A61P", which are two subclasses of this section, directed to preparations for medical, dental or hygienic purposes and therapeutic activity of chemical compounds or medicinal preparations, respectively. The search was carried out without limit of the date in which the patents were deposited or published. Based on this search strategy, 1198 patents were found, of which 508 were duplications. The inclusion criteria used were:

both preclinical and clinical studies that investigated and demonstrated biological activities, with the most diverse species of *Passiflora*, written in english, portuguese and spanish. Thus, 23 patents were selected for our critical analysis according to the objective of the study (Fig. **1**).

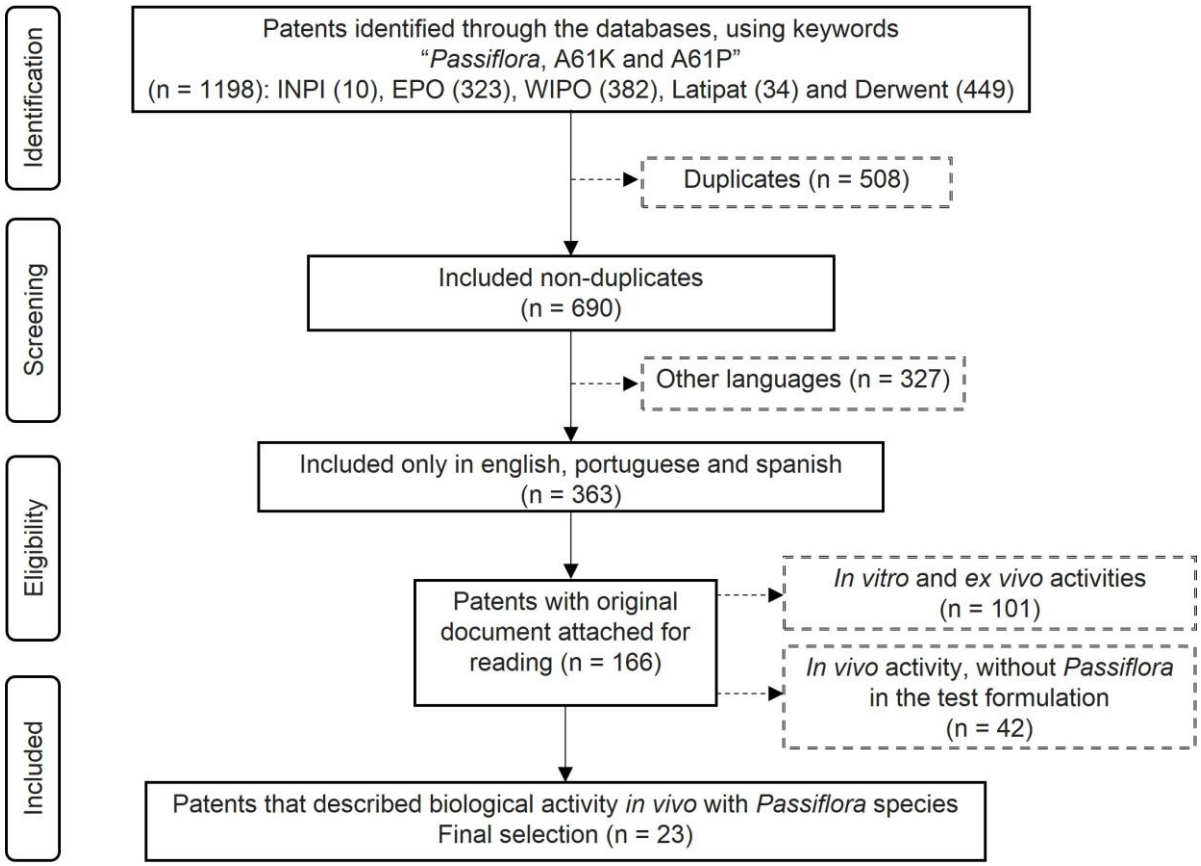

Fig. (1). Flowchart of studies included.

During the survey, patents were analyzed by year of publication, demonstrating that the first patent registration was filed in 1969, with publication in 1970, and, in the last 51 years, the year 2016 was the one that presented the largest number of publications, totaling 109 records, followed by 86 publications in the year 2020. The annual evolution of published patents with pharmacological activities of *Passiflora* is shown in Fig. **2**.

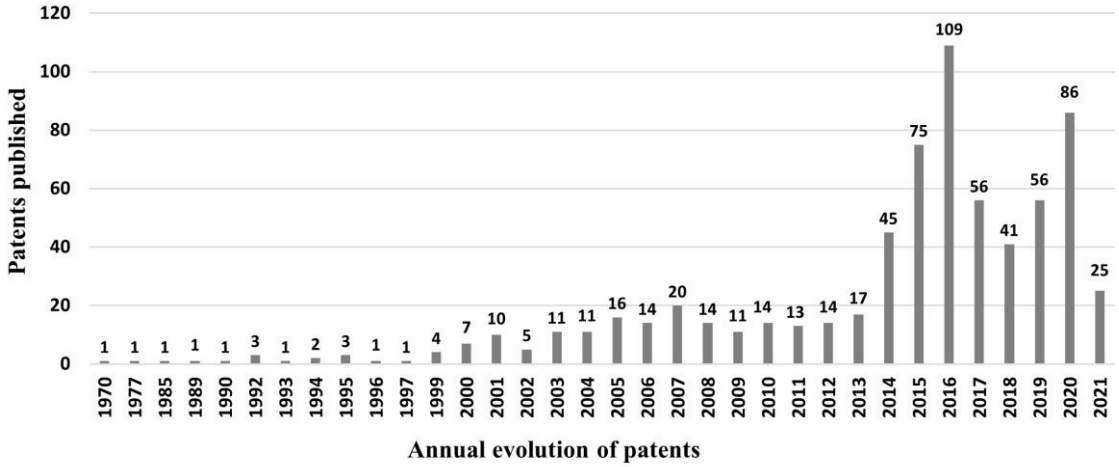

Fig. (2). Annual evolution of published patents on pharmacological properties with *Passiflora*, according to the keywords in the databases.

Regarding the requesting countries, it was noted that China showed the highest number of patent registrations, with 314 publications, followed by Japan, with 70.

In the meanwhile, Brazil published only 40 patents, as shown in Fig. **3**, describing countries applying for patents with *Passiflora* species.

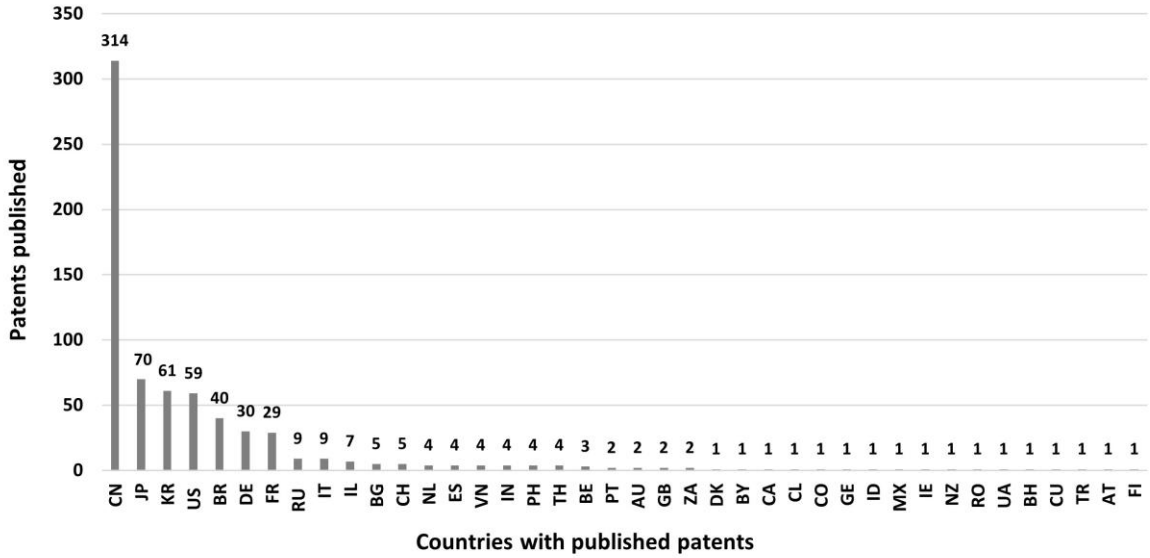

Fig. (3). Distribution of countries with patents published on pharmacological properties with *Passiflora*, according to the keywords in the databases.
(CN: China, JP: Japan, KR: Korea, US: United States, BR: Brazil, DE: Germany, FR: France, RU: Russia, IT: Italy, IL: Israel, BG: Bulgaria, CH: Switzerland, NL: Netherlands, ES: Spain, VN: Vietnam, IN: India, PH: Philippines, TH: Thailand, BE: Belgium, PT: Portugal, AU: Australia, GB: United Kingdom, ZA: South Africa, DK: Denmark, BY: Belarus, CA: Canada, CL: Chile, CO: Colombia, GE: Georgia, ID: Indonesia, MX: Mexico, IE: Ireland, NZ: New Zealand, RO: Romania, UA: Ukraine, BH: Bahrain, CU: Cuba, TR: Turkey, AT: Austria, FI: Finland).

Passiflora covers several wild species, being the majority of these, herbaceous, being able to present as shrubs and trees. Between Angiosperms, no other group show such a high leaf diversity, and its flowers show wide variation of size and color, with the crown and the perianth presenting diverse orientation and development [31]. Among the species of *Passiflora*, a total of 26 were researched and patented, for showing pharmacological activity, highlighting *P. incarnata* L., with 180 publications, followed by *P. edulis* and *P. wilsonii* Hemsl., with 173 and 89, respectively, including patents with association of different species of *Passiflora* in the same study. However, 83 publications did not specify the studied species, which, in a certain way, prevented a correct signaling of the greatest amount of species studied, as shown in Fig. **4**.

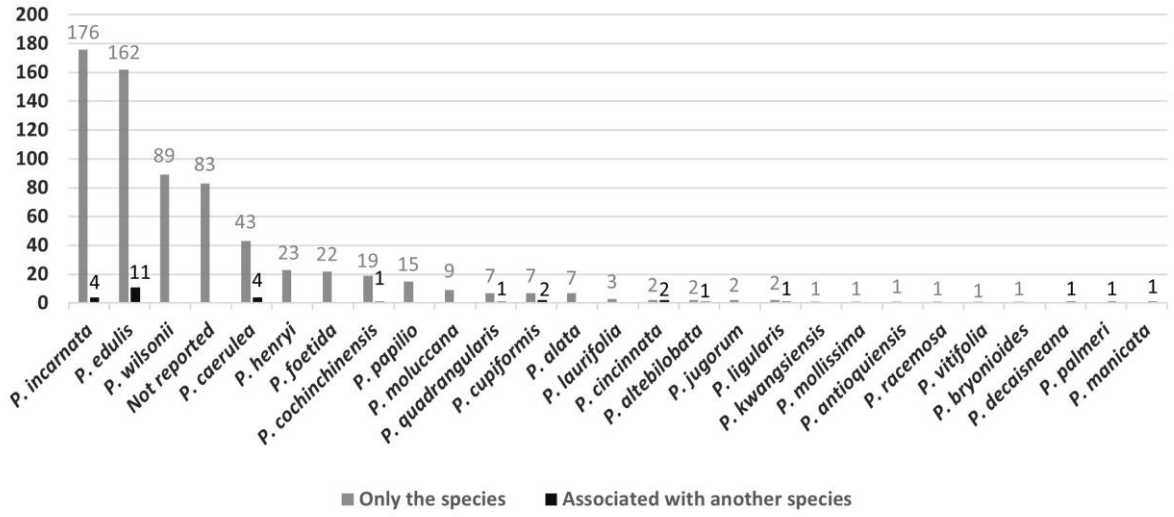

Fig. (4). Distribution of species of *Passiflora* in patents, according to the keywords in the databases.

3. PHARMACOLOGICAL ACTIVITIES WITH *PASSIFLORA*

Several published patents were observed related to the activities on the central nervous system (CNS), such as cognitive dysfunction and neurophysiological disorders, dermatological and cosmetic, treatment for alcohol and opioid abuse, through trials *in vitro*, *in vivo* and *ex vivo*, only with *Passiflora* species, or this combined with other vegetable species. Although, it stands out mainly, anxiolytic and depressant activities of the CNS, antimicrobial, as well as, anti-inflammatory and antinociceptive, also observed in several scientific studies.

3.1. Antianxiety and antidepressant activities

Depression is one of the most prevalent psychiatric disorders, affecting 25% of women and 12% of men throughout life. It is associated with a general disability and increased mortality [32]. Anxiety and depressive disorders are responsible for multiple somatic symptoms, such as gastritis, palpitation, tremor, insomnia or hypersomnia, significant weight loss or gain, as well as different psychosocial manifestations such as social withdrawal, depressed mood, suicidal ideation or attempted suicide [33], also presenting a greater significant impairment in the functioning of the work, psychosocial functioning and quality of life when compared to patients who do not suffer from these disorders [34]. These depressive disorders are commonly attributed to an imbalance in serotonergic neurotransmission [35].

In a study with *Passiflora* it was shown that 300 mg/kg, p.o. of *P. edulis* f. *edulis* (PEE) and, 100 and 300 mg/kg, p.o. of *P. edulis* f. *flavicarpa* O. Deg. (PEF), subpopulations of *P. eduli*s, reduced anxiety in the elevated plus maze test. As well as, 300 and 1000 mg/kg, p.o. of PEE, and 1000 mg/kg, p.o. of PEF, also induced antidepressant actions in the forced-swimming test. In this study, the administration of the extracts did not present alterations in the time of sleep [36].

In another study, it was observed that the six main flavonoid compounds isolated from the leaves of *P. edulis* "*flavicarpa*", lucenin-2, vicenin-2, isoorientin, isovitexin, luteolin-6-*C*-chinovoside, and luteolin-6-*C*-fucoside, were not detected in *P. edulis* "*edulis*", and that administration of 400 mg of each species, exhibited different activities, anxiolytic and sedative, respectively, concluding that species should be distinguished when pharmacological studies are carried out on them [37].

Researchers also demonstrated, in tests model for assessing antidepressant activity, as effects on the synaptic uptake of serotonin and forced-swimming-test, that the association of *P. incarnata* with another vegetal species, as extracts of *Hypericum perforatum* L., significantly increased the pharmacological potency of *H. perforatum*, on both models, suggesting that the antidepressant therapeutic effects of the species are only possible when combined with *P. incarnata*, in comparison, when used only mono-preparations of *H. perforatum*, demonstrating the importance of the species of *Passiflora* in the activity [35].

3.2. Antimicrobial activity

According to WHO, antimicrobial resistance is an increasingly serious threat to global public health. Infections caused by bacteria resistant to antibiotics are one of the major problems faced by medicine, because they require more complex and expensive treatments, and also are more difficult to diagnose and treat. Through WHO, encouraging and promoting the development and use of medicinal plant resources in the traditional system of medicine, had an increase, in the last decade, in the investigation of natural products as source of new bioactive molecules, important for antimicrobial activity and in the management of human infectious diseases. Although, natural plant products may also alter the effect of antibiotics, either increasing or decreasing antibiotic activity [38, 39].

In studies related to the antimicrobial activity and extracts of *Passiflora* species, evaluated the antimicrobial activity of hydroalcoholic extracts obtained from dry aerial parts (leaves, stem, bark, pulp, and seeds) of *P. cincinnata* Mast. [40], demonstrating that the extracts did not present antimicrobial activity of clinical relevance, showing a minimum inhibitory concentration (MIC) always greater or equal to 1024 μg/ml. Although, pulp and bark showed synergism with antibiotics, altering the *Pseudomonas aeruginosa* phenotype, Gram-negative bacteria, bacillary and aerobic, which was transformed from resistant to sensitive, when amikacin was used. Besides that, the extract of the pulp potentiated the effect of the beta-lactam antibiotics benzylpenicillin potassium and oxacillin, demonstrating that, the combined effect of the hydroalcoholic extracts of the bark and pulp of this species, and antibiotics, offers new therapeutic alternatives for the development of a pharmaceutical product based on multiple drugs.

