UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Jaciara Neves Sousa

Efeitos do extrato hidroalcoólico da folha de *Davilla elliptica* (Dilleniaceae) A. St.-Hil sobre parâmetros metabólicos de camundongos obesos induzidos por dieta

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Exame de defesa (Mestrado) apresentado ao Programa de Pós-graduação em Ciências em Saúde da Universidade Estadual de Montes Claros-Unimontes, como parte das exigências para a obtenção do título de Mestra em Ciências da Saúde.

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Orientador: Prof. Dr. Sérgio Henrique Sousa Santos

Coorientadora: Profa. Dra. Valéria Mafra Cota

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Unimontes

MESTRANDO(A): JACIARA NEVES SOUSA

TÍTULO DO TRABALHO: "Efeitos do extrato hidroalcoólico da folha de Davilla elliptica (Dilleniaceae) A. St.-Hil sobre parâmetros metabólicos de camundongos obesos induzidos por dieta"

ÁREA DE CONCENTRAÇÃO: Mecanismos e Aspectos Clínicos das Doenças

LINHA DE PESQUISA: Clínica, Diagnóstico e Terapêutica das Doenças

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“Não é o quanto fazemos, mas quanto de amor colocamos naquilo que fazemos. Não é o quanto damos, mas quanto de amor colocamos em dar”.

(Madre Teresa de Calcutá)

RESUMO

A obesidade é caracterizada pelo acúmulo excessivo ou anormal de gordura corporal, causada pelo desequilíbrio entre o consumo e o dispêndio energético. O uso de plantas com fins medicinais, para o tratamento, cura e prevenção de doenças, é uma das mais antigas formas de prática medicinal utilizada pela humanidade. Evidências acumuladas revelam o uso tradicional da *Davilla elliptica* como adstringente tônico, laxativo e diurético. Esta espécie apresenta uma grande diversidade de substâncias do metabolismo secundário, como flavonoides, saponinas, esteroides, taninos, cumarinas e triterpenoides, e são descritas por seus efeitos anti-inflamatórios, antimicrobianos e gastroprotetores. Nessa perspectiva, no primeiro capítulo dessa dissertação, apresentamos uma revisão narrativa sobre a *D. elliptica*, e seus aspectos farmacológicos. Em seguida, avaliamos o potencial terapêutico do extrato hidroalcoólico de folhas da *D.elliptica* sobre parâmetros metabólicos de camundongos obesos induzidos por dieta. Para isso, oito grupos de camundongos *SWISS* foram utilizados no experimento: controle (dieta padrão), dieta padrão + EA (fração de acetato de etila), dieta padrão + HE (extrato hidroalcoólico das folhas de *D. elliptica*), dieta padra + PL (pó da folha) e dieta hiperlipídica adicionada EA, HE e PL na dose de 0,26mg/kg/peso corporal, tratados por um período de quatro semanas. Durante o tratamento, foram avaliados peso corporal e ingestão alimentar. Ao fim do tratamento, testes de tolerância à glicose e sensibilidade à insulina foram realizados para avaliar o metabolismo glicêmico. Em seguida, os animais foram sacrificados por decapitação por guilhotina e amostras de sangue foram coletadas para análises bioquímicas da glicose, triglicérides e colesterol total. O estudo foi aprovado pelo Comitê de Ética e Pesquisa Experimental da Universidade Estadual de Montes Claros. Os resultados foram descritos em dois artigos. No primeiro, foram mostrados os efeitos terapêuticos e as propriedades biológicas da *D. elliptica.* Na estratégia de busca dos artigos, foi utilizado como descritor o termo "*Davilla elliptica",* de maneira que foram recuperados artigos, anais de congresso, livros, teses e dissertações. No segundo artigo, os principais achados mostraram uma redução da adiposidade e do peso corporal em animais alimentados com dieta hiperlipídica, já os animais alimentados com dieta padrão demonstraram melhoras nos parâmetros bioquímicos. Este é o primeiro estudo a investigar o papel da *D. elliptica* sobre os parâmetros metabólicos da obesidade. Análises futuras serão realizadas para investigar por quais mecanismos e vias de sinalização a *D. elliptica* atua sobre a obesidade.

Palavras-chave: Obesidade. Tecido adiposo. Planta medicinal. *D. elliptica*.

ABSTRACT

Obesity is a disease characterized by excessive accumulation of body fat caused by the imbalance between consumption and energy expenditure. The use of medicinal plants for the treatment, cure and prevention of diseases is one of the oldest forms of medicinal practice used by mankind. Cumulative evidence reveals the traditional use of *Davilla elliptica* as a tonic, laxative and diuretic astringent. This species exhibits a wide range of secondary metabolism substances such as flavonoids, saponins, steroids, tannins, coumarins and triterpenoids - described for their anti-inflammatory, antimicrobial and gastroprotective effects. In this perspective, in the first chapter, of this dissertation, we present a narrative review on *D. elliptica*, and their pharmacological aspects. Next, we evaluated the therapeutic potential of *D. elliptica* leaf hydroalcoholic extract on metabolic parameters of diet-induced obese mice. To this end, eight groups of *SWISS* mice were used in the experiment: control (standard diet); standard diet + EA (ethyl acetate fraction); standard diet + HE (hydroalcoholic extract of *D. elliptica* leaves); standard diet + PL (leaf powder) (0.26 mg/kg/body weight); control (high fat- diet); high fat- diet + EA; high fat- diet + HE; and high fat- diet + PL (0.26 mg/kg/body weight). The animals were treated for a period of four weeks. During the treatment, body weight and food intake were evaluated. At the end of treatment, glucose tolerance and insulin sensitivity tests were performed to assess glycemic metabolism. The animals were then sacrificed by guillotine decapitation and blood samples were collected for biochemical analyzes of glucose, triglycerides and total cholesterol. The study was approved by the Committee of Ethics and Experimental Research of the State University of Montes Claros. The results were described in two articles. The first showed the therapeutic effects and biological properties of *D. elliptica*. In the article search strategy, the term "*Davilla elliptica*" was used as descriptor, and literature sources consisted of articles, congress proceedings, books, theses and dissertations. In the second article, the main findings showed a reduction of adiposity and body weight in animals fed a high fat- diet, and animals fed a standard diet showed improvements in biochemical parameters. This is the first study to investigate the role of *D. elliptica* on the metabolic parameters of obesity. Future analyzes will be carried out to investigate by which mechanisms and signaling pathways *D. elliptica* acts on obesity.

Key words: Obesity. Adipose tissue. Medicinal plant. *D. elliptica*.

LISTA DE ILUSTRAÇÕES

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LISTA DE ABREVIATURAS E SIGLAS

OMS	Organização mundial de saúde
TAB	Tecido adiposo branco
IL-1β	Interleucina 1 beta
IL-6	Interleucina 6
TNF-α	Fator de necrose tumoral alfa
LDL	Lipoproteína de baixa densidade
HDL	Lipoproteína de alta densidade
TAM	Tecido adiposo marrom
UCP1	Proteína desacopladora 1
PNPIC	Política Nacional de Práticas Integrativas e Complementares
SUS	Sistema único de saúde

SUMÁRIO

1 INTRODUÇÃO

1.1 Obesidade

A obesidade está comumente associada a um aumento significativo da mortalidade e da redução da expectativa de vida de 5 a 10 anos (1-3). Caracterizada pelo acúmulo anormal de gordura corporal, a obesidade resulta do desequilíbrio entre o consumo e o gasto de energia (4). Segundo dados da Organização Mundial de Saúde (OMS), é uma epidemia global, um crescente problema de saúde pública (5).

Durante a obesidade, mudanças fenotípicas ocorrem no tecido adiposo, subsequente ao processo de inflamação (1, 6). O tecido adiposo branco (TAB) é um órgão multifatorial capaz de alterar suas dimensões, tamanho e estado inflamatório, em resposta ao estado nutricional (7-9).

A expansão anormal do tecido adiposo branco é responsável por provocar alterações na composição extracelular, vascularização, tamanho e estado inflamatório das células imunes infiltradas, aumentando níveis de ácidos graxos livres circulantes e fatores pró-inflamatórios como: fator de necrose tumoral alfa (TNF-α) e interleucinas, IL-1β, IL-6 (9, 10).

A obesidade é ainda associada à um quadro de dislipidemia, com aumento nos níveis da lipoproteína de baixa densidade (LDL), diminuição da lipoproteína de alta densidade (HDL) além de níveis elevados de triglicérides (1, 8) (Fig. 1).