Saravanan and Parimelazhagan [41] also investigated different extracts of the fruits of *P. ligularis* Juss., observing in their researches antibacterial activities against Gram (+) and Gram (-), and inhibition of fungal strains *Candida albicans* (14.85 mm) and *Aspergillus niger* (13.91 mm), in the disk diffusion method, indicating that the fruit pulp of *P. ligularis* may serve as a potential antimicrobial agent for the pharmaceutical industry.

3.3. Anti-inflammatory and antinociceptive activities

The inflammatory process is an immune defense mechanism, in response to various noxious stimuli, involving chemical and cellular infiltration of the affected areas. The response to these stimuli comprises several events, such as vasodilation, loss of function,

plasma extravasation, cell migration, edema, redness, heat, and release of several mediators, which is usually associated with pain [42, 43].

Studies have shown the effect of *Passiflora* species with anti-inflammatory and antinociceptive activity. Lavor *et al*. [10] observed that, in the hot plate test, the doses of 100, 200 and 400 mg/kg of the ethanolic extract of aerial parts (leaf and stem) of *P. cincinnata* increased reaction time, reducing painful behavior in mice, and that the antinociceptive mechanism probably involves central and peripheral pathways, comprising the path of opioid and muscarinic receptors, with influence of the potassium channels and the nitric oxide pathway. As well as, the extract inhibited the increase in edema volume after the administration of carrageenan and histamine. In the peritonitis test, acute pretreatment with the extract inhibited the leukocyte migration, with a reduction in the number of neutrophils, and total protein and nitric oxide, suggesting that the extract of aerial parts has antinociceptive, peripheral and central action, and is a potent inhibitor of the release of inflammatory process mediators.

Montanher *et al*. [44] also showed in their studies that 250 mg/kg of the lyophilized aqueous extract, obtained from leaves of *P. edulis*, inhibited leukocyte, neutrophils, myeloperoxidase, nitric oxide, TNF and IL-1, in carrageenan-induced pleurisy. The doses of 250-500 mg/kg also inhibited total and differential leukocytes, in pleurisy induced by bradykinin, histamine, or substance P, suggesting that several mechanisms, including inhibition of proinflammatory cytokines (TNF-α, IL-1), enzymes (myeloperoxidase) and mediators (bradykinin, histamine, substance P, nitric oxide), demonstrating to be responsible for the actions of *P. edulis*. As well as, Sasikala *et al*. [45] evaluated that 200 mg/kg of the ethanolic extract of leaves *P. foetida* L., exhibited analgesic activity in mice (13.50 ± 0.43 min), in hot plate method, in a reaction time of 20 min. And in the dose of 100 mg/kg, produced a highly significant anti-inflammatory effect (1.302 ± 0.079 ml), suggesting that this species also presents potential for analgesic and anti-inflammatory activities.

4. PATENTS RELATED TO PHARMACOLOGICAL ACTIVITIES WITH *PASSIFLORA* SPECIES

Several patents on pharmacological activities with *Passiflora* have been published, though only few in vivo assays, preclinical and clinical, were demonstrated. In Table **1**, are highlighted bioactivities, demonstrated *in vivo* assays, with different species of *Passiflora* as the only active constituent, or incorporated in formulations with other compounds.

Table **1**. Patents involving pharmacological activities with *Passiflora*.

Patent	Applicant	Inventor /year/ country	Compound	Indication/pharmacological profile	Route of administration	Dose/ concentration	Assay	Reference
US20150320815A1	Arabian German Medical Products Co WLL	Quadan F, 2017, BH	Composition with flower extracts of *P. incarnata* L., *Raphanus* sativus L., *Theobroma cacao* L. and *Crocus sativus* L.	Treatment of opioid and alcohol abuse	Oral	20 to 60 mg/kg**	Preclinical and clinical	[46]
WO2066041A1	Herbal Detox Therapy Aps; Bech SF	Bech SF, et al., 2002, DK	Formulation with extract of other species of plants and flower extract of *P. incarnata* L.	Treatment of opioid and alcohol abuse	Oral	40 mg/800 mg of the tablet*	Clinical	[47]
US2016271194A1	Campbell University	Jamerson BD; Breivogel CS, 2016, US	Flower aqueous extract of *P. incarnata* L.	Anti-smoking	Intraperitoneal	800 mg/kg*	Preclinical	[48]
US20070134189	Coty BV	Golz-Berner K, et al., 2007, DE	Composition of extracts of genus *Passiflora* L. (seed), *Papaver* L., *Myrtus* L. and *Mentha* L.	Dermatological/anti-wrinkles	Topical application	0.05 to 2% in weigh **	Clinical	[49]
US2009104174A1	Smith WP	Smith WP, 2009, US	Formulation with extract of other species of plants and *P. incarnata* L.	Treatment of anti-wrinkles and expression lines - Dermatological/cosmetic	Oral	50 mg/day**	Clinical	[50]

* Only extract of *Passiflora*; ** concentration or dose of extract of *Passiflora* in the formulation; *** Doses of the extract of *Passiflora* in different treatments; NR: Not reported.

(Continued)

Table **1**. (Continued).

Patent	Applicant	Inventor /year/ country	Compound	Indication/pharmacological profile	Route of administration	Dose/ concentration	Assay	Reference
BRPI0602106A	Aché Pharmaceutical Laboratories SA	Pianowski LF, 2008, BR	Hydroalcoholic extract of leaves of *P. incarnata* L.	Treatment of anxiety and insomnia	Oral	200 mg/kg*	Preclinical	[51]
WO2009047004A1	Farrington D; Farrington T	Farrington D; Farrington T, 2009, IE	Homeopathic complex, including extract of *P. incarnata* L.	Treatment for anxiety or anxiety-related symptoms or mental health, fear, phobias	Oral	2.07 %/ml; 2.07 to 6.67 v/v ***	Preclinical	[52]
WO2020162859	GMG Grand Medical Ilaclari Limited Sirket	Yusubov, N, 2020, TR	Formulation with actives of *P. incarnata* L., glycine, L-methyfolate Mg L-threonate	Treatment for somatoform disorders, anxiety or anxiety-related symptoms, migraine attacks	Oral	Formulations containing 125, 250, 500 and 1000 mg of *Passiflora* extract**	Preclinical	[53]
GB2381195A	Harvey CG	Harvey CG, 2003, GB	Formulation with plant extracts, including flowers of *P. bryonioides* Kunth	Anxiolytic activity/anti-stress	Topical application	NR	Clinical	[54]
WO2018132886A1	Carneiro RS; Morais VS	Carneiro RS; Morais VS, 2017, BR	Formulation with extract of *P. incarnata* L. and caffeine	Insomnia and stimulant	Oral	150 to 200 mg*	Clinical	[55]

* Only extract of *Passiflora*; ** concentration or dose of extract of *Passiflora* in the formulation; *** Doses of the extract of *Passiflora* in different treatments; NR: Not reported.

(Continued)

Table **1**. (Continued).

Patent	Applicant	Inventor /year/ country	Compound	Indication/pharmacological profile	Route of administration	Dose/ concentration	Assay	Reference
US2011268717A1	Hatipoglu B; Margrave R	Hatipoglu B; Margrave R, 2011, US	Composition of extracts of *P. caerulea* L., *P. incarnata* L., *P. edulis* Sims, or combination thereof	Neurological activity - invasive developmental disorder - symptoms associated with autism	Oral	10 to 400 mg*	Clinical	[56]
WO2007048356A1	International Health Center "La Pradera	Lorente RR, et al., 2007, CU	Formulation with extract of other species of plants and *P. incarnata* L.	Analgesic/migraines	Oral	1 to 20 mg in weigh (general dose: 1 tablet/day)**	Clinical	[57]
BRPI0816292	Natura Cosmetics S A; Univ Fed of Santa Catarina	Gesztesi JL et al., 2016, BR	Leaf aqueous extract of *P. alata* Curtis, enriched with vitexin-2 flavonoids "-*O*-rhamnoside	Anti-inflammatory and analgesic	Topical application	0.25%, 0.4%, 0.75% and 1%*	Clinical	[58]
US2007116664A1	Gonen S	Gonen S, 2007, US	Composition with extract of other species of plants and *P. incarnata* L.	Treatment of psoriasis and alopecia areata	Topical application	0.5-15%** in weigh	Clinical	[59]

* Only extract of *Passiflora*; ** concentration or dose of extract of *Passiflora* in the formulation; *** Doses of the extract of *Passiflora* in different treatments; NR: Not reported.

(Continued)

Table **1**. (Continued).

Patent	Applicant	Inventor /year/ country	Compound	Indication/pharmacological profile	Route of administration	Dose/ concentration	Assay	Reference
WO2005097153A2	Kemin Foods L. C. et al.	Foo LY, et al., 2005, NZ	Aqueous extract of the peel of the fruit of *P. edulis* Sims	Hypotensive, hepatotropic	Oral	10 and 50 mg/kg/day (preclinical), and up to 400 mg/day (clinical)*	Preclinical and clinical	[60]
BR102017027632A2	Federal University of São Francisco Valley	Almeida JRGS, et al., 2019, BR	Formulation with extract and flour of peel of fruit of *P. cincinnata* Mast.	Hypolipidemic	Oral	100 and 200 mg/kg, by test substance*	Preclinical	[61]
BR1020190185104	Federal University of Rio Grande do Norte	Bortolin RH, et al., 2021, BR	Formulation with extract of the pericarpo of *P. edulis* form flavicarpa degener.	Hypoglycemic activity	Oral	10% to 80% in formulation*	Preclinical	[62]
WO2019140479A1	Clever Health Pty Ltd	Healy CJ, 2019, AU	Formulation with extract of other species of plants and *P. incarnata* L.	Treatment of alcohol abuse	Oral	150 mg in composition/per tablet unit**	Clinical	[63]
US2019000902A1	Laboratoires Expanscience	Leclere BB, Bredif S, 2017, FR	Formulation with *P. edulis* Sims seeds	Antioxidant activity	Oral	NR	Clinical	[64]

* Only extract of *Passiflora*; ** concentration or dose of extract of *Passiflora* in the formulation; *** Doses of the extract of *Passiflora* in different treatments; NR: Not reported.

(Continued)

Table **1**. (Continued).

Patent	Applicant	Inventor /year/ country	Compound	Indication/pharmacological profile	Route of administration	Dose/ concentration	Assay	Reference
KR1020200001977	Soonchunhyang University Industry Academy Cooperation Foundation	Shin YS, et al., 2020, KR	Formulation with extract of flowers of *P. incarnata* L.	Amnesia and faille in memory	Oral	10 and 50 mg/kg*	Preclinical	[65]
NZ554762	Industrial Research Limited	Foo LY, 2008, US	Formulation with extract of peel of fruit of *P. edulis* Sims	Chronic asthma symptoms	Oral	150 mg/day (clinical)*	Clinical	[66]
US4942033	L'oreal	Aubert L; Anthoine P, 1985, FR	Formulation with extract of other species of plants and hydroalcoholic *P. incarnata* L.	Increasing capillary and venous resistance	Topical application	0.5% of *Passiflora* extract in formulation**	Preclinical	[67]
US2004166181A1	Shaklee Corporation	Hegenauer JC, et al., 2004, US	Formulation with extract of other species of plants and *P. incarnata* L.	Treatment for weight loss and inhibition the loss of lean body mass	Oral	0.4 ml (pulverization)**	Clinical	[68]

* Only extract of *Passiflora*; ** concentration or dose of extract of *Passiflora* in the formulation; *** Doses of the extract of *Passiflora* in different treatments; NR: Not reported.

The document deposited in 2015 [46] by Arabian German Medical Products Co W L L, refers to an activity for treatment of abuse and dependence on opioids and alcohol, or in the treatment of its symptoms and methods for its production. The invention is based on the combination of extracts obtained from aerial roots, seeds, or bulb of *Raphanus sativus* L. (2.5 parts), fruit of *Theobroma cacao* L. (3 parts), flower of *Crocus sativus* L. (1.5 parts), and flower of *P. incarnata* (2.5 parts). In the first test performed with the composition, after the administration of 40 mg/kg, orally (v.o), was observed a significant reduction in intraperitoneal self-administration (i.p) of morphine and heroin, showing good activity of the compounds at the opioid receptors. Increasing doses of the composition reduced the doses of morphine and heroin and stabilized the motor coordination of rats, according to the rotarod performance test, with the dose-dependent effect.

In the same study, the record of handling-induced convulsion (HIC) associated with ethanol abstinence, by hyperexcitability of the CNS, was also evaluated in rats. It was observed that, after administration of 20, 40 and 60 mg/kg of the composition, the higher doses significantly reduced the results of HIC, compared to animals treated with the vehicle. Although, 20 mg/kg showed no significant reduction. And, in a clinical trial for treatment of alcohol dependence with depressive disorder, patients received 40 mg/kg of composition, for 6 weeks, demonstrating a significant reduction of symptoms associated with alcohol abuse.

In another invention for treating symptoms of alcohol and drug detoxification, developed by Bech *et al*. [47], were produced tablets, presenting in its composition diverse species of vegetables, including 40 mg of flowers of *P. incarnata*, of a total of 800 mg, the unity. In the initial trial, made by men and women, it was proposed the mean intake of 76.5 tablets, on the first day of treatment, reducing to 16 units on the fifth day, as the symptoms were reducing. At the end of the fifth day of treatment, there was a reduction in the frequency of headache (from 29 to 18%), nausea (from 73 to 18%), gastric symptoms (from 47 to 4%), palpitations (from 69 to 9%) and sweating (from 93 to 33%). In general, patients described the composition as a better alternative to prescription drugs, due to the absence of drowsiness, euphoria, and other mood-altering effects.

Campbell University [48], in 2016, used the extract of *P. incarnata* to avoid withdrawal symptoms and desire during cessation of nicotine. In the analysis, wistar rats, males, received 0.4 mg/kg of nicotine, for 4 days, to observe nicotine sensitization, and were treated (i.p.) with 800 mg/kg of the aqueous extract of the flower of *P. incarnata*, to evaluate the time of habituation and the time for the peak effect of a dose of nicotine. The results

showed that the extract could antagonize the expression of motor sensitization by nicotine, indicating a decrease in signs of nicotine withdrawal.

Cosmetic and dermatological composition for anti-wrinkle treatment and expression lines, was patented by Golz-Berner et al., in 2007 [49]. The formulation consists of a silicone oil-W/S, with active components of extracts of the genus *Papaver* L., *Mentha* L., *Myrtus* L., and seeds of *Passiflora*, including *P. edulis*, *P. incarnata*, *P. laurifolia* L. and *P. quadrangularis* L., or mixtures of these species, presenting content of approximately, 0.05 to 2% of the total weight of the formulation. In the study, a clinical trial was performed with 21 individuals, men and women, with aged between 42 and 61 years, demonstrating that the combination of the four plant extracts achieved remarkable improvements in skin aging, in relation to fine-skin wrinkles. In another study, performed on 18 women with mixed dry skin for moisture testing, it was observed an increase of 41% of moisture with the complex of the invention, when compared to a cream without the composition of the extracts.