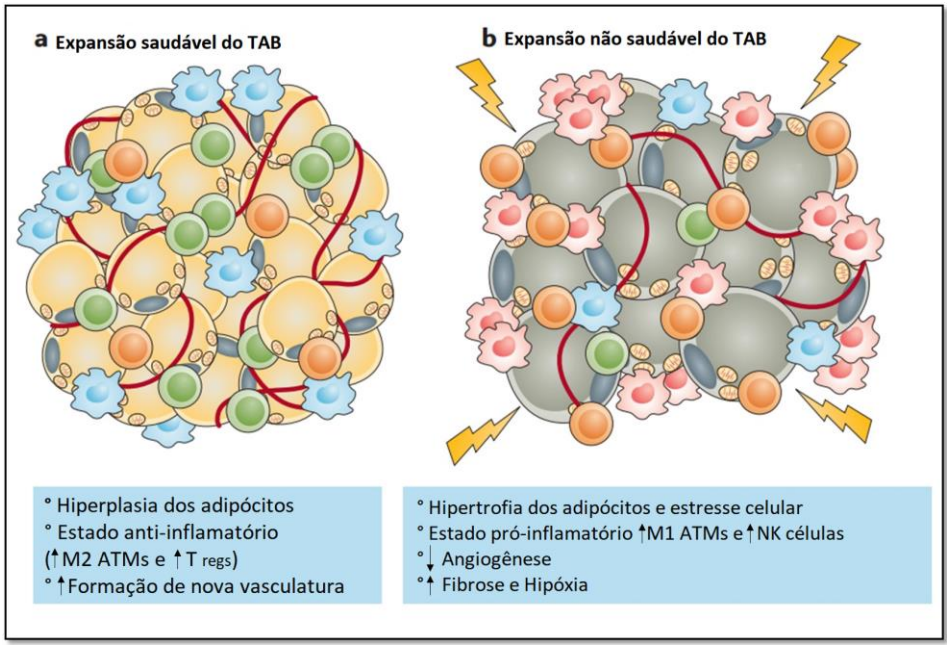

Figura 1: Expansão do tecido adiposo branco sobre condições fisiológicas distintas. a) Durante a expansão do TAB saudável, o tecido está em um estado anti-inflamatório, há

vasculatura suficiente para suportar a expansão e os adipócitos sofrem hiperplasia. b) TAB não saudável abriga adipócitos hipertróficos aumentados e um estado elevado de inflamação: macrófagos M1 pró-inflamatórios infiltrados. A formação de nova vasculatura ocorre para suportar o crescimento de TAB. Estes eventos contribuem para o desenvolvimento da resistência à insulina. Adaptado de Kusminski CM, Bickel PE, Scherer PE, 2016 (7).

Em mamíferos, além do tecido adiposo branco encontra-se o tecido adiposo marrom (TAM) que atua tanto no armazenamento de nutrientes como lipídios quanto dissipando energia em forma de calor em um processo chamado termogênese (11).

Os adipócitos castanhos são caracterizados por uma estrutura de gotículas lipídicas, multiloculares, com alto conteúdo de mitocôndrias e produção da proteína desacopladora 1 (UCP1) que está localizada na membrana mitocondrial interna (11).

A ativação de TAM está positivamente correlacionada com a quantidade desse tecido, o seu estado de ativação e fatores ambientais, tais como baixas temperaturas. Em humanos, a exposição repetida ao frio leva ao aumento da atividade do TAM. Esse aumento na atividade do TAM também tem sido fortemente correlacionado com aumentos induzidos pelo frio no gasto de energia pela termogênese sem tremores (12-17) (Fig. 2).

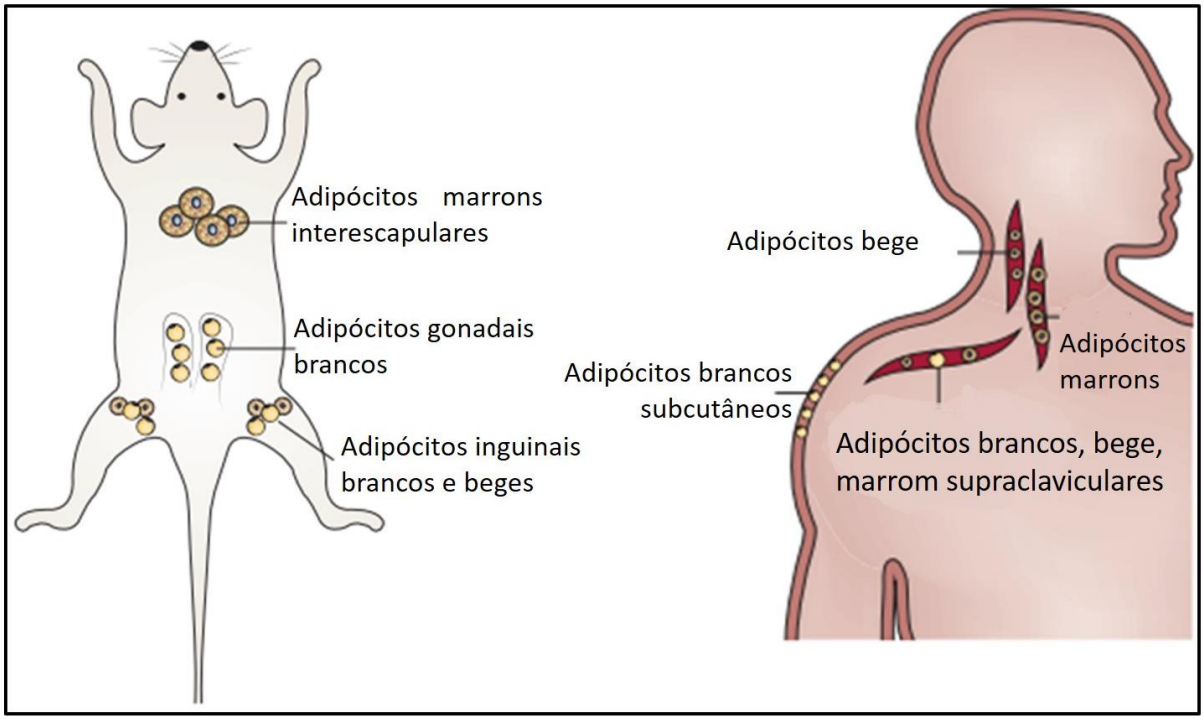

Figura 2: Locais anatômicos de deposição de adipócitos marrons, brancos e beges em camundongos e humanos. a) Em camundongos, o tecido adiposo marrom interescapular contém adipócitos marrons clássicos. O tecido adiposo branco do epidídimo contém

predominantemente adipócitos brancos. O tecido adiposo branco inguinal contém uma população mista de adipócitos brancos e beges, cujas proporções dependem do meio ambiente e dieta. b) Nos adultos humanos, a gordura subcutânea possui características do tecido adiposo branco clássico. O tecido adiposo marrom supraclavicular é composto por adipócitos brancos, marrons e beges, enquanto no marrom clássico o tecido adiposo pode ser encontrado no fundo do pescoço, próximo ao tecido muscular. Adaptado de Bartelt A, & Heeren J, 2014 (11).

Como alternativa para o tratamento e prevenção da obesidade, espécies de plantas do Cerrado têm sido alvo de interesse, alguns fatores poderiam explicar o interesse na pesquisa desse campo, a busca por terapias menos agressivas, resgatando o conhecimento tradicional da população e os avanços ocorridos na área da pesquisa científica (25).

1. 2 Cerrado

O bioma brasileiro Cerrado é a flora que apresenta maior riqueza de todas as Savanas do mundo. É um reservatório de potenciais espécies com atividades terapêuticas. Com mais de 10.000 espécies de plantas das quais 4.000 são endêmicas, o Cerrado é um *hotspot* global de biodiversidade (44). Embora seja fundamental para a conservação de espécies e para o suprimento de serviços ecossistêmicos, o Cerrado perdeu 88 milhões de hectares – Mha (46%) de sua cobertura vegetal nativa e apenas 19,8% permanecem inalterados (18).

O Cerrado compreende um tipo de vegetação associado a condições ecológicas especiais em que a vegetação de Savana domina, mas não é necessariamente exclusiva, sendo intercalada com matas ciliares ou matas de galeria, trechos de florestas semidecídua, ambientes úmidos, pântano e / ou pântanos (19, 20). No entanto, o cerrado *sensu lato* inclui uma variedade de fisionomias de campo limpo, campo sujo, cerrado *sensu stricto* e “cerradão” (19). Constitui uma formação florestal, em que a densidade de indivíduos lenhosos (árvores e arbustos) é uma das variáveis mais evidentes (21).

O Rio Pandeiros, localizado no interior do Norte de Minas Gerais, inicialmente chamado de “*Sertão*” por Auguste de Saint-Hilaire, comporta uma flora abundante em espécies com potenciais farmacológicos. Nesse contexto, a pesquisa histórica explana uma rica fonte de informações sobre o uso da biodiversidade brasileira. Mügge e colaboradores (2016) propõem que os frutos de algumas espécies nativas do Cerrado, como *Xylopia aromática*, *Davilla rugosa* e *Stachytarpheta jamaicensis*, possuem um alto valor econômico e são utilizadas para produzir renda às populações do interior de Minas Gerais. As espécies

Davilla elliptica, *Davilla papyracea* e *Davilla angustifólia* pertencentes ao gênero *Davilla* também foram registradas no Cerrado mineiro (22).

O Cerrado é detentor de expressivo potencial de plantas com fins terapêuticos, porém requer especial atenção, novas pesquisas e incentivos, objetivando o uso racional pela população (23).

1.3 Plantas medicinais

Plantas medicinais são utilizadas na medicina popular como medicamentos tradicionais e fonte de tratamento há milhares de anos (24).

O uso de plantas com fins medicinais, para o tratamento, cura e prevenção de doenças é uma das mais antigas formas de prática medicinal utilizada pela humanidade. Na década de 1990, a Organização Mundial de Saúde (OMS) notificou que 65-80% da população dos países em desenvolvimento dependiam principalmente das plantas como única forma de acesso aos cuidados básicos de saúde, sendo que sua utilização tem sido crescente em países desenvolvidos (25).

Diante do exposto, o termo “planta medicinal” é definido pela OMS como sendo “todo ou qualquer material vegetal que possui, em um ou mais órgãos vegetais, substâncias que podem ser utilizadas com fins terapêuticos ou que sejam precursores de fármacos semissintéticos”. Sendo relatado que as plantas do Cerrado são fonte de compostos de alto interesse biotecnológico, com aplicações nas indústrias alimentícias, cosméticas e farmacêuticas (26).