Smith, 2009 [50], patented a method to reduce the appearance of dynamic facial wrinkles, induced by stress, through the administration of extracts of plants with relaxing activities, combined or isolated, including the genus *Passiflora,* with a flavonoid and glycoside content of at least about 3% by weight of the extract. In the test, 25 individuals presenting stress received once a day, oral supplement in capsule form, composed by 150 mg of *Valeriana officinalis* L. and *Ziziphus zizyphus* (L.) H. Karst., 50 mg of *P. incarnata*, *Portulaca oleracea* L., *Ganoderma lucidum* (Fr.) Karst., *Solanum melongena* L., *Solanum lycopersicum* L. and *Mirciaria dubia* (Kunth) McVaugh, and 2 mg of *L*-treonin and adenosin. After three months of treatment, the individuals presented significant reductions in the lines and wrinkles evaluated, in addition to reducing stress levels, according to the survey.

Several studies have evaluated the effect of *Passiflora* species on central nervous system disorders, as observed in the patents that used the botanical extract for this pharmacological action.

Aché Pharmaceutical Laboratories S/A, in 2007, patented a standard powder of *P. incarnata* [51], which deals with a method of treatment for anxiety and insomnia, through the administration of a daily amount from 1 to 3000 mg of the product to the patient. The extract contains dextrin and 7% isovitexin, and is devoid of alkaloids, by means of the extractive process, performed at a constant temperature of 80 ºC, under stirring, for 3 h, of form that temperature and time provide the thermal degradation of a substantial part of the harmonic core alkaloids. Posteriorly, there was the cooling and removal of the alkaloids by ultrafiltration, for removal of the remaining alkaloids, obtaining a product free of harmful

substances, but maintaining its proper pharmacological action on anxiety and insomnia. In one study preclinical, 200 mg/kg the hydroalcoholic extract of leaves of *P. incarnata* was administered to mice, identifying that removal of alkaloids did not significantly affect anxiolytic activity. In an opposite way, the comparative study demonstrated that the presence or not of alkaloids provided the same therapeutic effect, with slight unexpected increase in the anxiolytic action of the product without alkaloids.

Patent with the same pharmacological activity and species was demonstrated by Farrington D. and Farrington T. [52], in 2009, through the invention of a homeopathic tincture, for the treatment of fear, phobias and symptoms related to anxiety or mental health disorders. Different treatments were tested in dogs suffering from symptoms of fear and anxiety, using fireworks or loud noise, to evaluate its effectiveness. Thus, after test, the reduction of fear and anxiety was observed, decreasing the need or dose of conventional medication.

Yusubov [53], in 2020, formulated a composition to assess the antidepressant effect, through a combined therapy with extract of *P. incarnata*, L-glycine, L-metilfolate and magnesium L-threonate, in different doses. The synergistic effect of this combination was demonstrated in a forced swim test, exhibiting anti-depressant activity, by decreasing immobility time, and the increase in swimming and climbing time, in mice treated with the formulations, when compared to the control group. Possibly, the presence of alkaloids and the effect on the GABAergic system, by the specie of *Passiflora*, can contribute to antidepressant activity.

Harvey, 2003, patented a formulation with the objective of reducing stress levels [54] through the use of an aqueous cream of topical use, composed of 5% of the orchid essence volume, 0.2% of essential oils and 7% of secondary floral essences, including flowers of *P. bryonioides* Kunth. In the test, the formulation was administered for one month, on the face of patients suffering from stress due to some disease, lifestyle, or for a temporary period of stress. After treatment, a reduction in stress level and improvement of mood was observed and reported by study participants.

Researchers also reported an invention, according to document WO2018132886-A1, published in 2018 [55], intended for individuals who present insomnia or need a calmer wake-up. The objective was to readjust the human biological clock, through the combination of one or more soothing substances, with one or more stimulating substances, characterized by being antagonistic active constituents that interact in specific biological functions and that are released at different times, absorbed in different ways and physically separated from each

other. For example, a capsule made up of *P. incarnata*, which is a soothing substance of quick release, absorbed by the stomach, and caffeine, as a late released substance, being absorbed in the intestine and promoting the awakening of the individual. In the clinical trial, carried out in three stages, with a duration of 21 days, each stage, including individuals without ingesting the formulation, placebo only, and the formulation, were analyzed several parameters, as hours of light sleep and total sleep, hours of REM sleep (rapid eye movement), agreed hours and how many times woke up, heart rate, and time that slept and what woke up. The results showed that, individuals who ingested the formulation obtained the desired initial effect (sedation), through the release of extract of *P. incarnata* in a short period of time, and, with the release of caffeine, after a pre-set time, increased the individual's overall metabolism and a more active awakening.

Compositions of extracts, including *P. incarnata*, *P. caerulea* L. and/or *P. edulis*, or combination thereof, with 80, 18 and 2% in composition, respectively, were patented by Hatipoglu and Margrave [56], in 2011, with the purpose of improving the neurological function in pervasive developmental disorder (PDD), such as autism, Asperger's disorder or Rett syndrome, restoring a balance between the inhibitory GABAergic pathways and excitatory glutamatergic pathways. In this study, patients with autism, aged between 4 and 22 years, were treated with a starting dose of 250 mg, twice a day, and for 12 weeks the patients were evaluated for baseline vineland adaptive behavior scale, routine metabolic profile, height, weight, blood pressure, heart rate, clinical global scale (CGI-S), social responsiveness scale, and aberrant behavior checklist, at the beginning and end of treatment, in addition to other additional analyzes. The results showed that the active supplementation with the extracts reduced the irritability score in 25% of patients, after 10 weeks of treatment. In another example of the same patent, patients between 5 and 8 years, with diagnosis of PDD, were treated with a formulation composed of 400 mg de purified extract of *P. incarnata*, 90 mg of *P. caerulea* and 10 mg of *P. edulis*, showing in their results, personality improvements, humor, self-control, empathy, and social functioning, reduced sensitivity to sound, better sleep patterns, decreased aggressiveness, best attention and eye contact, and reduced hyperactivity.

Lorente et al., 2007 [57], formulated a pharmaceutical composition to prevent and combat migraine, improving pain intensity indicators, the frequency at which crises occur and duration of migraine, as soon as the patient presents the crisis. The composition was obtained from fluid extracts of plant species, including *P. incarnata*, obtained starting from mixtures of distilled water and ethanol, with final alcoholic concentration between 30 and 70%. The

extracts from the formulation were analyzed for total phenol content, showing concentration between 1 and 5 mg. The composition in tablet form presented a concentration of *P. incarnata* between 1 and 20 mg in different treatments. Assays were performed with the administration of a tablet for three months, for men and women suffering from migraine, and, only women, who had migraine during the menstrual period. All the participants underwent medical examinations to classify the type of migraine and, after treatment, intensity, duration, and frequency of crises were evaluated. The results showed that the tablet, in most participants, reduced the frequency, duration, and intensity by more than 50%, and when these 3 parameters were combined, the improvement of the patient was 89%, on average, for only 3 weeks of treatment.

Gesztesi et al. developed a cosmetic formulation, with anti-inflammatory and analgesic activity, where *P. alata* Curtis leaf extract was incorporated, with a content of 16.46% of total flavonoids and 11.87% vitexin-2-*O*-rhamnoside, in different concentrations [58]. In one of the clinical trials, using protocols to evaluate the preventive and curative effect of skin irritations caused by the application of 10% lactic acid, a transient chemical irritation was caused in the nasolabial fold region, but characterized by a microinflammatory procedure, with initiation of signaling cascades for pain and irritation (erythema, redness), in volunteers, between 20 and 50 years old, who had a positive prick test in the nasolabial fold region. The formulations containing the extract in different concentrations, 0.25%, 0.4%, 0.75% e 1%, were compared to each other, with placebo and positive control (cortisone for dermatological use).

Using a pain scale for subjective assessment, the results showed that cosmetic formulations containing extracts of *P. alata*, at any of the tested concentrations, are effective in reducing the sensation of discomfort (pain, irritation, and itching), with its action not significantly different from that presented by the topical corticosteroid, demonstrating that the active ingredients present in the extracts are responsible for preventing and reducing irritation and pain.

Formulations with plant extracts, including *P. incarnata*, were searched by Gonen, 2007 [59], for treatment of psoriasis and alopecia areata, preventing, stopping or minimizing the loss of hair follicles. The formulation was composed of at least one phyto-corticosteroid, as the derivative of *Glycyrrhiza glabra* L. extract, in proportion 0.3%, *Zingiber officinalis* Roscoe (0.5-20%), *Foeniculum officinalis* All. (0.2-5%), *Salvia officinalis* L. (0.5-20%), *Lavandula angustifolia* Mill. (0.4-15%) and *P. incarnata* (0.5-15%). In one of the trials, the participants who suffered from various stages of baldness, received the composition, reporting

that hair loss was interrupted between 2 and 6 weeks, and there was capillary growth after four months of treatment. Participants with psoriasis also showed significant improvement in lesions. In another trial with participants suffering from alopecia areata, after treatment for a period of 6 months with the formulation, all subjects observed improvement in hair condition, paralysis of lost hair, growth, and thickening of new hair, confirming the effectiveness of the product.

Kemin Foods et al., in 2005 [60], suggested a product for the prevention and/or treatment of arterial hypertension, as well as, any other disease or disorder associated with elevated blood pressure, reduction of serum levels of nitric oxide, hepatoprotective and antioxidant activity, through the administration of the aqueous extract of the fruit peel of *P. edulis*. The analysis by high-performance liquid chromatography (HPLC) indicated in the peel extract several flavonoids, including quercetin, quercetin galactoside, quercetin glucoside, luteolin, luteolin glucoside, cyaniding-glucosides, catechin and epicatechin. In studies with rats, spontaneously hypertensive, the researchers have observed that the diets of these animals, supplemented with 50 mg/kg of the extract, reduced blood pressure compared to a control group. As, in rats fed with 10 and 50 mg/kg of the extract, the concentration of nitric oxide decreased in 40 and 65%, respectively, when compared to the group in which the extract was not administered. In the same study, in a randomized clinical trial performed with 30 hypertensive patients, between men and women, a reduction in systolic and diastolic blood pressure was observed at the individuals who received two doses of 2 mg/lb/day of the extract, and up to 400 mg/day for 4 weeks when compared to the group receiving a placebo.

Studies for lipid-lowering activity were developed by Almeida *et al*., 2019, using the ethanolic extract and the flour, obtained from peel of the fruit of *P. cincinnata* [61]. Through the LC-MS/MS technique, were identified in the extract, flavonoids such as vitexin, isovitexin, isoorientin-4'-*O*-glycoside, isoorientin and orientin, while in the flour, various antioxidant minerals, in addition to pectin. In a pre-clinical trial, a 15-day pre-treatment was performed with the test substances, at doses of 100 and 200 mg/kg, subsequent hyperlipidic induction with a single dose of 400 mg/kg of Triton WR-1339, and a last treatment similar to the initial one, observing a significant reduction in the total cholesterol, triglyceride, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol indexes, with significant increase in high density lipoprotein cholesterol, in two biochemical analyzes performed, 24 and 48 h after induction, with the two tested substances, when compared to the negative control group (treated with saline and subsequent hyperlipidic induction), positive control group (treated with fenofibrate and subsequent hyperlipidic induction), and the

healthy control group (treated with saline, without hyperlipidic induction). In the two biochemical analyzes performed, some parameters analyzed demonstrated that the test substances showed better results than the reference drug and healthy control group, suggesting a hypolipidemic and antioxidant potential of the extract and flour obtained from the species.

Studies to evaluate the treatment of diabetes mellitus, under clinical conditions of hypercholesterolemia and hypertriglyceridemia, were developed by Bortolin et al., 2021, using extract obtained from the pericarp of *P. edulis*, form *flavicarpa* Degener [62]. Compounds such as flavonoids, phenolic acids, saponins and terpenes were identified in the sample, using the thin layer chromatography technique (TLC). For the assessment of antidiabetic activity, initially an injection of streptozotocin (40 mg/kg) was administered in the animals, intrapenian, for induction of diabetes mellitus, verifying its presence by measuring blood glucose above 250 mg/dl, and through signs of polyphagia, polydpsia and polyuria. The results of the analysis showed the hypoglycemic activity of the extract, which was able to decrease blood glucose against experimental models of diabetes, induced by streptozotocin, highlighting, further, that the administered dose of the inducing substance was able to produce a total destruction of the pancreatic beta cells, followed by a severe resistance to insulin.

Other products developed, which include extracts of different species of *Passiflora* in its formulation, for treatment or prophylaxis of conditions associated with alcohol and drugs consumption and treatment of detoxification symptoms [63], antioxidant activity [64], amnesia and faille in memory [65], chronic asthma symptoms [66], capillary and venous insufficiency [67] and inhibition of muscle loss [68], were also evaluated in pre-clinical and/or clinical trials, as shown in Table **1**.

Some patents have also demonstrated important *in vitro* and *ex vivo* studies with different parts of species of *Passiflora*. The leaf extract of *P. incarnata* was incorporated in photoprotection formulation, topical use, carried out from nanoemulsions, microemulsions and micellar solutions [69], and a gelatine nanoparticle was also developed, comprising lyophilized dry extract of leaves and stem of *P. alata* (2 to 5%), and gelatin (95 to 98%), to be incorporated into pharmaceutical compositions, mainly formulations with antiulcer activity [70].

The seed extract of *P. edulis* was researched for prevention or treatment of disorders or diseases of the skin, use in combating oxidative stress, and for prevention and treatment of adipose tissue disorders [71], in the production of antibiotic with microbicidal action against several bacteria and fungi of clinical interest, as *Staphylococcus aureus*, in different

presentations, as a capsule, solution, syrup, tablets and gel, for human and veterinary use [72], as well as, researches identified the presence of high content of the phenolic compound piceatannol (0.01 to 50% by mass), for the purpose of incorporating it into a cosmetic, a drug, or as a functional food [73]. In seeds of another species, *P. incarnata*, was also evaluated the action on the modulation of gene expression and effect on gene expression in normal human fibroblasts [74].

Flavored capsules to decrease the absorption of lipids and carbohydrates, the basis of chitosan and flour of the peel of *P. edulis*, were also patented, suggestive of hypolipidemic and hypoglycemic activity [75].

Study for anti-wrinkle treatment and expression lines were developed by Leclere Jacques, 2008 [76], using the association of hydroglycerinated extract of aerial parts of *P. incarnata* and *Anchusa arvensis* (L.) M. Bieb. in ganglion cells removed from the dorsal root of rats. The extracts were evaluated against the skin sensitivity and relaxation, induced by neuropeptides, presenting excellent activity, with reduction of 25% in release of substance P, up to 23% in release of calcitonin gene-related protein (CGRP), up to 28% in the fixation of substance P on your receptor at the level of human keratinocytes in cultured, and up to 27% in acetylcholine release, compared to untreated control.

5. DISCUSSION

Generally, the present patent review involved different species of *Passiflora*, acting in diverse pharmacological activities as the only active constituent, or associated with formulations with other compounds, vegetables or not. There is a growing interest in this genus due to the commercial value of some species, for its edible fruits, such as juices and use in various food products [77], as well as by biological activities, due to the presence of secondary metabolites, such as flavonoids, phenols and, alkaloids [78], and others several phytochemicals identified in the species [9]. This is also demonstrated, as regards the use of some species in folk medicine, with proven therapeutic properties.

In Brazil, the fruit has great economic importance, being responsible for approximately 60% of its world production [79], being the yellow passion fruit, *Passiflora edulis* Sims f. *flavicarpa* Degener, the most important variety cultivated in the country for commercial purposes. The peel of the fruit, considered as a by-product, can contain numerous

valuable substances such as pigments, sugars, organic acids, flavorings, dietary fiber and other bioactive compounds with antioxidant and antimicrobial activities [80].

Plants play an important role in human civilization, as they have been used for various purposes in different fields such as medicine, nutraceuticals, perfumery, beverages, fragrances, cosmetics and dyeing industry. This is observed in the various patents published with species of *Passiflora*, suggestive for incorporation into cosmetic, pharmaceutical, and food formulations.