A utilização de plantas medicinais, como recurso terapêutico alternativo ou adicional, tem aumentado consideravelmente e têm sido utilizadas no tratamento de vários problemas de saúde e este vasto consumo tem sido sob a forma de pó, chás, óleos ou formas associadas (27).

No Brasil, a Política Nacional de Práticas Integrativas e Complementares (PNPIC), aprovada em 2006, segue as recomendações preconizadas pela OMS e se propõe a normatizar, adequar e implantar o uso de plantas medicinais como fitoterápicos no Sistema Único de Saúde (SUS), com o objetivo de reduzir custos, aumentar as formas de terapias e resgatar os conhecimentos tradicionais da população (28).

Em 2010, foi criada a Farmácia Viva, responsável por todo o processamento das plantas medicinais desde o cultivo até a comercialização. Diversos municípios brasileiros estão inseridos nesse programa. A secretaria do Estado de Saúde de Minas Gerais percebeu a

necessidade de conhecer as diferentes atividades praticadas para tratamentos realizados na rede pública de saúde do estado a partir da Resolução nº 1.885, de 27 de maio de 2009 e implantou estratégias preconizadas pela PNPIC no SUS/MG com o intuito de melhorar a qualidade de vida da população, por integração multidisciplinar entre a cultura popular e a ciência (28).

O uso das plantas medicinais cresceu substancialmente, estes produtos naturais continuam a ser um complemento terapêutico, ou mesmo a base, para o tratamento de doenças (29).

1.4 Gênero *Davilla*

O gênero *Davilla* é definido, segundo Kubitzki (1971), por apresentar margens flexionadas, não alatas, das sépalas mais internas, sobrepostas pelas sépalas internas adjacentes (30). É amplamente distribuído e tem como outros exemplos de espécie a *D. kunthii, D. nitida* (Vahl.) *Kubitzki, D. rugosa* (Poir.), com distribuição geográfica no Neotrópico. Diferenciando-se das outras espécies do gênero, que são ocorrentes no cerrado, a *D. flexuosa* é geograficamente encontrada em áreas com solos arenosos, como os existentes nas áreas da planície quaternária litorânea, comumente denominada Restinga (31, 32). A classe de compostos comumente encontrada em todos estes gêneros é a classe de flavonoides, conforme mostrado na Tabela 1.

Estudos demonstram que algumas espécies do gênero *Davilla*, como *D. rugosa, D. kunthii, D. nítida,* apresentam propriedades adaptogênicas, ansiolíticas, antimicrobianas, antioxidantes e antiulcerativas (33, 34). Ensaios *in vivo* e *in vitro* evidenciam que os extratos de *D. nitida* e *D. kunthii* promovem uma inibição de bactérias multirresistentes das espécies *Pseudomonas spp., Enterobacer spp., Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus saprophyticus,* bem como, levedura *Candida albicans,* respectivamente (35, 36). Adicionalmente, atividades adaptogênicas foram ainda observadas para a espécie *D. rugosa*, com efeito protetor contra úlceras em modelos experimentais in vivo (33, 34).

Diante da importância da espécie *Davilla elliptica* e seus potenciais efeitos farmacológicos já descritos, é de fundamental importância a análise do seu potencial anti-inflamatório sobre a obesidade e suas comorbidades associadas, de forma que a *D. elliptica* poderá contribuir para a identificação de novos alvos terapêuticos capazes de atuar simultaneamente nos fatores de risco desencadeantes da doença, permitindo um melhor prognóstico e tratamento de doenças relacionadas à obesidade.

Tabela 1. Descrição das principais espécies do gênero *Davilla* e a relação de compostos químicos com sua atividade biológica.

Espécie	**Parte vegetativa**	**Fração**	**Substâncias**	**Atividade biológica**	**Autor**
D. flexuosa St. Hill.	Folhas	Hexano; acetato de etila	Miricetina; quercetina miricetina 3-ramnosídeo; miricetina 3'- ramnosídeo α-tocoferol	Não descrito	David *et al*., (1996) (37)
		Hidroalcoólico; clorofórmio; clorofórmio/acetato de etila; acetato de etila; acetato de etila/ etanol; etanol; etanol/água.		Efeitos estimulantes; Atividade intestinal; Atividade motora e ansiolítica.	Guaraldo *et al*., (2000) (38)
D. rugosa Poiret	Hastes	Hidroalcoólico; clorofórmio; clorofórmio/acetato de etila; acetato de etila; acetato de etila/etanol; etanol; etanol/água.	Saponinas; flavonoides e mucilagem	Anti-úlceras gástricas.	Guaraldo, L.; Sertie, J. A. A.; Bacchi, E. M. (2001) (39)
		Hidrometanólico; hexano; clorofórmio; acetato de etíla	Friedelina; sitostenona ácido betulínico; narigenina e quercetina 4'-O-metil taxifolina	Não descrito	David *et al.*, (2006) (40)
	Partes aéreas	Hidroalcoólico	Alcaloides; flavonoides; saponinas; polifenóis/taninos; cumarinas; lignanas	Atividade adaptogênica; Atividade antioxidante; Efeito moderado antiúlcera;	Mendes, F. R.; Tabach, R.; Carlini, E. A. (2007) (41)
D. kunthii A. St. -Hil.	Folhas	Etanólico		Atividade antimicrobiana e antioxidante	Nascimento *et al.*, (2016) (42)
Davilla nitida (Vahl) Kubitzki	Casca	Etanólico	Terpenos Flavonoides Taninos	Atividade antibacteriana	Perim *et al*., (2018) (43)

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o potencial do extrato hidroalcoólico das folhas da *Davilla elliptica* sobre parâmetros metabólicos de camundongos obesos submetidos a uma dieta hiperlipídica.

2.2 Objetivos específicos

2.2.1 Caracterizar as classes de compostos presentes nas folhas da espécie *D. elliptica*.

2.2.2 Avaliar o efeito da administração do extrato hidroalcoólico e das frações das folhas de *Davilla elliptica*, nos diferentes grupos de estudo.

2.2.3 Avaliar a regulação glicêmica por meio de testes de sensibilidade insulínica e tolerância à glicose.

2.2.4 Mensurar os níveis plasmáticos lipídicos, dosando especificamente os níveis de triglicérides e colesterol total.

2.2.5 Mensurar os níveis de glicose sanguínea.

5 PRODUTOS

5.1 Produto 1: *Davilla elliptica (Dilleniaceae) A. St.-Hil. medicinal, pharmacological and phytochemical aspects: a review,* formatado segundo as normas para publicação do periódico Journal of Ethnopharmacology.

5.2 Produto 2: *Effects of oral treatment with Davilla elliptica leaf extract on diet-induced obese mice,* formatado segundo as normas para publicação do periódico Journal of Ethnopharmacology.

5.1 PRODUTO 1

Davilla elliptica (Dilleniaceae) A. St.-Hil. medicinal, pharmacological and phytochemical aspects: a review

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ABSTRACT

Ethnopharmacological relevance: Davilla elliptica (Dilleniaceae) is a shrub that occurs naturally in the Brazilian Savannah (Cerrado). *D. elliptica* and its relative specie *D. rugosa* have been traditionally used in Brazilian folk medicine on tea preparing, infusions, and decoctions from leaves and roots for gastritis, ulcers and diarrhea treatment.

Aims: The present study aimed to provide a comprehensive review on ethnopharmacological uses, botanical aspects of *Davilla elliptica,* as well as research findings about phytochemistry characterization, *in vivo* and *in vitro* pharmacological studies using extracts, fractions and/or isolated compounds that support its potential therapeutic to treat several human diseases.

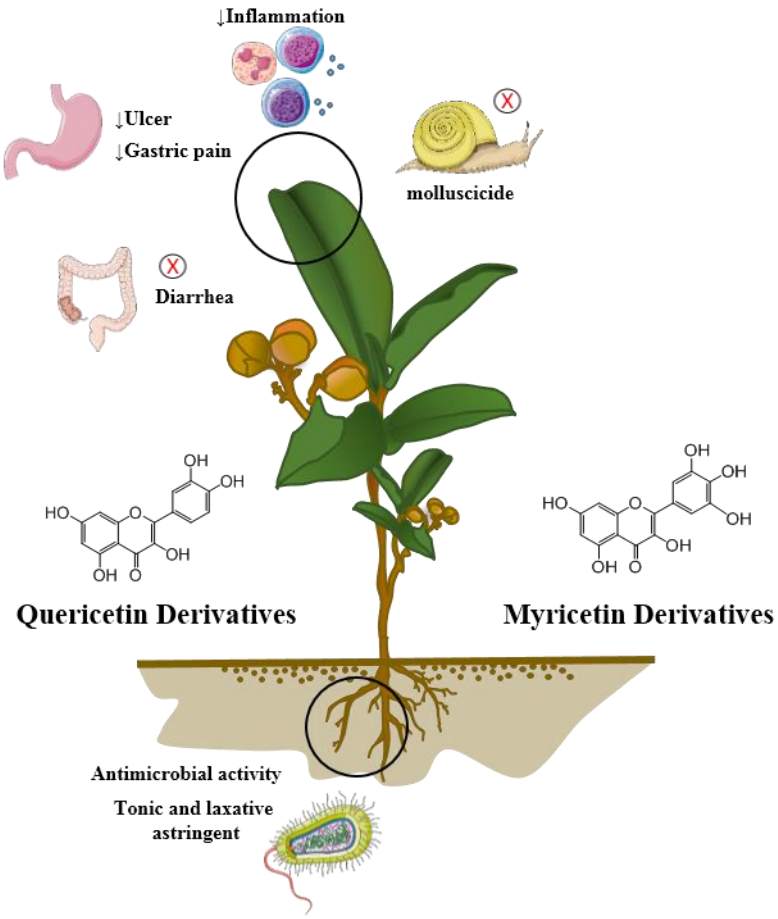
Materials and methods: The relevant and updated reports about *D. elliptica* were retrieved from the scientific databases Web of Science, Google Scholar, Scopus, SciFinder, PubMed,

Scielo, and ScienceDirect. Additional information derived from un-published resources and other literature sources such as books, Ph.D. and MSc thesis was also considered after a critical review.

Results: Ethnopharmacological reports of *Davilla elliptica* revealed the use of infusions from roots with astringent, tonic and purgative effects, whereas leaves have been used as anti-inflammatory, anti-ulcerative, and to treat diarrhea and gastric disorders. Also, fresh leaves have been used in baths to treat swellings. Phytochemical studies has led to the isolation of relevant secondary compounds such as terpenes, flavonoids derived from quercetins and myricetins, a higher concentration of tannins, and others. *In vitro* assays using crude extracts and/or isolated fractions of leaves and roots from *D. elliptica* have evidenced antibacterial, antioxidants and antitumoral effects, while *in vivo* studies have proven antinociceptive or gastroprotective effects from hydroalcoholic extract. Concerning toxicological effects of extracts and fractions from *D. elliptica*, one report showed slight mutagenicity potential in *Salmonella*, while no significant genotoxic effect was detected in mice. However, further studies with long-term and repeated administration and/or the use of higher concentration of extracts are needed to ensure the safety of the therapeutic use of *D. elliptica*.

Conclusion: This review summarizes current knowledge about ethnomedicinal use of *D. elliptica*, provides relevant information about its botanical aspects, current taxonomy and distribution and highlights main findings from research studies related to its phytochemical characterization, toxicology and efficacy of crude extracts, fractions and isolated compounds of *D. elliptica* with relation to their therapeutic potential. We found scientific evidences that corroborates the popular use of *D. elliptica* to treat several human diseases, particularly those related to gastric disorders and inflammation. However, these studies were preliminary, based on *in vivo* studies using animal models, which requires further investigation in preclinical and clinical trials to confirm these therapeutic effects. Also, studies underlying molecular mechanisms involved with mechanism of action of the bioactive components and tests using different fractions or isolated compounds are still lacking, open great opportunities for future studies.

Graphic abstract



Keywords: ethnomedicine; phytochemistry; inflammation; antioxidant; antimicrobial.

Abbreviations

COX-1, Cyclooxygenase-1; GST, Glutathione S-transferase; H_2O_2, Hydrogen peroxide; IC, inhibitory concentration; iNOS, Inducible nitric oxide synthase; LD, lethal dose; LPS, Lipopolysacharides; MIC, Minimal inhibitory concentration; MMP-9, Metallo-9-matrix proteinases; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H- tetrazolium bromide; NF-Kb, Factor nuclear kappa B; NO, Nitric oxide; NO_2, Nitrogen dioxide; SOD, Superoxide dismutase; TNF-α: Tumor necrosis factor alpha; WHO,World Health Organization.

Chemical compounds studied in this article

(–)-epicatechin (PubChem CID: 72276); gallic acid (PubChem CID: 370); kaempferol (PubChem CID: 5280863); quercetin (PubChem CID: 5280343); isoquercetin ou quercetin-3-glucoside (PubChem CID: 5280804); quercitrin ou quercetin-3-rhamnoside (PubChem CID: 5280459); hyperoside ou quercetin-3-O-β-D-galactopyranoside (PubChem CID: 5281643); guajavarin ou quercetin-3-O-α-L-arabinopyranoside (PubChem CID: 5481224); quercetin-3-

O-α-L-rhamnopyranoside (PubChem CID: 40486293); myricetin (PubChem CID: 5281672); myricetin-3-arabinoside (PubChem CID: 21672568) myricitrin ou myricetin-3-O-α-L-rhamnopyranoside (PubChem CID: 5281673); myricetin 3-galactoside ou myricetin-3-O-β-D-galactopyranoside (PubChem CID: 5491408); myricetin-3-O-β-D-glucopyranoside (PubChem CID: 5318606); myricetin-3-O-(2″-O-galloyl)-α-L-rhamnopyranoside (PubChem CID: 5316590); 3-O-galloylmucic acid ou myricetin-3-O-(3″-O-galloyl)-α-L-rhamnopyranoside (PubChem CID: 101248998); myricetin-3-O-(2″-O-galloyl)-β-D-galactopyranoside (PubChem CID: 101114351); (6S)-vomifoliol ou (6S,7E)-6,9-dihydroxy-4,7-megastigmadien-3-one (PubChem CID: 12444927); rutin (PubChem CID: 5280805).

1. Introduction

Natural products derived from plants have been considered a primary source of chemical diversity with therapeutic purposes by traditional medicine and have prompted the development of many drugs over the past century (Veeresham, Ciddi, 2012). Throughout the history, the folk use of plants has been well documented. Plant extracts have been used in the form of natural remedies such as teas, potions and oils with many of these bioactive compounds still being unidentified (Dias, Urban and Roessner, 2012; Gurib-Fakim, 2006). According to the World Health Organization (WHO), the plant-based traditional medicines have been widely disseminated worldwide, especially in developing countries where population commonly use herbs for primary health care (Akerele, O., 1993). It has been estimated that approximately 80% of 122 plant derived drugs developed exert similar or related effects based on the original ethnopharmacological knowledge (Fabricant and Norman, 2001). However, nearly 10% of the total biodiversity of higher plant species have been systematically explored (Cragg and Newman, 2005), open a wide opportunity to the discovery of new drug leads using plants as source, especially in Brazil, that has one of the richest flora worldwide.

In Brazil, The Brazilian Savannah, also known as Cerrado, has a high biodiversity, with approximately 10.000 plant species of which 4.000 are endemics, which characterize this biome as a hotspot (Myers et al., 2000). From these plant species stands out the medicinal plants. Among those, *Davilla elliptica* used in popular medicine for the treatment and prevention of some diseases, with proven pharmacological effects. Furthermore, this species presents important molecules with evidenced biological activities.

In this sense, this study presents a comprehensive review about the therapeutic potential of *Davilla elliptica*, starting from its geographic distribution, botanical

characterization and the current knowledge about its ethnomedicinal use. Following, we summarized the relevant research findings related to its phytochemical characterization, and scientific evidences supported by *in vitro* and *in vivo* experiments which corroborate the bioactive effects attributed to *D. elliptica* in traditional practices and other bioactive effects recently reported. Finally, discussions for directing future research were also proposed.

2. Materials and methods

Data on the *Davilla elliptica* botanical, and phytochemical description, and traditional and pharmacological use, published up to November 2018, were retrieved from the scientific literature in the following databases: Web of Science, Google Scholar, PubMed, Scielo, and ScienceDirect, in addition to the universities repositories that contain thesis and congress presentations. The term "*Davilla elliptica*" was used as a descriptor. The data was selected after a critical paired reading of the main findings to ensure more reliability of the data. The scientific names and botanical descriptions were validated in the following websites: www.theplantlist.org and floradobrasil.jbrj.gov.br/reflora. The molecules illustrations were carried out in the ChemDraw software (version 12.0), while the scheme pictures were created by the authors with the resources available in the smart.servier.com website. Photos of the studied species were accessed from the author's personal archives. The data recovery and molecules registry were assessed at pubchem.ncbi.nlm.nih.gov.

3. Botanical description and distribution Phenotypic characterization

Dilleniaceae is a family of plants with 14 genders, which comprise about 500 species with different arboreal, shrub and liana habits. Among them is the *Davilla* genus, with 20 species widely distributed in neotropical regions, being found in the central Savannah in Brazil. Within these species is *Davilla elliptica* A. St.-Hil, popularly known as "*lixeirinha*", "*lixeira*", "*bugre*", "*sambaibinha*", "*muricizinho*", "*cipó-caboclo*", "*cipó-de-carijó*", "*pau-de-bugre*" and "*lixinha*". The name "*elliptica*" is derived from the elliptical morphological aspect of their leaves, which have sinuous and slightly serrated edges, rough on the adaxial surface and hairy in the abaxial surface (Bustamante et al., 2004; Horn, 2009; Jácome et al., 2010; Santos and Ferreira, 2012). It has shrub and sub-bush habits, with ramifications of 0.6 cm up to 3 m in height, as shown in Figure 1. A number of idioblasts and a high mucilage content have also been reported to this species, and these characteristics were responsible for distinguishing *D. elliptica* from its relative species *D. rugosa* (Jácome et al., 2010).

This species to *Davilla elliptica* is characterized by phytochemical compounds presence, such as myricetin and quercetin rhamnoside. The specific name, *D. elliptica*, refers to the leaf of elliptical ambit a characteristic present in most of the species of the genus *Davilla* (Gurni and Kubitzki, 1981; Horn, 2009).