Most of the drug prescriptions in the world were mainly derived from herbal source, due to the presence of numerous phytochemicals. Plants develop different bioactive molecules, making them a rich source of various types of medicinal compounds [81]. Faced with this, Brazil is considered an enormous potential for the development of new drugs, because they hold much of the world's biodiversity, learning about 24% of biodiversity, which comprises more than 45,000 species of higher plants of the total existing in the planet [4]. The country also stands out in the number of patents published with species of *Passiflora*, when compared to other countries. Although, it shows an unsatisfactory number, when studies show Brazil with the largest number of scientific publications related to anxiolytic and depressive activity, one of the most researched actions with the different species [82].

It is also observed that, even with the presence of a large variety of phytochemicals in this genus, there are only a few reports on pharmacological research, being the majority related to CNS depressant effects, still needing more scientific studies with this genre, with toxicity tests, pharmacological tests *in vivo*, and possible investigation of the mechanism of action, for better scientific evidence of its safety and efficacy.

Researchers have also increasingly sought the development of new drugs, through of constituents of natural origin, mainly of vegetable origin, committing, however, with the sustainable development and rational exploitation of brazilian biodiversity. The data demonstrated reinforce the importance of the use of composition based on plant extracts, especially of the genus *Passiflora*, by various pharmacological actions observed in patents that showed the potential of the species, demonstrating that a combination of different bioactive compounds is a good defense strategy against several diseases.

It is observed in the published documents that notable advances were obtained with the development of a variety of formulations based on different species of *Passiflora*, in different presentations, such as capsule, solution, syrup, tablets, gel, aerosols, cream, powder, paste, ointment, among others, and that this is a vast field of research with great prospects, which can contribute with different and innovative approaches, for various pharmacological

treatments, favoring the development of new products more effective and safe, compared to formulations already in use by the population.

Although, few patents have demonstrated pharmacological assays, *in vivo*, and confirmed the active principle responsible for pharmacological action, this review found preclinical and clinical studies, which tested a variety of formulations under various pharmacological conditions, presenting, at times, better responses than the drug used as reference. Suggesting so, the potential of the genus, and a possible reduction of conventional medicines, with consequent reduction of the side effects.

This study has as one of its strengths the provision of an overview of the growth trends in a given area or product of interest to the researched genus. As well as, several scientific studies do not demonstrate all the technological information, for the purpose of protecting your inventions, not providing, thus, better understanding of the tests performed, what is best observed in a patent.

Regarding the limitations of this review, some authors decide for the initial publication in scientific articles, so that different forms of treatments, for different pathologies, and are not immediately patented, and are not clearly detailed. As well as, the identification and inclusion of patents can only occur after the 18-month confidentiality period that patent offices grant inventors, limiting, thus, the expression of new data important for research, in the surveyed period.

In this study, the number of patents observed without pharmacological assay, shows that it is still a field of research to be explored, aiming at the development of new therapeutic products based on *Passiflora*, for better prognosis of certain diseases and providing, thus, a better quality of life to patients. In this way, there is a need for the industry of pharmaceuticals, cosmetics or nutraceuticals products, to continue following the expressive and surprising discoveries by researchers around the world, for better pharmacological applicability of the species.

CONCLUSION

In conclusion, we can observe the great potential of *Passiflora* species for obtaining phytoproducts, with extracts of the various species and parts of *Passiflora* presenting different biological activities, possibly due to the presence of chemical constituents such as flavonoids *C*-glycosides. Despite the large number of scientific publications, the number of products

obtained with the genus is still small, needing that this gap needs to be filled. For this, more scientific studies are needed in order to determine the active constituents and the possible mechanism of action responsible for these biological activities, for better technological applicability.

CONSENT FOR PUBLICATION

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CONFLIT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CAPÍTULO 2

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Physicochemical, phytochemical characterization, and assessment of hypolipidemic activity of *Passiflora cincinnata* Mast. in hyperlipidemia induced by Triton WR-1339

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Abstract

Passiflora cincinnata Mast. is a native species from the Caatinga biome, popularly used for several therapeutic purposes in folk medicine, although there are few biological studies with this species. The objective of this work was to perform a physicochemical analysis, and identification and quantification of chemical constituents present in the fruit peel of *P. cincinnata*, as well as evaluate the toxicity and hypolipidemic activity *in vivo*, and possible mechanism of action, through of molecular dynamics simulations. The pulverized vegetable drug was submitted to physicochemical assays, according to Brazilian Pharmacopoeia. The chemical composition of lyophilized aqueous extract (AE-Pc) was assessed by high-performance liquid chromatography coupled to diode array detector (HPLC-DAD). It was analyzed acute oral toxicity, and the hypolipidemic profile of the extract (given orally: 100 and 200 mg/kg) was established using the *in vivo* model, with analysis 24 and 48 h, after hyperlipidemic induction with Triton WR-1339 (400 mg/kg). Physicochemical tests showed parameters within acceptable limits for pharmacopeial standards. The presence of vitexin, orientin and isoorientin in the AE-Pc was confirmed using HPLC-DAD. No clinical signs of toxicity were observed in the animal studies. Treatment with AE-Pc in induced hyperlipidemic mice showed a reduction in plasma levels of total cholesterol (TC) and an increase in high-density lipoprotein (HDL-c), statistically significant ($p < 0.05$), when compared to Triton group, while the triglycerides indices (TG) did not show a significant response. The molecular docking showed stability of the compounds, mainly vitexin, in practically the entire simulation, when associated with LCAT. The present study suggests that the peel of the fruit of *P. cincinnata* has a lipid-lowering action, probably due to chemical compounds present in the sample, presenting potential for the development of phytopharmaceuticals.

Keywords: *Passiflora cincinnata*; Physicochemical characterization; Herbal medicine; Therapeutic potential; Flavonoids.

Abbreviations: HPLC-DAD, high-performance liquid chromatography coupled to diode array detector; TC, Total Cholesterol; TG, Triglycerides; LDL, Low-density lipoprotein; VLDL, Very low-density lipoprotein; HDL, High-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; HMG-CoA reductase, hydroxy-methylglutaryl-CoA reductase CVD, Cardiovascular disease; AE-Pc, lyophilized aqueous extract of *Passiflora cincinnata*.

1. Introduction

Hyperlipidemia, characterized by high levels of lipids and/or lipoproteins in the blood, and oxidative stress, are the main risk factors for atherosclerosis and cardiovascular disease, currently considered the leading cause of death in western countries (Kumar et al., 2011; Ntchapda et al., 2015). Studies show that some medicinal plant metabolites act by inhibiting enzymes of the cholesterol biosynthesis and absorption, such as hydroxy-methylglutaryl-CoA reductase (HMG-CoA reductase), as well as lipogenic enzymes (glucose 6-phosphate dehydrogenase and malic enzyme), also acting on other enzymes in the lipid metabolism, as lipoprotein lipase and lecithin-cholesterol acyltransferase (LCAT) (Anila and Vijayalakshmi, 2002; Jung et al., 2006).

Plants have been used by the population as a therapeutic alternative in the treatment of various diseases, due to the presence of several phytochemicals present in its constitution. According to the World Health Organization (WHO), over 80% of the population depend on traditional medicine for their basic health care, reaching over 4.5 billion people in developing countries (Klan et al., 2018). In this perspective, Brazil presents itself with great prominence, for having one of the richest floras on the planet, seizing about 20% of the world's plant biodiversity, with forests owning a significant number of native and endemic species with therapeutic potential (Santos et al., 2018).

It stands out among medicinal plants, the genus *Passiflora* L., presenting about 600 species (Wosch et al., 2015), including the species *Passiflora cincinnata* Mast., popularly known as “maracujá-do-mato” and “maracujá-da-Caatinga”, which is widely distributed in the Caatinga biome. It is a species adapted to the semi-arid climate, resistant to water deficit and pathogens, with varied uses, as ornamental plant, for fruit production, and for medicinal purposes, presenting activities proven scientifically, as antibacterial, antioxidant (Siebra et al., 2018), antinociceptive and anti-inflammatory (Lavor et al., 2018).

It is a polymorphous species that, among several features, presents itself entirely glabrous, with cylindrical or subangular stem, simple leaves, dark green in color, flowers pale pink to violet, with crown filaments showing deep purple color. Its fruits have format ovoid or oblong, with peel of coloring straw-green and, sometimes, yellowish, having deformable consistency (Oliveira and Ruggiero, 2005).

In the last decade, notable advances have been made with the development of a variety of drugs used as lipid-lowering agents, with increasing potential for reducing serum lipid

concentrations and treating hyperlipidemia, especially low-density lipoproteins (LDLs) (Ballantyne et al., 2019). However, the search for new drugs for the treatment of atherosclerosis has greatly valued the role of nutraceuticals and medicinal plants that help to reduce triglyceride, cholesterol and atherogenic lipoproteins (Mahdavi et al., 2020), because, although the drugs are effective, also feature many side effects.

Natural products and their derivatives represent over 50% of all drugs used in clinical practice worldwide (Wannes and Marzouk, 2016), however, the increasing use of herbal medicines in a indiscriminate way, often because it is considered safe, in parallel with the lack of high quality studies in this area, raises many questions about the effectiveness and safety of these drugs (Castro et al., 2014). Therefore, standardization on plant drug control is an essential step in ensuring the quality of raw material, needing to meet pharmacopeial criteria, exact amount to be incorporated into formulations, according to the concentration of active substances present, knowledge about the use of their pharmacological properties and plant toxicology, enabling greater security regarding drug identification, quality of your extracts and its reproducibility (Alberton et al., 2001; Gross et al., 2019).

Therefore, the aim of the study was to analyze parameters for the quality control of future herbal medicines obtained from the fruit peel of *P. cincinnata*, evaluating also the toxicity and hypolipidemic potential in mice, with investigation of the possible mechanism of action of the activity.

2. Materials and methods

2.1. Plant material

The fruits of *P. cincinnata* were collected in the city of Uauá (coordinates: S 9º49'20.82’’; W 39º 37’59.34”), State of Bahia, Brazil, in May 2018. The samples were identified by a botanist Prof. Dr. Jose Alves de Siqueira Filho, and a voucher specimen (#22870) was deposited at the Herbarium Vale do São Francisco (HVASF) of the Federal University of San Francisco Valley (UNIVASF). The seeds were removed, and the fruit peels was dried at 45 °C for 72 h, in an oven with air circulation and renewal, Ethic Technology brand, model 420-6TD, and after, pulverized in a knife mill, brand Solab, model SL-31, and weighed on a scale, model Mark S 520. All procedures for access to genetic patrimony and associated traditional knowledge were carried out, and the project was registered in SisGen (Register #ABD9AA7).

2.2. Quality control of the plant drug

The quality control of the powder of the peel of the fruit of *P. cincinnata* was evaluated in terms of physicochemical analysis, through determination of particle size, loss by desiccation, total ash, acid insoluble ash, sulfated ash, alcohol extractable substances, and determination of the foam index, following the recommendations of the Brazilian Pharmacopoeia (Brazil, 2019).

2.2.1. Particle size analysis

On a stainless steel granulometric analysis sieve, Bronzinox brand, the sample was subjected to forced passage by vibration, 25 g of the sample, through sieves and collector previously weighed, with mesh opening corresponding to 850, 600, 500, 425 and 250 µm, using vibrating sieve, in vibration, for 15 min. After, the fractions retained in each sieve were weighed, and the percentages of passage fractions (PF), and of retention (FR) were calculated. The values represent the average of 3 determinations and are expressed in percentage (%, p/p).

2.2.2. Determination of loss by desiccation

In a crucible previously weighed and desiccated, 2 g of the sample were weighed. Posteriorly, the sample was dry in an oven, at a temperature of 105 °C, for 5 h, and after cooled in desiccator, submitted to new weighing, up until get constant weight. The values obtained represented the average of 3 determinations and were expressed as percentage (%, p/p).

2.2.3. Determination of total ash

The sample was incinerated in a muffle. 3 g of the sample, with progressive increase of temperature, up until reach 600 ± 25 °C, with subsequent weighing. The values obtained represented the average of 3 determinations and were expressed as a percentage.

2.2.4. Determination of acid insoluble ash

It was added 25 ml of hydrochloric acid 7% (p/v) to the resulting ash of the analysis of content of total ash. The mixture was boiled for 5 min, and washed with heated distilled water, up to neutral pH. Posteriorly, were transferred to crucibles and taken to the muffle, up until reach 500 °C. Then, has been cooled in a desiccator and weighed, for the determination of the percentage of acid insoluble ash. The values obtained represented the average of 3 determinations and were expressed as a percentage.

2.2.5. Determination of sulfated ash

For determination of sulfated ash, 1 g of the sample was moistened with sulfuric acid in two steps and incinerated in muffle, with progressive increase of temperature, up until reach 800 °C. Then, was cooled and weighty, and incineration was repeated for another 15 min, weighing again. The values obtained represented the average of 3 determinations and were expressed as a percentage.

2.2.6. Determination of extractable substances in alcohol

Using the cold extraction method, it was added 100 ml of ethanol to 4 g of the sample, leaving macerate exhaustively for 6 h, and stirring at 30 min intervals. Posteriorly, the sample was left in rest for 18 h, filtered, and transferred 25 ml of the filtrate to a crucible previously weighed. Then, it was performed solvent evaporation and insertion of the crucible in an oven for 6 h, at 105 °C, with subsequent cooling in desiccator, and weighted. The values obtained represented the average of 3 determinations and were expressed as a percentage.

2.2.7. Determination of the foam index

It was transferred 1 g of the sample to an erlenmeyer, adding, then, 10 ml of heated distilled water. The mixture was kept on a heating plate for 15 min. Then, the solution was cooled and transferred to a 100 ml volumetric flask, completing the volume with distilled water, and distributed in 10 test tubes, in successive series of 1 to 10 ml, with the final volume of the liquid, adjusted to 10 ml, with the same water. Posteriorly, the tubes were shaken and left to stand for 15 min, for later measurement of foam height.

2.3. Obtaining extracts from the fruit peel of P. cincinnata

2.3.1. Obtaining the crude ethanolic extract (EtOH-Pc)

The sample was macerated with EtOH (99.9%), in 1:10 ratio (plant material:solvent, m/v), performing the procedure for 12 days, with solvent exchange every 3 days, to obtain the EtOH-Pc, according to studies of Sampaio (2017).

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2.3.2. Obtaining aqueous extract by infusion and decoction

Infusion was prepared by the addition of the sample (1 g of the peel powder) to 125 ml of boiling distilled water, followed by 10 min of incubation at room temperature. For decoction, 1 g of the sample was added to 125 ml of distilled water and kept boiling for 10 min, according to studies of Sampaio (2017).

2.4. High-performance liquid chromatography coupled to diode array detector (HPLC-DAD) analysis

The extracts obtained were filtered and, posteriorly, analyzed by high-performance liquid chromatography coupled to diode array detector (HPLC-DAD) to investigate of the concentration of analytical markers, in order to determine the concentration in µg of the respective chemical marker per mg of the extracts.

The analysis of phenolic profile of the extracts of fruit peel of *P. cincinnata* was performed by HPLC-DAD according to the study of Oliveira (2018). The samples were investigated to determine the concentration in µg of the respective chemical marker per mg of the extracts, of flavonoids orientin, isoorientin and vitexin, chemical markers of the genus *Passiflora*.

The samples were prepared in triplicate, at a concentration of 1 mg/ml, using an octadecylsilane column (250 x 4.6 mm, 5 µm, C-18, Agilent®) and a Shimadzu® LC-20 chromatograph equipped with a quaternary pump system model LC - 20A DVP, degasser model DGU - 20A, PDA detector model SPD - 20AVP, oven model CTO - 20ASVP, automatic injector model SIL - 20ADVP, and controller model SCL - 20AVP coupled to a diode array detector (DAD). For quantification of analytical markers, calibration curves were

performed with concentration ranging from 4 to 200 µg/ml for each of the standards. The data obtained were processed using the software Shimadzu® LC solution 1.0 (Japan).