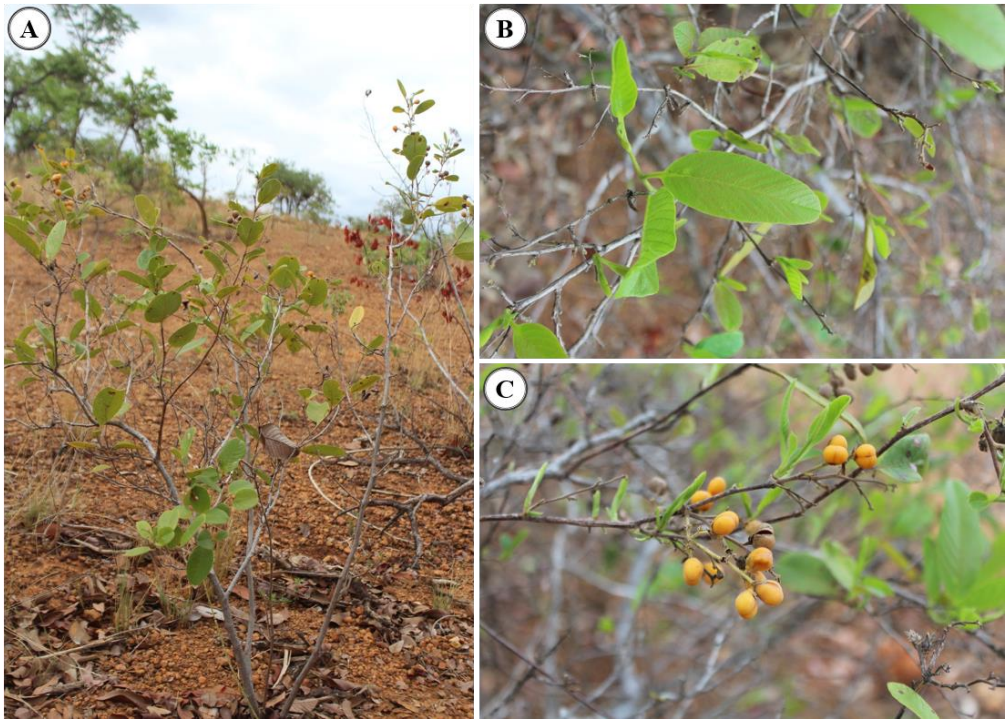


Figure. 1 Phenotypic characteristics of *D. elliptica* showing young shrub (**A**), leaves (**B**) and mature fruits e ripe and dry fruits (**C**).

Regarding the *D. elliptica* vegetative development varies, it presents variations according to the environment: from evergreen, with continuous growth and brevideciduous behavior in which is inserted, justifying the differences found in the literature. A study by Silvério and Lenza (2010) evidenced an evergreen, with continuous growth and brevideciduous behavior, while a seasonal and deciduous growth was also reported by others (Lenza and Klink, 2006). In contrast, Lenza and Klink (2006) reported this species also as evergreen, but with seasonal and deciduous growth. Buds also occur in the dry season and may be less frequent in rainy conditions (Lenza and Klink, 2006; Silvério and Lenza, 2010). Leaf senescence occurs between September and October, when the dry season ends. During this period, the Budding occurs in dry season and may be less frequent in rainy conditions, whereas leaf senescence occurs at the end of this period (Lenza and Klink, 2006; Silvério and Lenza, 2010). After that, plants remain without leaves for approximately two weeks. The

same variations observed for the leaves were verified in the flowers, which arise in low-temperature months (17 to 18 °C), between January and May. The fruiting, on the other hand, occurs in the dry season, indicating that the species has mechanisms to overcome water stress (Gurni and Kubitzki, 1981; Horn, 2009). Fruit maturation occurs mainly during rainy periods and can be seen in the dry season. Seed dispersal occurs through the dry and rainy period.

The environmental variations in which the species is distributed impactly directly in the anatomy, interfering in the height, fruit biomass, and leaves width and length. Regarding plant height, the individuals found in Veredas regions are higher, as well as with higher population density and flowering anticipation as compared to the Cerrado environment, where leaves length and width are higher. Regarding the fruits number, no significant differences were evidenced between the observed phytophysiognomies (Santos and Ferreira, 2012; Rocha Filho et al., 2006).

Concerning its distribution, studies have been shown that *D. elliptica* is an endemic shrub vegetation widely distributed at the Cerrado biome that occurs along a latitudinal gradient of about 1500 km, being found in areas such as cerrado *stricto sensu*, savannah and rupestrian field. In general, the environmental conditions of the cerrado are favorable for the occurrence of the species of the family Dilleniaceae, and *D. elliptica* is among the main representatives species both in frequency and in abundance. Besides of cerrado, *D. elliptica* is also found in the Chaco of Bolivia, with an estimated occurrence of more than 20,000 km^2 in a fragmented area concomitantly with agricultural crops such as soybean (Pereira, 2014).

Jácome et al. (2010) demonstrated for the first time in their studies an important pharmacognostic characteristic of the species, which presents increased idioblasts number followed by a high mucilage content, being these characteristics responsible for distinguishing *D. elliptica* from its relative species *D. rugosa* (Jácome et al., 2010).

4. Fruits and seeds production

The *D. elliptica* produces fruits and flowers protected by two synaparic capsules, which remain closed during maturation and exposes the flower in the pollination period. After the ovary fecundation, the synaparic capsules are closed until the end of the seeds maturation period. Each fruit produces a maximum of two seeds (Iubatã P. Faria et al. 2003).

In a study carried out in areas of the Cerrado *stricto sensu* and rupestrian fields, the proportion of intact seeds was 66% in the rupestrian fields and 29% in the Cerrado. This species is responsible for 32% of the seeds aborted in these two areas. Based on these findings, we conclude that the seeds abortion is the main determinant factor for the

development deficit of this plant in a few Cerrado regions, although the flowers predation rates in the rupestrian field and Cerrado *stricto sensu* correspond to 31% and 3%, respectively. Lepidoptera and Coleoptera larvae represent the main species responsible for the *D. elliptica* herbivorism (Iubatã P. Faria et al. 2003).

5. Distribution

The *D. elliptica* distribution in the Cerrado biome evidenced that this species is an endemic bush of shrub vegetation that occurs along a latitudinal gradient of approximately 1500 km, as the Cerrado *stricto sensu*, Savannah, and rupestrian field. The *D. elliptica*, with a wide distribution, is among the main Cerrado representatives, both in frequency and abundance. In general, the Cerrado environmental conditions are favorable to the occurrence of species from the *Dilleniaceae* family (Pereira, 2014).

Therefore, this species was collected in several disturbed environments, allowing it to be included in the low-risk species classification according to the International Union for Conservation of Nature criteria (Fraga, 2012).

Due to environmental factors such as population growth or competitor's interactions, the absence of this species can be observed in small geographic areas that could be favorable to its development. Therefore, it is verified that *D. elliptica* occurs in isolated areas, indicating that its dispersion is not a limiting factor (Pereira, 2014).

6. Population dynamics

Studies performed by Aquino et al. 2007 in areas of the Cerrado, demonstrated that specimens of *D. elliptica* presented an accentuated decrease in density and baseline area from between 1995 and 2002 when a high mortality rate was registered for this species. However, the seedlings recruitment registered in the *D. elliptica* vegetal community reached 12% year, opposing the high mortality rates. Felfili (1995) explained these findings by reporting that abundant species in the gallery forests are subjected to increased mortality and recruitment rates (Aquino et al., 2007; Felfili, 1995).

In this context, it is possible to observe that during the dry season, the occurrence of fires is frequent. The fire is responsible for altering the structure, flower composition, and plants recruitment, leading to a decreased trees density, thus changing the vegetal composition (Medeiros and Miranda, 2005; Felfili et al., 2000; Woods, 1989).

Studies report that *D. elliptica* is in great number in all Cerrado areas hit by fire. In the community dynamics, this species is responsible for recruiting individuals in 4.8%, despite being more sensitive to mortality by fires, totalizing 16.5% of dead individuals. In contrast,

this species presents high abundance in natural regeneration, with a relative density greater than or equal to 1, evidencing its potential to undergo intervention through a sustainable management regime (Barreira et al. 2002; Fiedler et al., 2004; Ribeiro et al., 2012).

7. Ethnopharmacological studies

In popular medicine, *D. elliptica* is widely disseminated by its medicinal use applications, as shown in Figure 2. Leaves have been used in the preparation of teas to treat gastric, inflammation, diarrhea and ulcer pain whereas fresh leaves have been used in baths to treat lymph nodes and testicular swellings. Roots infusion have been employed as tonic and laxative astringent. Other properties have been reported in ethnomedicinal practices such as its general use as , sedative, diuretic, contraceptive, antiseptic, and in the treatment of hemorrhoids, hematomas and hernia (Azevedo et al., 2007; Bonacorsi et al., 2013; Campos et al., 2013; Kushima et al., 2009; Michelin et al., 2005; Rodrigues and Carvalho, 2001). Aphrodisiac, healing and emollient properties were also reported previously, but in this study *D. elliptica*, *D. brasiliana*, *D. cearensis* and other species were depicted as synonyms of *D. rugosa*, which makes it difficult to know from which species of *Davilla* these properties were attributed. However, more recently, a comprehensive phylogenetic study confirmed that these species are distinct each others, and a taxonomic revision of *Dilleniaceae* family was proposed (Fraga et al., 2012).

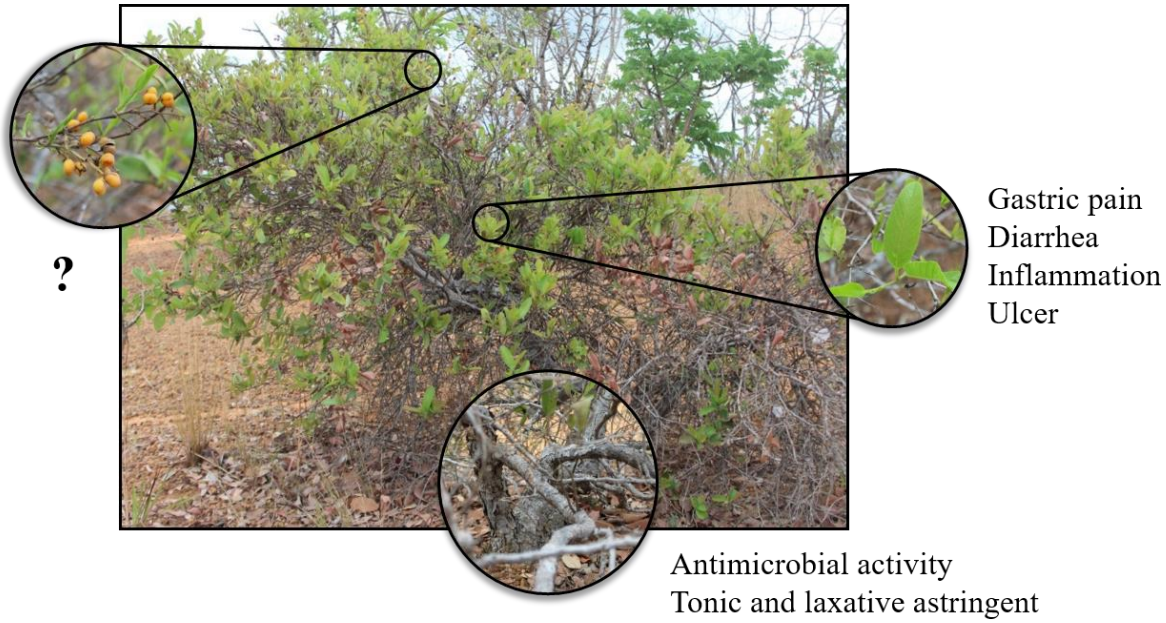


Figure 2. Effects ethnobotanical observed in popular medicine from different parts of *Davilla elliptica* in the treatment of diseases.

Additionally, Mendes and contributors reported other *D. elliptica* properties, including healing, emollient and aphrodisiac. However, the authors considered *D. elliptica*, *D. brasiliana*, and *D. cearensis*, among other species as synonyms of *D. rugosa.* However, Fraga (2012) presented a phylogenetic classification and taxonomic review of the *Davilla Vand.* (Dilleniaceae), reporting the distribution, etymology, taxonomy, typification and characterizing species of the genus, as distinct species (Mendes et al., 2005; Fraga, 2012).

8. Phytochemistry

Phytochemical studies have been reporte devidenced important compounds classes in *D. elliptica*, such as triterpenes, steroids, catechins, flavonoids, saponins, phenolic acid derivatives, coumarins, condensed tannins and tannins, the latter being in higher concentrations (Carlos et al., 2005; Soares et al., 2005). Also, phytochemical characterization using high-performance liquid chromatography, gas chromatography coupled to mass spectrometry, confirmed by certified standards or the nuclear magnetic resonance technique were able to identify compounds such as epicatechin, gallic acid, kaempferol, quercetin derivatives, myricetin derivatives, and rutin (Biso et al., 2008). Analyzes have demonstrated compounds such as epicatechin, gallic acid, kaempferol, quercetin derivatives, myricetin derivatives, and rutin, as detailed in Figure 3 (Biso et al., 2008; Campos et al., 2013; Kushima et al., 2009; Azevedo et al., 2015; Rinaldo et al., 2006; Rodrigues et al., 2008). These findings showed that myricetin-3-O-α-L-rhamnopyranoside was reported as the main flavonoid present in this species (Kushima et al., 2009). As expected, seasonal variations were found to have has a significant influence on the presence of secondary metabolites, where the content of flavonoids and tannins the rainy season, in an analysis of ten grams of dry leaf, quantified in 0.91% and 7.20%, respectively. On the other hand, analyzes with leaf samples collected during the dry period presented a total flavonoid and tannin content of 1.20% and 9.89%, respectively (Jácome et al., 2010; Soares et al., 2005).

Although differences were found for flavonoids and tannins in detriment of seasonality, the comparison for the results found is made difficult due to methodological differences, using different proportions of dry mass of the plant for the analyzes. This, in part, explains the gross values between the periods, in addition to the environmental factors already described and recognized as secondary decomposing modulators in vegetables.

These compounds have antimutagenic, antineoplastic, antinoceptive, anti-inflammatory, anti-bacterial and gastroprotective effects and are found in different species. A limiting factor in the study of these compounds is the lack of nomenclature standardization,

thus hindering the data recovery in the literature. In the present review, the most common names used in the literature were used, besides providing different recurrent synonyms, as well as the general characteristics that may be accessed in the molecules registry in the PubChem.

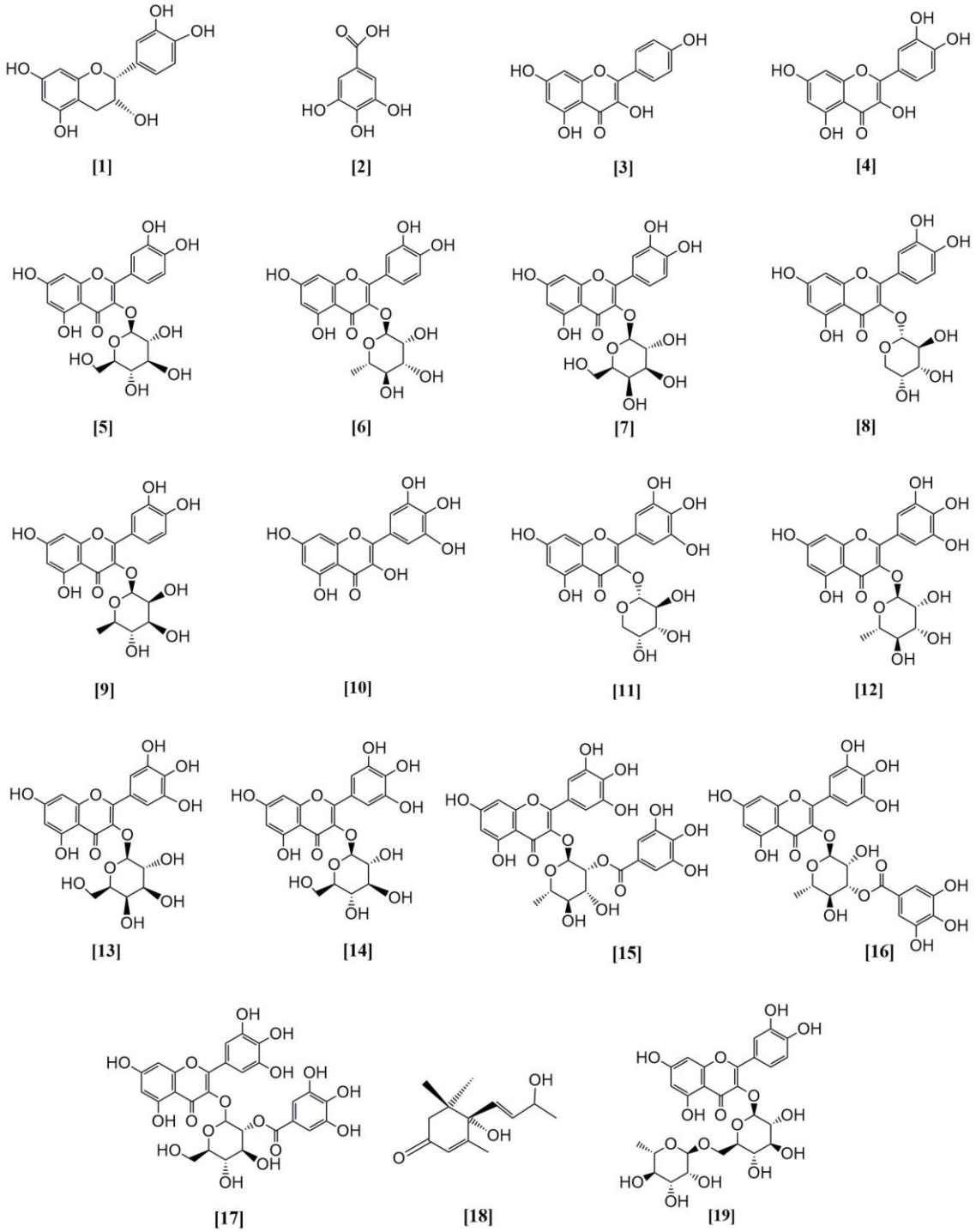


Figure 3. Phytoconstituents molecular structure of different vegetative parts of *Davilla elliptica*. [**1**] (–)-epicatechin, [**2**] gallic acid, [**3**] kaempferol, [**4**] quercetin , [**5**] quercetin-3-glucoside, [**6**] quercetin-3-rhamnoside, [**7**] quercetin-3-O-β-D- galactopyranoside, [**8**]

quercetin-3-O-α-L-arabinopyranoside, [**9**] quercetin- 3-O-α-L-rhamnopyranoside, [**10**] myricetin, [**11**] myricetin-3-arabinoside, [**12**] myricetin-3-O-α-L-rhamnopyranoside, [**13**] myricetin-3-O-β-D-galactopyranoside, [**14**] myricetin-3-O-β-D-glucopyranoside, [**15**] myricetin-3-O-(2″-O-galloyl)-α-L- rhamnopyranoside, [**16**] myricetin- 3-O-(3″-O-galloyl)-α-L-rhamnopyranoside, [**17**] myricetin- 3-O-(2″-O-galloyl)-β-D-galactopyranoside, [**18**] (6S,7E)-6,9-dihydroxy-4,7- megastigmadien-3-one, and [**19**] rutin.