Two solutions were used as a mobile phase: Solution A: 0.1% (v/v) formic acid, and solution B: acetonitrile with 0.1% acid formic. The gradient system in which the samples were analyzed are described in Table 1, with a flow of 0.4 ml/min. The analytical standards and samples were injected at a volume of 10 µL and detection was performed in DAD at a wavelength of 340 nm.

The equation obtained on the calibration curve, for the quantification of these markers for each type of extract, is detailed below:

Eq. (A.1): Vitexin ($y = 37420x - 137628$; $R^2 = 0.9993$). Quantitative results were expressed in vitexin equivalents.

Table 1
Gradient system used in the analysis through HPLC-DAD.

	Time (min)	**Solution A (%)**	**Solution B (%)**
Linear gradient	0-40	85-50	15-50
Linear gradient	40-45	69-0	31-100
Isocratic	45-50	0	100
Linear gradient	50-55	0-85	100-15
Isocratic	55-65	85	15

2.5. Freeze-drying

The powder of the peel of the fruit of *P. cincinnata* was subjected to a decoction, after the result of the qualitative and quantitative analysis of the chemical compounds present in the extracts, by HPLC-DAD, and the decoct was stored in a container with a lid, for freezing, for a period of 48 h. The frozen samples were subjected to drying, by freeze-drying, in the LS300 equipment (Terroni®), at a vacuum pressure of approximately 10 µmHg, for 72 h, with an average condenser temperature of -46 ºC. Subsequently, the lyophilized samples were stored in a freezer for further analysis of the concentration of chemical markers.

2.6. Animals

In all experiments with animals, were used Swiss mice (*Mus musculus*) between 6 and 8 weeks of age (21-43 g). The animals were kept in a temperature-controlled room at 25 ± 1 °C, with a 12/12 h light/dark cycle (the light was turned on at 6:00), with food and water provided *ad libitum*. The experimental protocols were approved by Committee on Ethics in Animal Use of UNIVASF (protocol number 001/281119) and conducted according with the Conselho Nacional para o Controle de Experimentação Animal (CONCEA, Brazil).

2.7. Acute toxicity analysis

The acute toxicity was performed in mice male and female, which were divided into 2 groups with both sexes, with 12 animals per group. The negative control group received only the vehicle (saline 0.9%) orally (gavage) and the other group received 2 g/kg of the lyophilized aqueous extract (AE-Pc). The dose tested was based on the protocol proposed by the ANVISA (Brazil, 2013) and highest dose evaluated in pharmacological activity. After administration of the treatments, the animals were observed continuously during the 14 days of the experiment, to analyze of any physical signs of toxicity, such as writhing, breathing difficulties, or mortality. The consumption of water, food, and weight of the animal were evaluated daily.

After euthanasia, the organs, lung, heart, stomach, spleen, liver, kidney and pancreas, were analyzed macroscopically, observing appearance, texture and possible signs of toxicity, in addition to the relative weight of each organ. All apparent characteristics and significant, or abnormalities, were recorded.

2.8. Hypolipidemic analysis

Lipid-lowering analysis was performed according to Kumar et al. (2012), and Sikarwar and Patil (2012), with slight modifications. Male mice were divided randomly into 5 groups of 12 animals. The animals were treated for 15 days, daily, orally (p.o., by gavage), of the following form:

1. Group I (Normal Control - NC): treated with normal diet and saline 0.9%
2. Group II (Triton): treated with normal diet and saline 0.9%
3. Group III (Fen): treated with 100 mg/kg of fenofibrate

4. Group IV (AE-Pc 100): treated with 100 mg/kg of AE-Pc
5. Group V (AE-Pc 200): treated with 200 mg/kg of AE-Pc

After 15 days of treatment, being 1 h after the last administration of the substances, the acute hyperlipidemia was induced in all groups, except in Group I, through the application intraperitoneally of 400 mg/kg of Triton WR-1339 (Sigma-Aldrich) dissolved in saline 0.9%. A final treatment was performed in half of the animals of each group, 26 h after hyperlipidemic induction. The blood collection, for assessment of biochemical parameters and analysis of lipid-lowering activity, was performed 24 and 48 h after induction. However, the last collection was carried out only in the animals in which it was administered the last treatment.

2.9. Biochemical parameters analysis

The animals were anesthetized intraperitoneally (i.p.) with ketamine (100 mg/kg) and xylazine (10 mg/kg) and were submitted to blood collection by cardiac puncture. The collected blood was conditioning into tubes without anticoagulant, and serum samples were obtained by centrifugation at 3000 rpm for 5 min. Total cholesterol (CT), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c) were analyzed in Cobas integra 400 plus equipment, with enzyme colorimetric kit, of the Labtest Diagnostics SA®.

2.10. Molecular dynamics

Molecular dynamics simulations were performed to estimate the flexibility of interactions between the LCAT enzyme and flavonoids, using GROMACS 5.0 software (Berendsen et al., 1995; Abraham et al., 2015). The topology of the ligands was prepared using the generator of ATB topology (Malde et al., 2011), applying the GROMOS96 54a7 force field. The enzyme topology was prepared using the GROMOS96 54a7 force field in the GROMACS. Molecular dynamics simulation was performed using the SPC water model of point charge, extended in a cubic box (Bondi, 1964). The system was neutralized by the addition of ions (Cl^- and Na^+), and minimized to remove bad contacts between complex molecules and solvent. The system was also balanced at 300K using the V-rescale algorithm at 100ps, represented by NVT (constant number of particles, volume, and temperature),

followed by equilibrium at 1 atm of pressure, using the Parrinello-Rahman algorithm as NPT (numeric particles, constant pressure and temperature) up to 100ps. DM simulations were performed in 5,000,000 steps at 10ns. To determine the flexibility of the structure and what if the complex is stable close to the experimental structure, the root-mean-square deviation (RMSD) of all Cα atoms was calculated relative to the starting structures. Root-mean-square fluctuation (RMSF) were also analyzed to understand the role of residues near the receptor binding site. RMSD and RMSF graphics were generated in Grace software and protein and ligands were visualized in UCSF Chimera (Pettersen et al., 2004).

2.11. Statistical analysis

The results of the *in vivo* assays are presented as mean ± standard error of mean (S.E.M.). Statistical comparisons of the data were performed by one-way analysis of variance (ANOVA), followed by Tukey's test. In all cases, differences were considered significant when $p < 0.05$. All statistical analyses were performed using the software GraphPad Prism version 6.01 (GraphPad Prism Software Inc., San Diego, CA, USA). For the analysis of identification of constituents in the sample and physicochemical characterization, the data obtained were treated using the software Shimadzu® LC solution 1.0 (Japan) and OriginPro8®, respectively, and the data are presented as mean ± standard deviation (S.D).

3 Results

3.1. Physicochemical assays

The sample retention in the analyzed sieves demonstrate that the powder of the peel of *P. cincinnata* does not have a homogeneous size, with predominant retention in the sieve of mesh 850 µm, representing an average of 34.66 ± 2.69% of the total material (Figure 1).

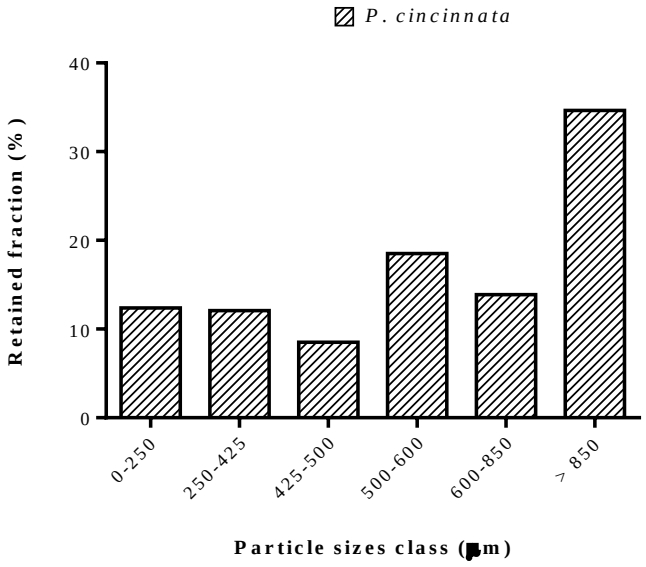

Fig. 1. Particle size distribution histogram of the fruit peel of *P. cincinnata*.
0-250 = 12.37 ± 2.79%; 250-425 = 12.08 ± 0.62%; 425-500 = 8.52 ± 0.21%; 500-600 = 18.50 ± 0.77%; 600-850 = 13.87 ± 0.07%; > 850 = 34.66 ± 2.69%. The values show mean ± S.D. N = 3.

Starting from the calculation of the percentage of the passing fraction and retained fraction, it was also possible to determine the mean particle size in 584 µm (Figure 2).

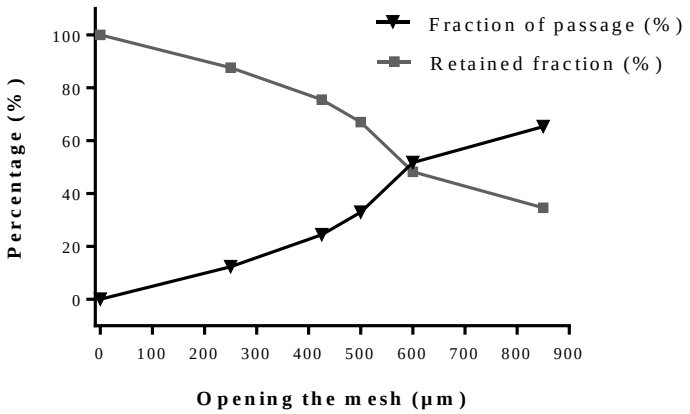

Fig. 2. Retention and passage curves of the fruit peel of *P. cincinnata*.

Other results of the physicochemical assays, such as loss by desiccation, total ash, acid insoluble ash, sulfated ash and extracted substances in alcohol, are presented in Table 2.

Table 2
Results from the physicochemical assays that were developed with the fruit peel of *P. cincinnata*.

Physicochemical assays	Content (%)
Loss by desiccation	11.40 ± 0.20
Total ash	6.32 ± 0.19
Acid insoluble ash	1.11 ± 0.08
Sulfated ash	6.37 ± 0.02
Extractable substances in alcohol	9.24 ± 0.10

Values are expressed as mean ± S.D. N = 3.

In the test for determination of the foam index found a height less than 1 cm in all test tubes. Thus, the foam index was < 100, showed negative result for the presence of saponins in the sample of *P. cincinnata*.

3.2. HPLC-DAD analysis

The analyzes of the extracts by HPLC-DAD indicated the presence of the flavonoids orientin, isoorientin and vitexin, as shown in Table 3.

Table 3
Concentration of orientin, isoorientin and vitexin in aqueous and ethanolic extracts of fruit peel of *P. cincinnata*.

Extractes (type of preparation)	Orientin (µg/ml)	Isoorientin (µg/ml)	Vitexin (µg/ml)
Ethanolic	5.63 ± 0.05	--	3.93 ± 0.10
Aqueous/Infusion	7.92 ± 0.08	--	3.73 ± 0.02
Aqueous/Decoction	9.28 ± 0.29	9.75 ± 0.24	9.25 ± 0.02

Values are expressed as mean ± S.D. N = 3.

Because it has a higher content of these compounds identified, compared to other extractions, the decoction was chosen to proceed with the pharmacological assays, passing, initially, through a freeze-drying, and posteriorly, by the analysis of chemical constituents, to evaluate the possible loss of the compounds. The concentration and percentage of degradation of the analytical markers, after this process, are shown in Table 4.

Table 4
Concentration and percentage of degradation of orientin, isoorientin and vitexin in aqueous extract by decoction, lyophilized, of fruit peel of *P. cincinnata*.

Chemical constituents (µg/ml)	**Content (%)**	**Degradation by freeze-drying (%)**
Orientin	8.64 ± 0.01	6.90
Isoorientin	8.79 ± 0.03	9.85
Vitexin	8.65 ± 0.02	6.49

Values are expressed as mean ± S.D. N = 3.

The representative chromatograms of the decoct and lyophile are shown in the Figure 3. The major peak shown in the chromatogram introduced himself impure, thus, the compound cannot be identified. Three more peaks were observed, with retention times at 21.05 (1), 22.20 (2), and 26.67 (3) min. The analysis of ultraviolet spectrum indicated that probably these peaks correspond to flavonoids isoorientin, orientin and vitexin, respectively, chemical markers of species of *Passiflora*, when comparing retention times and maximum absorption spectra in analytical standards (data not shown).

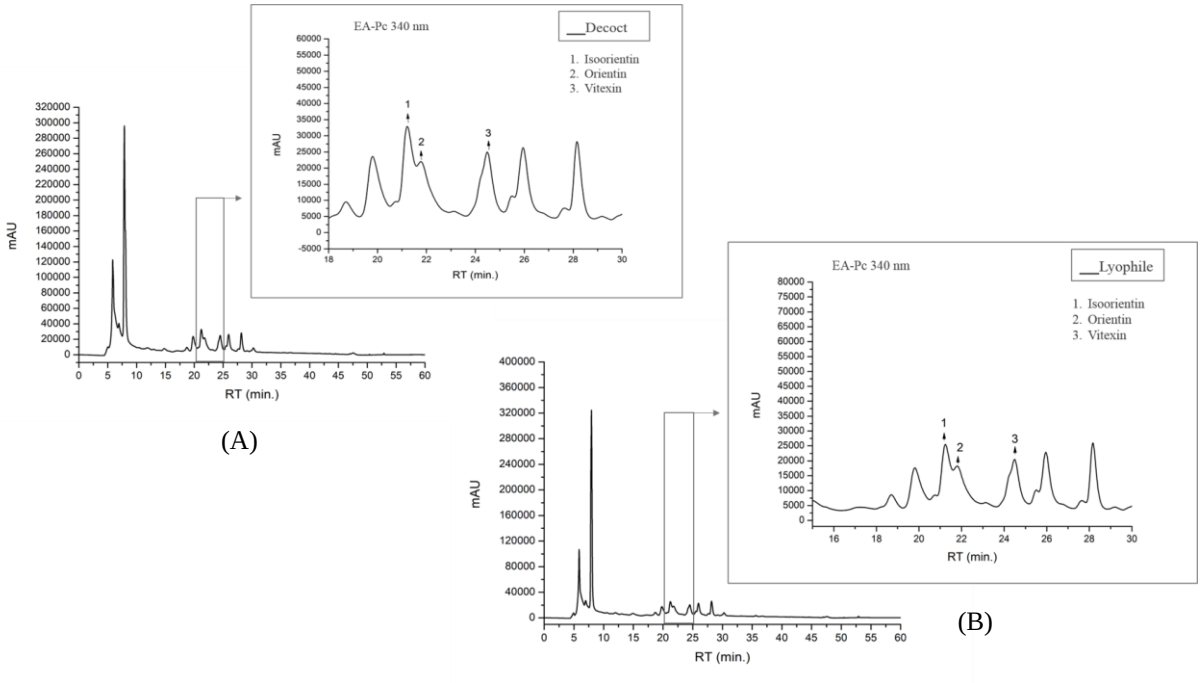

Fig. 3. HPLC chromatogram of the decoct (A) and lyophile (B) of the fruit peel of *P. cincinnata* (AE-Pc).

3.3. Acute toxicity analysis

No physical and behavioral signs, characteristic of toxicity, were observed, according to the evaluation parameter used. The body weight, and the average consumption of water and feed of the mice was not significantly changed with the single dose of the treatment, during the 14 days of analysis (Table 5). Not were reported any macroscopic alterations in the organs of the animals, presenting consistency and color normal, and preserved architecture, and no case of death were reported. Thus, the results indicate the non-toxic nature of the aqueous extract of *P. cincinnata* (AE-Pc) (data not shown).

Table 5.

Effect of AE-Pc in a single dose of 2 g/kg and saline 0.9% orally, in the average consumption of water and feed, initial weight, final weight and body weight variation of the animals.

Groups	**Parameters**				
	Water consumption (g)	**Feed consumption (ml)**	**Initial weight (g)**	**Final weight (g)**	**Body weight variation (g)**
Saline 0.9%	52.93 ± 1.12	33.89 ± 0.85	34.00 ± 0.77	36.50 ± 1.22	2.50 ±1.45
AE-Pc 2g/kg	43.93 ± 0.77	28.46 ± 0.60	28.17 ± 1.55	31.92 ± 1.48	3.75 ± 2.14

Values are expressed as mean ± S.E.M. N = 12.

3.4. Effect of aqueous extract of P. cincinnata on lipid profile in mice

3.4.1. Plasma levels of total cholesterol (TC) in mice

The administration of Triton significantly increased the plasma concentrations of TC in the group only induced (Triton), after 24 h of hyperlipidemic induction, with an increase of 86.9% ($p < 0.05$), when compared to the normal control (NC). Pretreatment with AE-Pc at a dose of 100 mg/kg (100) reduced serum TC levels by 36.1% ($p < 0.05$), compared to the Triton group, while the group treated with 200 mg/kg of the sample (200) did not present significant results in this analysis. It was also observed that the animals that received the doses of the extract did not present significant differences in the levels of TC, in relation to that demonstrated in the normal control group (Figure 4A).

In the 48 h analysis, there was a significant reduction ($p < 0.05$) of 38.4% in the TC levels in the Triton group, when compared with the same group, in the 24 h analysis. However, this group still showed an increase of 39.6% ($p < 0.05$), when compared to the normal control group. In contrast to the 24 h analysis, the pre-treatment and one last treatment performed 26 h after Triton injection, promoted a significant reduction ($p < 0.05$) in the groups treated with 100 and 200 mg/kg, in 28.9 and 39.9%, respectively. The treated groups also showed no significant difference when compared to the normal control group, which received only saline 0.9% (Figure 4B).

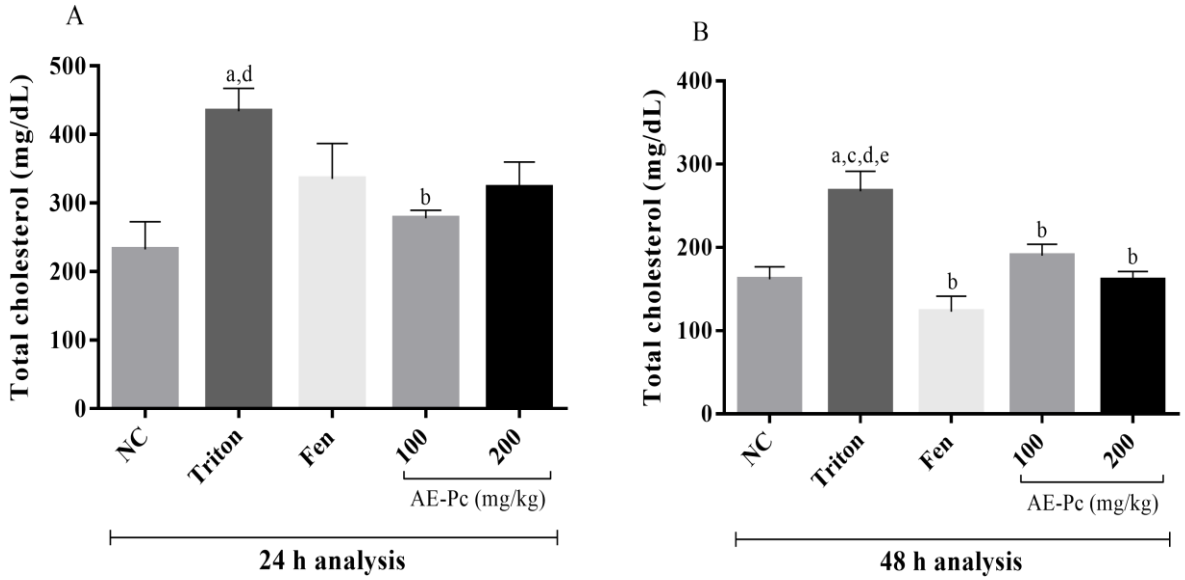

Fig. 4. Effects of pretreatment with AE-Pc (100 and 200 mg/kg/day for 15 days), and a single treatment after hyperlipidemic induction on total cholesterol (TC) in induced hyperlipidemic mice, in the 24 (A) and 48 h (B) analyzes. Values are expressed as mean ± S.E.M., n = 6. [a] $p < 0.05$ compared to the normal control group; [b] $p < 0.05$, compared to Triton group; [c] $p < 0.05$, compared to fenofibrate group (Fen), [d] $p < 0.05$, compared to the 100 mg/kg of AE-Pc, and [e] $p < 0.05$, compared to the 200 mg/kg of AE-Pc. The analysis was performed by ANOVA, followed by Tukey's.

3.4.2. Plasma levels of triglycerides (TG) in mice

Plasma levels of TG significantly increased ($p < 0.05$) in 1382.9% in the Triton group, after 24 h of hyperlipidemic induction, when compared to the normal control group. The pretreatment with doses of 100 and 200 mg/kg of AE-Pc did not significantly reduce the plasma concentration of TG, compared to the group only induced (Figure 5A).

In the analysis of 48 h there was a significant reduction ($p < 0.05$) of 60.6% in TG levels in the Triton group, when compared to the same group, to 24 h analysis. However, that same group, kept an increase ($p < 0.05$) of 851.4% compared with the normal control group. As well as in the 24 h analysis, the pretreatment of 15 days and a single treatment after hyperlipidemic induction, showed no significant reduction in plasma TG levels in animals treated with the AE-Pc, in the two doses administered (Figure 5B).

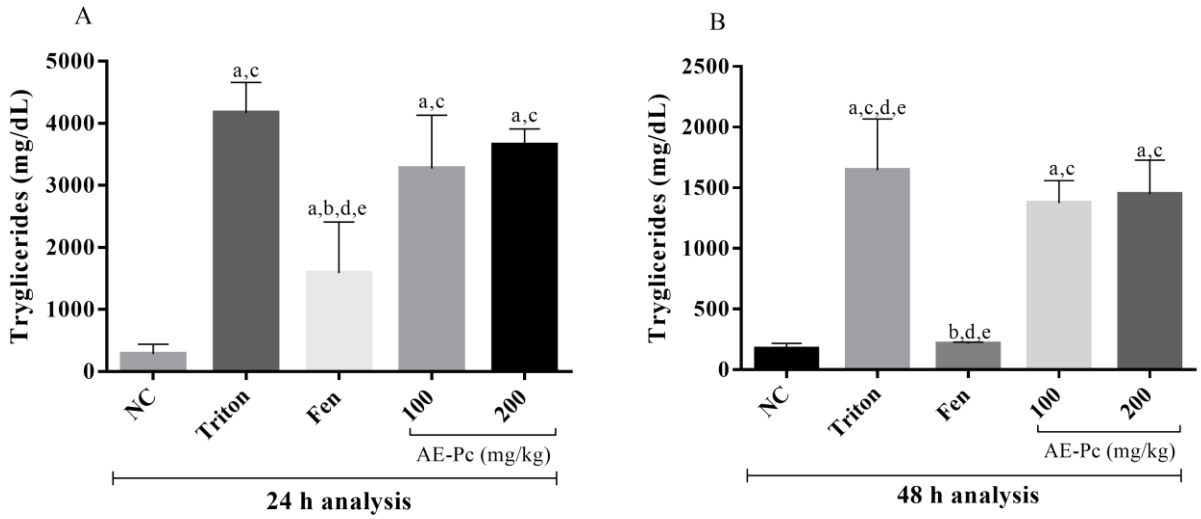

Fig. 5. Effects of pretreatment with AE-Pc (100 and 200 mg/kg/day for 15 days), and a single treatment after hyperlipidemic induction on triglycerides (TG) in induced hyperlipidemic mice, in the 24 (A) and 48 h (B) analyzes. Values are expressed as mean ± S.E.M., n = 6. [a] $p < 0.05$ compared to the normal control group; [b] $p < 0.05$, compared to Triton group; [c] $p < 0.05$, compared to fenofibrate group (Fen), [d] $p < 0.05$, compared to the 100 mg/kg of AE-Pc, and [e] $p < 0.05$, compared to the 200 mg/kg of AE-Pc. The analysis was performed by ANOVA, followed by Tukey's.

3.4.3. Plasma levels of high-density lipoprotein (HDL-c) in mice

Plasma levels of HDL-c decreased significantly ($p < 0.05$) in 85.9% in the Triton group, after 24 h Triton injection, when compared to NC. The pretreatment with the two doses of the extract (100 and 200 mg/kg) showed a significant difference ($p < 0.05$) in plasma HDL-c, when compared to the group Triton, in 265.3 and 289.8%, respectively. The groups treated with the extract no showed significant difference with the reference drug (Figure 6A).

In the 48 h analysis, the plasma levels of HDL-c in the Triton group maintained a significant reduction ($p < 0.05$) in 74.6%, when compared to NC. Different from the 24 h analysis, the pre-treatment and a last treatment performed 26 h after Triton injection, the two doses of AE-Pc didn't promote a significant increase, when compared to the group only induced (Figure 6B).

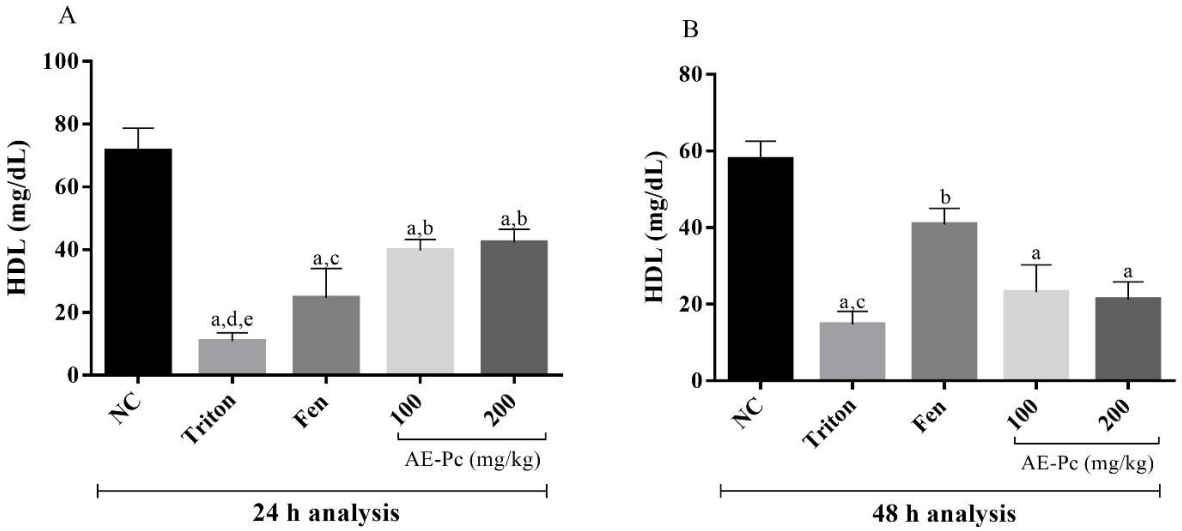

Fig. 6. Effects of pretreatment with AE-Pc (100 and 200 mg/kg/day for 15 days), and a single treatment after hyperlipidemic induction on high-density lipoprotein (HDL-c) in induced hyperlipidemic mice, in the 24 (A) and 48 h (B) analyzes. Values are expressed as mean ± S.E.M., n = 6. [a] $p < 0.05$ compared to the normal control group; [b] $p < 0.05$, compared to Triton group; [c] $p < 0.05$, compared to fenofibrate group (Fen), [d] $p < 0.05$, compared to the 100 mg/kg of AE-Pc, and [e] $p < 0.05$, compared to the 200 mg/kg of AE-Pc. The analysis was performed by ANOVA, followed by Tukey's.

3.5. Molecular dynamics simulations

The RMSD analysis showed that the compounds in general showed stability in practically all the molecular dynamics simulation (Figure 7), with higher fluctuations in some moments. However, the compounds considered more stable were vitexin, isovitexin and isoorientin-4'-*O*-glycoside. This suggests that these molecules tend to remain in the active site, even in the presence of different factors, such as solvent and ions.

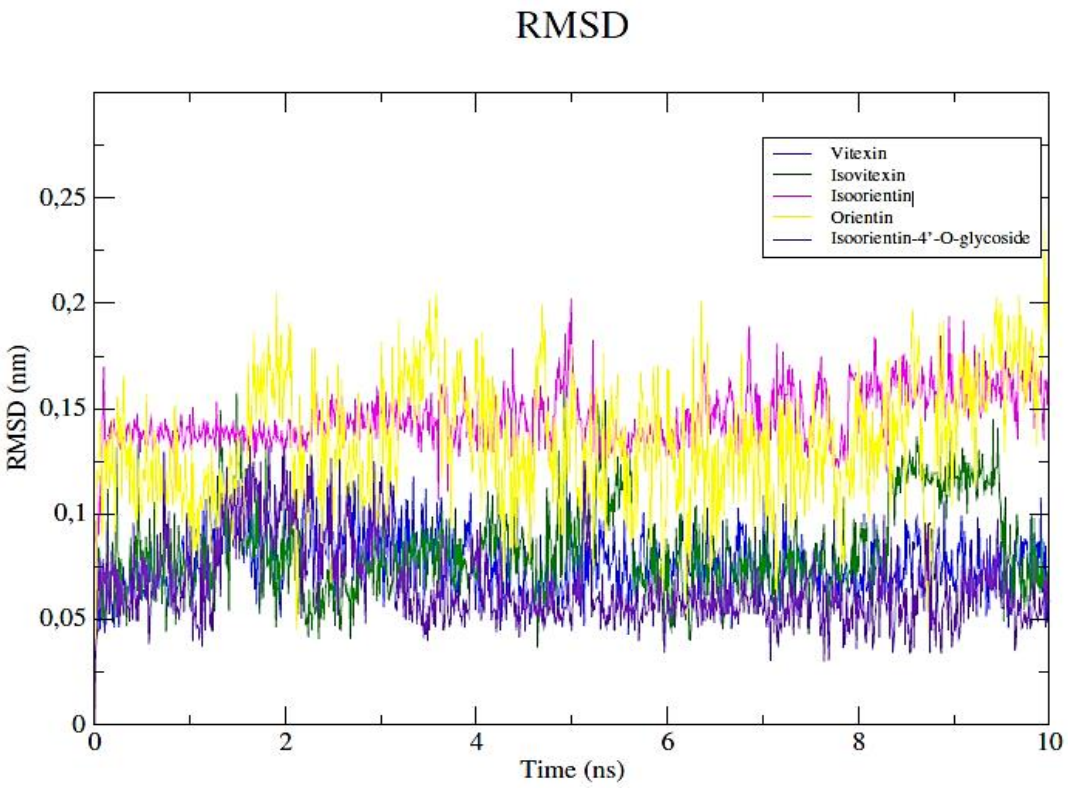

Fig. 7. RMSD of Cα atoms of flavonoids complexed to LCAT enzyme.

To understand the flexibility of residues and aminoacids that contribute to LCAT conformational change, the root-mean-square fluctuations (RMSF) of each amino acid in the protein were calculated. Residues with high RMSF values suggest more flexibility, and low RMSF values reflect less flexibility. Considering that amino acids with fluctuations above 0.3 nm contribute to the flexibility of the protein structure, it was found that among the 398 amino acids that make up the protein, only residues Ser86, His329, Ala397 and His398 contribute to the proteins conformational change (Figure 8).

Thus, the protein structure was considered little variable and, therefore, stable. The amino acids where were observed peaks above 0.3 nm, that is, that contribute to the conformational change of the protein, are not part of the site and, therefore, do not impair the binding of compounds in this region.