9. Allelopathic activity

Besides the above-mentioned biological properties, phytotoxicity from extracts of *D. elliptica* was also observed to weed species. An inhibitory effect of 58% and 60% of the growth in coleoptiles of wheat were detected using 0.8 mg/mL of the methanolic extract derived from leaves and bark, *D. elliptica* respectively. In general, different extracts from leaves of *D. elliptica* (methanolic, acetonic or aqueous) resulted in significant inhibition of germination rate of weeds, with slight variations according to the plant species (Candido, 2016).

Significant variations were observed during the growth of wheat coleoptiles, under the activity of extract from different *D. elliptica* vegetative parts. The observed growth was proportional to the dose of the ethyl acetate fraction, extracted from the leaves of the species under study. The concentration adjustment from 0.2 mg/mL to 0.8 mg/mL, augmented the inhibition activity of 58% in wheat coleoptiles. However, the stem methanol extract (0.8 mg/mL), reduced 60% of the coleoptiles wheat growth (Candido, 2016).

The results obtained with the extracts application depend on the season when the extract was collected. In this context, the *D. elliptica* leaves extracts significantly decreased the lettuce seeds germination. The inhibition caused by the *D. elliptica* extracts was 75.9% (Gatti, 2008). For the gergelim germination, the *D. elliptica* extracts lead to a germination delay, when seeds were used from two different seasons (Gatti, 2008).

The *D. elliptica* leaves extract seems to be a potent phytotoxic that acts on the growth and development of *E. heterophylla* and *P. maximum* (known as "*amendoim-bravo*" and "*capim-colonião*") (Candido, 2016). The different acetonic, methanolic and aqueous extracts of *D. elliptica* leaf inhibited the percentage of germination, mean time and average seed germination speed of *P. maximum*. On the other hand, this result was not observed in the germination of *E. heterophylla*. The leaves methanolic extracts were more sensitive when administered on the seeds of "*capim-colonião*," especially in the radicular system where it

was observed a greater inhibition rate 70% in the concentration of 2 mg/mL, results compared to the commercial herbicide GOAL 59% (Candido, 2016).

The aqueous extract was responsible for 35% of the “capim-colonião” plantules that exhibited stunted roots. This result differed significantly from ethyl acetate (21%), acetone (15%) and methanolic (13%) acetate extracts (Candido, 2016).

Comparing the results of entropy for the leaves extracts collected in the dry and rainy seasons, it is possible to observe that the values obtained in the dry season were Higher, suggesting a higher greater synchrony on seed germination under the influence of these extracts (Candido, 2016).

For a better understanding by the reader, the inhibition related results 70%, 75.9% should be standardized.

10. Molluscicidal activity

Several human and domestic animals parasitic diseases have mollusks as intermediate hosts, such as snails of the genus *Biomphalaria*, the intermediate host of *Schistosoma mansoni*. Another example is fasciolosis that affects cattle, sheep and goat, significantly increasing the costs for the food industry. This disease has snails of the genus *Lymnaea* as the intermediate host in the parasite life cycle. In this scenario, with the aim to find natural products with molluscicidal activity, hydroalcoholic leaves extracts from two Brazilian Cerrado plant species, such as *D. elliptica* and *D. nitida*, were tested against snails of the genus *Lymnaea* (Gardioli et al., 2017). Although hydroalcoholic extracts of *D. nitida* and *D. elliptica* also presented molluscicidal activity against the *L. columella* species, extracts of *D. elliptica* were more prominent, triggering lethal molluscicides effects as soon as 6h after treatment using 100 mg/ml of hydroalcoholic extract concentration.

In contrast, *D. nitida* reached the same result only after 24 h of treatment. At the same concentration, a lethal dose of 50 (LD50) was determined within 24 h of exposure with extract of *D. elliptica*, resulting in 66% of mollusks deaths, whereas 33% were detected when extract of *D. nitida* was used. In addition, the hydroalcoholic extracts of *D. elliptica* showed an ovicide effect against *L. columella*, with an inhibitory effect of 60% after 15 days of treatment. This study seemed to reveal a potential effect of hydroalcoholic extracts of *D. elliptica*, being higher than its relative specie *D. nitida*, however the authors failed to perform statistical tests to assess the significance of the results.

Gardioli aimed to evaluate the molluscicide activity of the *D. elliptica* and *D. nitida* extracts human parasitic infection control through assessment of hydroalcoholic extract of

these species leaves on the motility and viability of mollusks in intervals of 2, 6, 12 and 24h, in final concentrations of 20, 25, 50 and 100 mg/mL (ppm). The results obtained by the authors point the *D. elliptica* extract as a potential agent in the control of snail's populations. Data point to an inhibitory concentration dose of 100 (IC) in a concentration of 100ppm in 6 hours, in contrast, the same dosage may be observed in the treatment with *D. nitida* extract; however, the latter extract IC was determined in 24 hours.

The 50 lethal doses of the *D. elliptica* species was determined on a 24 hours exposure period, resulting in 66% of mollusks deaths. These data counteract itself in the analysis performed with the *D. nitida* extract that registered a 33% death rate. Although the chemical composition analysis confirms the compounds to be similar between the two species. The *D. elliptica* presented better results; thus, in these perspectives, the authors suggest the synergism among diverse metabolites is responsible for such an effect, or even the majority concentration of determined metabolite. We hypothesize that this effect might be attributed to a specific active principle predominant in the extract.

Oviposition evaluation tests were used to test the inhibitory power of the studied extracts, confirming that the *D. elliptica* hydroalcoholic extract was capable of inhibiting 60% of the oviposition in a 15-days period, as compared to the negative control, evidencing the extract capacity to act as a molluscide agent in spawns, leading to the mollusk death (*Lymnaea columella).* No results were observed in further dosages. The results were not contrasted; however, it was possible to verify the statistical significance of the exposed data (Gardioli et al., 2017).

11. Antibacterial activity

Analysis of the antimicrobial potential of leaves crude ethanolic extracts were evaluated, presenting efficient bactericidal and antimicrobial activities against gram-positive, gram-negative bacteria and *C. albicans*. Gram-positive strains are shown to be more sensitive to crude ethanolic extracts than gram-negative strains. It is suggested that these extracts action on these microorganisms occurs due to the synergism between tannins, flavonoids, and saponins (Soares et al., 2009).

The minimum inhibitory concentration (MIC), pointed in analysis performed with the crude ethanolic extract of the *D. elliptica* leaves, from a specific region in the Cerrado, is 0.37 mg/mL for the Gram-positive bacteria (MIC from 0.37 to 0.74 mg/mL for gram-negative bacteria and 0.37 mg/mL for *C. albicans* yeast). The *D. elliptica* crude ethanolic extract obtained from leaves in another Cerrado region presented MIC varying from < 0.02 mg/mL to

0.74 mg/mL for gram-positive bacteria, from <0.02 mg/mL to 11.9 mg/mL for gram-negative bacteria, and <0.02 mg/mL for yeast (*C. albicans*) (Soares et al., 2009).

Hypothetically, these variations occur at the expense of the chemical compounds concentrations present in the leaves, which may or may not be present in greater or lesser quantities, as the same species may undergo phenotypic variations between regions other than Cerrado. The results reported in this study (which were obtained by the agar dilution method) are not accompanied by details of how the plaques were read; thus, we may not affirm precisely the data inference, as they were not contrasted by statistical analysis.

In another antimicrobial assay using the *D. elliptica* extract in *Mycobacterium fortuitum*, the minimal inhibitory concentration (MIC) was 125 μg/mL, using dichloromethane as an extraction agent. However, the MIC values superior to 500 μg/mL were observed for the same plant species, *D. elliptica,* when polar extraction agents were used (i.e., ethanol and methanol). However, the study does not justify the absence of positive controls, and the obtained results were not statistically analyzed (Arantes et al., 2005).

Lopes et al., also reported *in vitro* antimicrobial effects of *D. elliptica* using chloroform extract of leaves tested against *Mycobacterium tuberculosis.* They found the plant extract was efficient as an antimycobacterial agent with a minimum inhibitory concentration of 62.5 μg/mL (Mascia Lopes et al., 2007). As mentioned by the authors, the extract was less effective as compared to the reference medication, the isoniazid (MIC = 0.03 μg / mL), because only the extract was tested.

Michelin et al. (2005) showed that methanolic extracts from *D. elliptica* leaves and bark presented antimicrobial activity against *Bacillus subtilis, B.cereus, Shigella* spp. and *Candida albicans,* but only the leaves methanolic extract was effective against *Enterococcus faecalis* and *Salmonella* spp. on the other hand, leaves and barks chloroform extracts applied on eight microorganisms species did not display antimicrobial activity. However, the leaves ethanolic extracts showed to be more efficient inhibiting the following six microorganisms' species, *B. subtilis, B. cereus, E. faecalis, Shigella* spp., *Salmonella* spp. and *C. albicans,* while the ethanolic bark extracts evidenced efficiency in four species *B. subtilis, B. cereus, Shigella* spp. and *C. albicans* (Michelin et al., 2005).

Although the results evidence satisfactory effects, it is not possible to quantitatively compare the mean significance of the different types of extracts tested, since statistical methods were not applied.

12. Gastroprotective activity

Pharmacological assays performed with ethanolic extracts (100 g/kg) from *D. elliptica* aerial parts inhibited 46% of ulcerogenic lesions caused by HCl/ethanol solution (Rinaldo et al., 2006). This can be justified by the presence of tannins, which are potent peroxide radical scavengers in addition to interacting with mucus proteins, improving their cytoprotective effect, forming a protein coating on the gastrointestinal mucosa (Okuda, 2005). This protective effect can still be attributed to the interaction of compounds such as polyphenols that act to strengthen the mucosal gastric barrier. These findings corroborate the popular use of *D. elliptica* as a gastroprotective compound (Kushima et al., 2009).