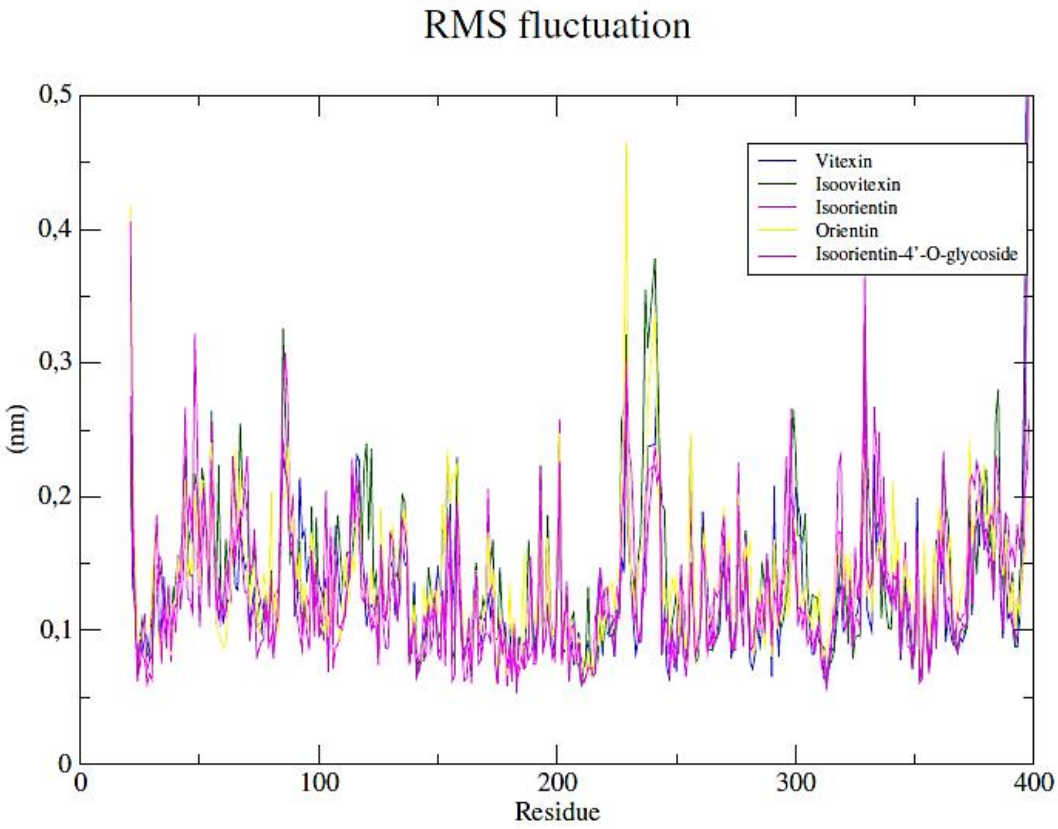

Fig. 8. RMSF of the Cα atoms of each amino acid of LCAT complexed to flavonoids.

In Figure 9, it is observed that the vitexin, compound with the best evaluation in the stability complexed to LCAT, presented several bonds and interactions, highlighting hydrogen bonds with the amino acids Met29, Tyr31, Thr39, Asn45, Leu48, Pro49 and Cys54; van der Waals interactions with amino acids Cys30, Gly51 and Trp55; and hydrophobic interactions with Met29 and Phe38 amino acids.

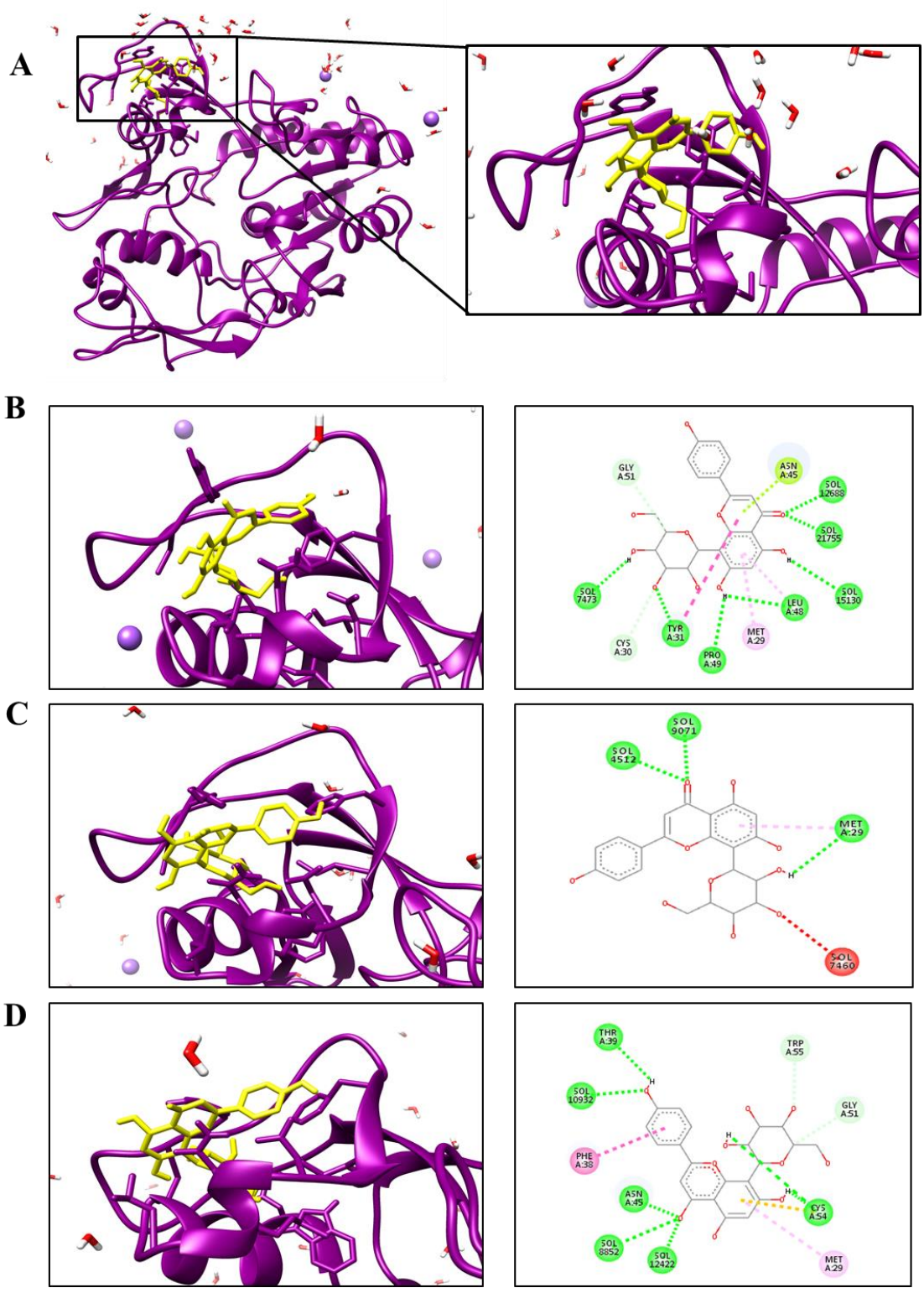

Fig 9. Molecular dynamics simulations during 1000ps. A – Structure of the LCAT complex with vitexin, before simulation, B – 200ps, C – 600ps and D – 1000ps. Hydrogen bonds are highlighted in dark green; van der Waals connections are highlighted in light green; hydrophobic interactions are highlighted in pink, electrostatic interactions are highlighted in orange, and steric interactions are highlighted in red.

4. Discussion

Hyperlipidemia is a metabolic and endocrine disorder, defined as abnormal lipid metabolism, being able to present elevations in triglyceride levels (TG), cholesterol containing cholesterol ester, and free cholesterol in plasma or serum, being a risk factor for atherosclerosis and cardiovascular disease, with probability of cardiovascular events or death, especially when associated with other additional risks, such as type 2 diabetes and cerebrovascular disease (Wake et al., 2019; Gao et al., 2020).

Drugs used to treat hyperlipidemia, such as statin, fibrates, link resin and niacin, have some toxic and side effects, such as hyperuricemia, gastric irritation, abnormal liver function. Fibrate, the drug used as a positive control in this study, among other side effects, it can cause, mainly, liver damage, with consequent elevation of aminotransferases (ALT, AST). Thus, in long deadline or large doses, become inadequate, and the development of new effective lipid-lowering drugs based on natural sources, with non-toxic substances and no side effects, becomes essential (Duraipandiyan et al., 2016; Gao et al., 2020; Xie et al., 2021).

This study had as objective evaluate the hypolipidemic activity of lyophilized AE-Pc from the fruit peel of *P. cincinnata*, as a medicative alternative, analyzing important parameters for obtaining of better quality control of this phytopharmaceutical.

Considering the increasing use of species of *Passiflora* for various pathologies, and pharmacological research already developed with the *P. cincinnata*, the determination of pharmacognostic parameters for species of the genus, through physicochemical analysis, is of great importance, for better quality control of herbal medicines (Souza-Moreira et al., 2010). Thus, particle size evaluation becomes a necessary parameter to be established, since the division of plant drug particles has a direct influence in extractive dissolution processes, beyond solvent for extraction, extraction methods, plant metabolism, and time of collection (Guizzo et al., 2015; Verma and Shukla, 2015), and also in its conservation. Very large diameters (greater than 0.8 mm) can make it difficult the penetration of the extractor liquid in the cells, as well as, the very pulverized plant drug (smaller than 0.2 mm) increases contact surface of grains, forming clusters and causing problems in stability, due to humidity adsorption (Alberton et al., 2001; Amarante et al., 2011).

In this study, the results obtained in the granulometric analysis rated the powder of the fruit peel of *P. cincinnata* as a coarse powder (Brazil, 2019). This result show good prospect for the extraction of bioactive compounds of the plant drug, and in the conservation and shelf life of the product, important for obtaining of a phytomedicine.

The loss by desiccation is a parameter that is related to the stability of active constituents of plant material, once excess moisture benefits microorganism contamination, hydrolysis, and enzymatic activity, with consequent deterioration of its bioactive constituents (Couto et al., 2009). Thus, the fruit peel of *P. cincinnata* showed a humidity range within the official allowed limits (11.40 ± 0.20), which is in the range of 8 to 14% (Silva et al., 2016), as was shown in a study with other *Passiflora* species (Cazarin et al., 2014).

The content of total ash is crucial for quality control, since it quantifies non-volatile inorganic substances that may be present as constituents or contaminants in the vegetable drug. Presenting in large quantities, it could be due to problems during harvesting, cleaning and processing the material (Couto et al., 2009; Guizzo et al., 2015). The study result indicated a value of total ash inside the limit set by the Brazilian Pharmacopoeia, which is at most 14% (Brazil, 2019).

The analysis of acid-insoluble ash indicates the amount of siliceous constituents in the vegetable drug (Couto et al., 2009), while the content of sulfated ash refers to the non-volatile residue for incineration in the presence of sulfuric acid. Considering that the values of sulfated ash and acid-insoluble ash for fruit peel of *P. cincinnata* were not found in the researched literature, these data can provide subsidies for the quality control of this vegetable raw material.

The foam index is an important analysis for the identification of saponins in the sample and vegetable drug quality. This constituent has already been identified in leaves and stems of *P. edulis* and *P. alata* (Yoshikawa, 2000; Reginatto et al., 2001). However, in this study, this constituent was not identified in the fruit peel of the specie studied.

The physicochemical results of the research demonstrate that the studied species presents a good quality of the raw material for the development of future phytopharmaceuticals, being an important step for pharmacological tests. Although, environmental changes such as seasonality, circadian rhythm, temperature, humidity, extractive system, storage time and temperature, among other factors, can also change the active pharmaceutical input, qualitatively and quantitatively, modifying, thus, the reproducibility and effectiveness of the finished product (Sobrinho et al., 2012).

Various chemical compounds have already been identified in different species and parts of *Passiflora*, such as saponins, alkaloids, and the flavonoids orientin, isoorientin, vitexin, isovitexin, isoorientin-2”-*O*-glucoside, vicenin-2,6,8-di-*C*-glycosylchrysin, vitexin-2-*O*-rhamnoside and vitexin-2-*O*-xiloside, mainly flavonoids *C*-glycosides, in the species *P. edulis* var. *flavicarpa*, *P. edulis* var. *edulis*, *P. alata*, *P. setacea*, *P. tripartita* var. *mollissima*,

P. quadrangularis, *P. manicata*, *P. morifolia*, among others (Zucolotto et al., 2011; Costa et al., 2016; Wosch et al., 2017). In studies with the ethanolic extract of aerial parts, fruit peels and flower of *P. cincinnata*, the presence of isoorientin, isovitexin, vitexin was also identified, through analysis by high-performance liquid chromatography coupled to mass spectrometry (HPLC-DAD-MS/MS) (Lavor et al., 2018; Leal et al., 2018). In the present study, the presence of vitexin, orientin and isoorientin were also confirmed in the aqueous extract of the peel of *P. cincinnata*, by HPLC-DAD, confirming previous studies.

After analyzing the chemical composition, the determination of the degree of toxicity is the first step that must be carried out in the research, to evaluate a new drug or herbal medicine, owing be performed through pre-clinical trials, with different stages of time of exposure of the animal to the tested substance, as single or acute dose, repeated, subchronic or chronic dose (Garrido et al., 2011). The evaluation of toxicity also allows investigating the action of the substance on the central and autonomic nervous system, through of pharmacological behavioral screening (Almeida et al., 1999).

The choice for the freeze-drying process, in the extract for administration to animals of the study, this was due to the fact that some studies show that, compared to other drying methods, freeze-drying causes less damage to the properties of the fruits, as it includes crystallization and sublimation at reduced pressure (Caparino et al., 2012; Shuen et al., 2021), as well as, exhibit a greater retention of phenolic compounds, due to the absence of heat and formation of ice crystals, that break the cell structure, aiding in the extraction of metabolic compounds (Shih et al., 2009; Shuen et al., 2021).

To assess the safety profile of AE-Pc, acute toxicity was assessed for 14 days after administration in a single dose of 2 g/kg of AE-Pc, orally, to determine the LD_{50}, which aims to determine the lethal dose of a substance that kills half of the animals in a test group (Botham, 2004), thus, providing data on the degree of toxicity of the substance, development of guidelines for the selection of a safe dose, and to carry out pharmacological tests, subsequently. The administration in a single dose of the extract, was not able to promote changes in the toxicity parameters, not showing deaths, as well as behavioral changes, in the evaluated animals, during the proposed observation period, demonstrating that the substance did not cause toxicity, and suggesting that the AE-Pc has $LD_{50} > 2$ g/kg, when administered orally. The results obtained corroborate a clinical study with another species of *Passiflora*, *P. edulis* f. *flavicarpa*, which also did not show signs of toxicity, side effects or presence of death, with longer exposure time to the substance (Medeiros et al., 2009).

Passiflora species have been evaluated as hypolipidemic agent in some experimental studies (Ramos et al., 2007; Janebro et al., 2008), mainly the flour of the fruit peel, in *P. edulis* f. *flavicarpa* Deg., using different models of hyperlipidemia. In this study, Triton WR-1339 (400 mg/kg) was used as a model for inducing hyperlipidemia, observing in the 24 h of induction, a perturbation in the lipid profile, with an increase of TC, TG, and decrease in levels of HDL-c, when comparing the Triton group with the normal control group. After 48 h of induction, it was observed an improvement in biochemical parameters, although, in relation to the first analysis, comparing the only induced groups, the levels remained altered. These results corroborate studies that also used the same model of hyperlipidemia induction (Kumar et al., 2012; Zarzecki et al., 2014).

Triton WR-1339 is a nonionic surfactant, that promotes disturbances in lipid metabolism, because it inhibits the activity of lipoprotein lipase, blocking, thus, the absorption of triacylglycerol-rich lipoproteins and providing a difficulty in its blood removal, stimulates liver function in biosynthesis of cholesterol, through the increase in HMG-CoA reductase activity, with likely cholesterol efflux from body tissues (including the liver) into the circulation, as well as, it causes dissociation of apolipoprotein A-I (apoA-I) and apoC-II from HDL (Souza et al., 2017; Khlifi et al., 2019; Bouhlali et al., 2020), thus corroborating the results of the study, that promoted a marked increase in the TC and TG indices, and a significant decrease ($p < 0.05$), in the HDL-c levels, in the animals of the study.

This surfactant promotes hyperlipidemia in a short period, attaining its peak in 24 h, with return of the levels considered basal, 72 h after induction, and it has been very utilized in this experimental model, often for screening of lipid-lowering drugs, natural or chemical (Bertges et al., 2011; Zarzecki et al., 2014; Khlifi et al., 2019). In this study, were observed the effects of the dose of Triton-WR on plasma lipids, showing the viability of using it in trials with acute hyperlipidemia. After results of the analysis, by the method used, it was not possible to perform the calculation of VLDL-c and LDL-c, due to the plasma concentration of TG being above of 400 mg/dL, in the two investigated collections (24 and 48 h), being able to present an inaccuracy in the result (Friedwald et al., 1972).

The pre-treatment of 15 days (100 and 200 mg/kg) with AE-Pc of the peel of the fruit of *P. cincinnata* promoted a significant reduction ($p < 0.05$) in the plasma levels of CT, in the less dose, in addition to increasing levels of HDL-c (100 and 200 mg/kg), in the two doses administered. Although it was not able to decrease the levels of TG, in this experimental protocol, after 24 h of induction, in comparison to the group only induced.

With a single treatment post-induction, AE-Pc reduced plasma levels of TC, in the two doses administered, although not promote improvement in the TG and HDL-c indices, in this last analysis.

The lipid-lowering activity of AE-Pc, after pre-treatment and one last treatment after hyperlipidemic induction, may be due to bioactive compounds present in the sample, specifically flavonoids vitexin, isoorientin and orientin, that are already known for demonstrate antioxidant and anti-inflammatory activities, and is also already being evaluated in studies investigating its effect on the risks of atherosclerosis, in induced hyperlipidemic animals. Studies have shown the action of these flavonoids in reducing in TC, LDL-c, atherogenic index, inflammatory mediators (IL-1β, IL-6, TNF-α), antiatherogenic markers, and reduced of the hepatic activity of HMG-CoA reductase, as well as increasing of the HDL-c, antioxidant enzymes (SOD, GPx, CAT), and increased resistance of LDL-c to lipid peroxidation (Orrego et al., 2009, Belguith-Hadriche et al., 2016; Shaoli et al., 2017; Banerjee et al., 2019; Seyedana et al., 2019; Lei et al., 2020; Li et al., 2021).

Studies also have shown that vitexin and orientin reduces the accumulation of lipid droplets, inhibiting the accumulation of triglycerides and cholesterol, in serum and liver, in addition to suppressing lipogenesis, by negative regulation of the expression of the activated receptor γ by peroxisome proliferator (PPARγ), that regulates the expression of numerous genes involved in lipid metabolism (Kim et al., 2010; Inamdar et al., 2019).

In this study, the mechanism of action of lipoprotein metabolism was evaluated by molecular docking, what is a computational procedure widely used to evaluate interactions of a ligand (drug) with binding sites of the target protein (receptor), being important for the discovery of a drug, in the optimization of the most adequate molecule, being able to reduce time and resources during research (Muhammad et al., 2021). The dynamics allows characterizing the behavior of small molecules at the binding site of target proteins, as well as elucidating fundamental biochemical processes (Meng et al., 2011).

After observing, in the hyperlipidemic animals treated with AE-Pc, a significant increase of the HDL-c, which plays an important role in regulating blood lipids, simulation was carried out, through molecular docking, of the interaction of flavonoids and LCAT, which is the key enzyme to reduce free cholesterol levels in blood cells, through of the esterification of free cholesterol from plasma lipoproteins, through the HDL-LCAT system (Miller et al., 2016).

The docking result suggested that the compounds remained stable within the active site of LCAT, keeping up bound, even under the action of factors such as water molecules and

moving protein, as they are capable of forming hydrogen bonds, which are stronger and harder to break. This result becomes important for showing that vitexin, present in the extract, can act to increase the activity of the LCAT, being able change the concentration of free cholesterol, in the HDL fraction, to esterified cholesterol, thus promoting the reverse transport of cholesterol.

The result of this study also suggested that there was inhibition of cholesterol biosynthesis, observed in the analysis of total cholesterol, possibly due to the presence of bioactive compounds, identified in the aqueous extract of the peel of *P. cincinnata* fruit.

Polyphenols may have a possible effect on the prevention of atherosclerosis, making liver cells more efficient for removing of LDL-c in the blood, by increasing the density of the LDL-c receptor in the liver, and by binding to apolipoprotein B (Ibrahim et al., 2016), which is associated with atherogenic lipoproteins, such as chylomicrons remnant, VLDL, LDL and Lp (a) (Forti and Diament, 1991).

Studies have shown that compounds with character less hydrophilic, such as isoorientin, for example, are more available for the lipid structure of LDL and, therefore, they protect lipids from oxidation, being a potential candidate for use as an antiatherogenic agent (Orrego et al., 2009). As also, an indication of the antioxidant behavior of vitexin, it is due to the presence of glycoside in the C-8 position of the A-ring, which influences the hydrogen donation capacity of the B-ring, and computed molecular characteristics imply that these flavonoids are capable to donate electrons instead of capturing them (Praveena et al., 2013).

Although, the antioxidant capacity of a natural product extract will depend essentially on the bioavailability of the specific mixture of compounds present, and their synergistic interactions to produce the antioxidant response at the cellular level (López-Alarcón and Denicola, 2012).

The results found in the study show that, even if in some analyzes of the lipid profile, the extract did not act effectively, not being observed improvement in the lipid profile, the bioactive compounds present in the AE-Pc, even in small concentrations, showed the ability to suppress total cholesterol and increase HDL-c, in hypercholesterolemia models, suggesting that these bioactive compounds, especially vitexin, or the combination of them, promotes a lipid-lowering effect, and validates the use of the aqueous extract lyophilized of the fruit peel *P. cincinnata* as a therapeutic alternative to prevent and/or treat hyperlipidemia.

5. Conclusions

The results obtained in the preliminary physicochemical analysis of the fruit peel of *P. cincinnata* were within acceptable limits, according to the pharmacognostic parameters evaluated, presenting good quality of the raw material. The AE-Pc of the specie, in the administered dose, was not able to cause clinical signs of toxicity and death in the study animals, and attesting to their use for pharmacological studies. It is suggested that the analyzed substance have hypolipidemic potential, due to the presence of flavonoids vitexin, orientin and isoorientin in its constitution, which, in molecular docking dynamics, showed stability, especially vitexin, when associated with the LCAT enzyme. In this way, the results allow to validate the traditional use of *P. cincinnata* in the prevention and/or treatment of hyperlipidemia, demonstrating that this species presents great potential to be used as herbal medicine. However, there is a need for further studies to determine the possible mechanism of action, *in vivo*, responsible for this effect.

Conflict of Interest

The authors declare that they have no conflict of interest.

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CONCLUSÃO GERAL

Passiflora é um gênero que tem sem sido muito estudado para diversas atividades farmacológicas, devido à presença de importantes compostos bioativos, principalmente *C*-glicosídeos, o que tem aumentado o número de patentes relacionadas às espécies desse gênero, podendo ser considerada uma importante ferramenta para o desenvolvimento de novos produtos.

Embora seja evidenciado um número elevado de publicações de patentes no mundo todo, relacionadas à atividade farmacológica, a quantidade de depósito de patentes pelo Brasil ainda é pequena, mesmo apresentando um grande número de publicações científicas com o gênero.

Dentre as espécies desse gênero, *P. cincinnata* é uma espécie pouco estudada em termos farmacológicos, mas com grande potencial para a pesquisa. Neste estudo, a casca do fruto da espécie passou por diversos testes físico-químicos, que demonstraram uma matéria-prima de boa qualidade, dados importantes para dar continuidade à obtenção do extrato, realização de testes biológicos posteriores, e consecução de um protótipo de fitoterápico. Na amostra foi identificada a presença de isoorientina, orientina e vitexina, em extrato aquoso, corroborando estudos que já mostraram a presença desses compostos em diversas espécies e partes de *Passiflora*.

A análise da toxicidade não foi capaz de provocar sinais clínicos de toxicicidade e mortes, nos animais submetidos à avaliação da toxicidade aguda. Com isso, a planta em teste foi caracterizada como sendo de natureza atóxica, na dose administrada, o que a certifica para a realização de estudos farmacológicos.

Com relação aos efeitos farmacológicos, a avaliação da atividade hipolipidêmica do extrato aquoso, em camundongos hiperlipidêmicos induzidos, demonstraram ótimos resultados, através de reduções nos índices de CT e aumento de HDL-c, quando comparado ao grupo de animais hiperlipidêmicos induzidos, sem nenhum tratamento com o extrato da espécie estudada.

O mecanismo de ação dessa atividade foi observado por dinâmica de docking molecular, demonstrando que os compostos bioativos identificados no extrato, apresentaram estabilidade em praticamente toda a simulação, principalmente a vitexina, quando associado à enzima LCAT, sugerindo que espécie possui atividade hipolipidêmica, possivelmente pela presença dos flavonoides encontrados na casca desse fruto.

Os resultados obtidos sugerem que o extrato aquoso liofilizado da casca do fruto de *P. cincinnata* é um agente promissor e tem valor biotecnológico no tratamento da hiperlipidemia, no entanto é necessário estudo com maior tempo de exposição do extrato analisado, para confirmação do efeito observado, e estabelecimento do mecanismo de ação, *in vivo*, para viabilidade clínica.

PERSPECTIVAS

O desenvolvimento de fitoterápicos que sejam eficazes, seguros, de fácil acesso e com uma quantidade mínima de efeitos colaterais, tende a ter um grande impacto econômico e social, uma vez que diminuem os gastos e podem aumentar a adesão à terapêutica, sobretudo pelos pacientes resistentes ao uso de medicações que sejam recorrentes, tornando-se imprescindível o desenvolvimento de outras potencialidades terapêuticas que minimizem os efeitos adversos de medicamentos comumente utilizados pela população.

Assim, espera-se que os extratos de cascas do fruto de *P. cincinnata* sejam obtidos a partir de uma matéria-prima que passou por etapas de padronização sobre o controle da droga vegetal, desde a sua colheita, assim como, seus constituintes químicos devem estar quantificados de forma padronizada, de forma a obter um insumo farmacêutico ativo que ofereça garantia de qualidade, apresente constância, e que seja eficaz em sua ação hipolipidêmica, com potencial biotecnológico para superar os efeitos adversos de formulações convencionais.

Sugere-se avaliar o perfil de segurança de *P. cincinnata*, através de teste com maior tempo de exposição do extrato, assim como, outras pesquisas relacionadas ao tratamento das dislipidemias, a partir dos flavonoides isolados da casca de *P. cincinnata*, ou maior concentração do extrato.

Sugere-se também que os resultados da pesquisa sejam publicados em periódicos científicos e contribuia como nova opção terapêutica para as dislipidemias e suas complicações.

Por fim, recomenda-se propor melhorias para o destino das cascas, de forma a não ser considerada mais um resíduo industrial, podendo ser utilizada na elaboração de novos produtos que trazem benefícios à saúde.

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ANEXO A - Declaração da Comissão de Ética no Uso de Animais (CEUA) da UNIVASF

MINISTÉRIO DA EDUCAÇÃO
MINISTÉRIO DE CIÊNCIA, TECNOLOGIA E INOVAÇÃO
UNIVERSIDADE FEDERAL DO VALE DO SÃO FRANCISCO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Certificado de autorização

Certificamos que a disciplina intitulada: "**Desenvolvimento e validação de método analítico para extratos de *Passiflora cincinnata* Mast. e avaliação da atividade hipolipidêmica e antioxidante *in vivo***", registrada com o nº **0001/281119**, sob a responsabilidade de **Ana Ediléia Barbosa Pereira Leal** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Universidade Federal do Vale do São Francisco - UNIVASF, em 28/11/2019.

Finalidade	( ) Ensino (x) Pesquisa Científica
Vigência da autorização	08/01/2020 a 08/03/2020
Espécie/linhagem/raça	*Mus musculus*
Nº de animais	120
Peso/Idade	25 a 40g/ 08 a 10 semanas
Sexo	96 M e 24 F
Origem	Biotério da UNIVASF, Campus Petrolina-PE, Centro

Em: 17/01/2020

KARINE VIEIRA ANTUNES
Coordenadora da Comissão de Ética no Uso de Animais
CEUA/UNIVASF

ANEXO B - Comprovante de submissão do manuscrito 1

Submission Acknowledgement | BMS-CTMC-2022-14
1 mensagem

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Reference#: BMS-CTMC-2022-14

Submission Title: Pharmacological activities of the genus Passiflora (Passifloraceae): a patent review

Dear Dr. Almeida,

Thank you for your submission to "Current Topics in Medicinal Chemistry(CTMC)". It will be sent to the Editor-in-Chief for his initial provisional approval. Once this is obtained it will be passed on to our Author Support Services department (BASS), for an initial assessment of the manuscript. BASS is a specialized department that helps authors and editors to make sure that the manuscript becomes ready for submission into the peer-review process. The manuscript is being processed on the clear understanding that it contains original work that has neither been published earlier nor has it been simultaneously been submitted for publications elsewhere. In case this is not so, then kindly let us know immediately.

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ANEXO C - Comprovante de submissão do manuscrito 2

Saudi Journal of Biological Sciences

Physicochemical, phytochemical characterization, and assessment of hypolipidemic activity of Passiflora cincinnata Mast. in hyperlipidemia induced by Triton WR-1339

--Manuscript Draft--

Manuscript Number:	
Article Type:	Original Article
Keywords:	Passiflora cincinnata; Physicochemical characterization; Herbal medicine; Therapeutic potential; Flavonoids
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Abstract:	Passiflora cincinnata Mast. is a native species from the Caatinga biome, popularly used for several therapeutic purposes in folk medicine, although there are few biological studies with this species. The objective of this work was to perform a physicochemical analysis, and identification and quantification of chemical constituents present in the fruit peel of P. cincinnata, as well as evaluate the toxicity and hypolipidemic activity in vivo, and possible mechanism of action, through of molecular dynamics simulations. The pulverized vegetable drug was submitted to physicochemical assays, according to Brazilian Pharmacopoeia. The chemical composition of aqueous extract (AE-Pc) was assessed by high-performance liquid chromatography coupled to diode array detector (HPLC-DAD). It was analyzed acute oral toxicity, and the hypolipidemic profile of the extract (given orally: 100 and 200 mg/kg) was established using the in vivo model, with analysis 24 and 48 h, after hyperlipidemic induction with Triton WR-1339 (400 mg/kg). Physicochemical tests showed parameters within acceptable limits for pharmacopeial standards. The presence of vitexin, orientin and isoorientin in the AE-Pc was confirmed using HPLC-DAD. No clinical signs of toxicity were observed in the animal studies. Treatment with AE-Pc in induced hyperlipidemic mice showed a reduction in plasma levels of total cholesterol (TC) and an increase in high-density lipoprotein (HDL-c), statistically significant ($p < 0.05$), when compared to Triton group, while the triglycerides indices (TG) did not show a significant response. The molecular docking showed stability of the compounds, mainly vitexin, in practically the entire simulation, when associated with LCAT. The present study suggests that the peel of the fruit of P. cincinnata has a