Pilote trials to define *D. elliptica* methanolic extract dosages on an experiment of colitis induced by trinitrobenzenesulfonic acid in male Wistar rats, demonstrated that the 500 mg/Kg dosage promotes the increase of colon injuries in 47%. These data are in agreement with Kushima et al., who verified a protective effect for methanolic extracts in gastric ulcer induced by HCl (0.3 M) and ethanol (60%) for the same dose (Kushima et al., 2009). Similar findings were observed for the treatment of gastric ulcers, where the D. elliptica ethanolic extract (500 mg/Kg) induced 65% more protection as compared to the negative control, a protective effect similar to the obtained with lansoprazole (69%), the standard drug used in the treatment of ulcers (Kushima, 2006).

In gastric lesions induced by stress, the *D. elliptica* treatment was gastroprotective in the dosages of 250 and 500 mg/Kg, protecting the gastric mucosa in the proportions of 83 and 75%, respectively. This same dosage demonstrated a significant wound healing activity in acetic acid-induced lesions, being capable of decreasing the size of the injury as compared to the control group (animals treated with saline), thus displaying more effective effects than the cimetidine (100 mg/Kg). It is argued that the extract elongates the mucosa glands localized in the regeneration area, findings not observed in the treatments with saline and cimetidine (Kushima, 2006).

In summary, the *D. elliptica* gastroprotective effect in different models of gastric lesions induction, confirm the popular knowledge regarding this species therapeutic properties. This extract has tannins fractions that present a gastroprotective impact, and its activity is associated with the nitric oxide and increased COX-1 expression. The ethanolic extract gastroprotective activity is related to the sulfhydryl groups and wound healing activity with reduction of the lesion area, but not altering the cellular proliferation (Kushima, 2006).

Aimed to comprehend the *D. elliptica* mechanisms in the gastric disorders, Vieira and cols evaluated the presence of elements such as phosphorus, selenium, chlorine, potassium,

calcium, titanium, chromium, magnesium, iron, nickel, copper, zinc, rubidium, and bromine (Vieira et al., 2018).

These chemical elements are responsible for exerting essential biological activities in the living organisms. The Ca, K and Zn, act in the diseases prevention and control, regenerating the damaged gastric mucosa, thus corroborating the popular use of *D. elliptica* to treat gastric disorders (Desideri et al., 2010; Koo, 1994; Watanabe et al., 1995).

The *D. elliptica* ethanolic extract S concentration is 3.019 µg/g. This element, along with Mn, Fe and Cu play essential functions in the amino acids metabolism, where Cu is responsible for exerting functions related to growth, blood cells production, and iron transportation, whereas Mn and Fe are responsible for exerting essential immunomodulatory activities. The Cr chemical element act on the glucose, lipid and carbohydrate metabolism. Low Cr concentrations are associated with the development of cardiovascular diseases (Komarnisky et al., 2003; Marmiroli et al., 2008; Vieira et al., 2018).

The P element participates in metabolic processes and biological activities as part of organic molecules and component of enzymatic reactions, participating in the growth of the tissues and renew. Furthermore, the Cl element participates in the acid-base balance and osmotic stability among the cells (Desideri et al., 2010; Marmiroli et al., 2008; Nielsen, 2009).

13. Anti-inflammatory and antioxidant effect

D. elliptica might be considered as a potential target to be used as a chemopreventive therapeutic agent, which can be perceived according to the data shown. The *D. elliptica* hexane fraction of the aerial parts had an inhibitory effect on NF-κB and COX-1 (Endringer et al., 2010). The aerial parts hydroalcoholic extract reduced the nociceptive response in the inflammatory phase via formalin test in mice, which suggests the involvement of the nitriding pathway. These results demonstrated that the extract presented an anti-inflammatory effect, with efficiency similar to diclofenac (Azevedo et al., 2007).

Concerning the isolated compounds, myricetin-3-O-β-galactoside, isolated from the hydroalcoholic extract of the aerial plant parts, in concentrations of 0.26 and 0.78 mg/kg evidenced a reduction in paw edema induced by intraplantar injection of carrageenan in mice knockouts for the enzyme inducible nitric oxide (iNOS -/-) to confirm the involvement of iNOS in the antinociceptive and anti-inflammatory mechanisms of action. This bioactive effect is attributed to the nitric oxide synthesis inhibition, mainly via iNOS. In the same study, the animals motor signs assessment did not display any changes (Azevedo et al., 2015).

The ethyl acetate and methanol fractions derived from the crude leaf extract showed a significant reduction in licking time in the formalin test late phase performed in animal models of paw edema induction (Jácome et al., 2010). Bioguided fractionation of the leaf methanol fraction resulted in the isolation of myricetin-3-O-β-galactopyranoside, which produced significant inhibition in formalin-induced nociception at a concentration of 0.26 mg/kg. According to these findings, it is possible to infer that the antinociceptive effect of the *D. elliptica* species is attributed to the myricetin-3-O-β-galactopyranoside component, which justifies its popular use for the attenuation of pain and inflammation (Campos et al., 2013).

A study performed by Kushima et al. (2009), showed that the methanolic extracts from the *D. elliptica* aerial parts orally administered in the following concentrations: 125, 250 and 500 mg/kg were not effective to reduce paw edema after carrageenan test. This effect was attributed to the tannins bioavailability in the extract. An evaluation of this extracts pharmacologic effect *in vivo* showed that the polymeric protoantocianidines do not cross the intestinal wall and are not digested in the stomach (Kushima et al., 2009). However, the intraperitoneal administration in the concentrations of 7.81, 15.62 and 31.25 mg/kg caused a persistent reduction in the edema that was perceived 1 hour after application and resisted up to 4 hours. Differences were not observed among doses; however, the lowest doses presented late effect as compared to the others. Furthermore, investigating the anti-inflammatory effect of the tannin fraction, the 15.62 mg/Kg extract administration caused a 50% reduction in leukocyte recruitment induced by lipopolysacharides (LPS) application in the post-capillary venules of the mice cremaster muscle (Nishijima et al., 2015).

The application of a tannins fraction (15.62 mg/kg) (via intraperitoneal administration) on an animal model evidenced 33% inhibition of paw edema; however, the leaves methanolic extract in the same dosage was capable of inhibiting 65% (Nishijima et al., 2015). As discussed by the authors, the extract is responsible for exerting a better anti-edematogenic activity as compared to the isolated tannins fraction. Therefore, the observed inhibition might be associated with the extract chemical constituent's synergism (Nishijima et al., 2015).

Another study performed to evaluate anti-edematogenic activity evidenced the tannins as efficient to inhibit leukocytes recruitment, consequently reducing edema. The decreased metallo-9-matrix proteinases (MMP-9) activation was also evidenced when mice were submitted to the *Bothrops jararaca* venom (Nishijima et al., 2015). Corroborating these findings, Nishijima et al. (2009), reported that the flavonoids responsible for the venom neutralization against the *B. jararaca* hemorrhagic activity are kaempferol, quercetin,

quercetin-3-O-β-D-galactopyranoside, quercetin-3-O-α-L-arabinopyranoside and myricetin (Nishijima et al., 2009).

However, a decreased MMP-9 activity in the dermis compromised with poison + *D. elliptica* methanolic extract and a decreased pro-MMP-2 and MMP-9 activity in the compromised dermis in the group poison + *D. elliptica* tannins fraction (Nishijima, 2010).

The tannins seem to be responsible for decreasing 50% of the leucocytes "rolling" activity during the inflammatory process induced by LPS, where the *D. elliptica* extract chemical constituents must be the responsible agents with anti-inflammatory properties. (Nishijima, 2010).

Exploring the *D. elliptica* leaves biological effects on *Helicobacter pylori*-induced oxidative stress at the concentrations of 5, 50 and 100 μg/mL, a dose-dependent improvement was observed. The concentration of 100 μg/mL was efficient, presenting antioxidant activity above 80% (Bonacorsi et al., 2013), which was corroborated by an *in vitro* study that used *D. elliptica* leaves methanolic extract (250 μg/mL) on *H. pylori* (Kushima et al., 2009).

The investigation of other oxidative stress parameters, verified that the production of hydrogen peroxide (H_2O_2) evaluated in immunological assays was not significantly increased in both leaves ethanol and methanolic extracts, however, both extracts showed a moderate release of nitric oxide (NO) by approximately 28 μM (Carlos et al., 2005). Concerning the chloroform fraction, it was verified a dose-dependent production stimulation of H_2O_2, NO and tumor necrosis factor alpha (TNF-α) in *in vitro* macrophage culture (Mascia Lopes et al., 2007). Once the MTT data was not reported in the study, it is not possible to make inferences regarding cell viability. Also, the reagents used were not fully described in the methods section.

It was also noticed that both methanolic and ethanolic extracts induced a marked TNF-α production, although the methanolic extract showed to be more efficient, producing almost five times more release of this cytokine as compared to the ethanolic extract. In summary, the results showed that methanolic and ethanolic extracts stimulated the production of NO and TNF-α, demonstrating immunostimulatory activity in mice, by modulating the activation of macrophages (Carlos et al., 2005).

Carli et al. (2009) found that leaves methanolic extract exerted immunosuppressive activity on LPS stimulated macrophages, presenting better results than the standard cis-Pt drug used in the treatment of cancer, which showed no NO formation inhibitory activity (Carli et al., 2009).

The main findings regarding the *D. elliptica* effects on gene expression modulation are summarized in Figure 4.

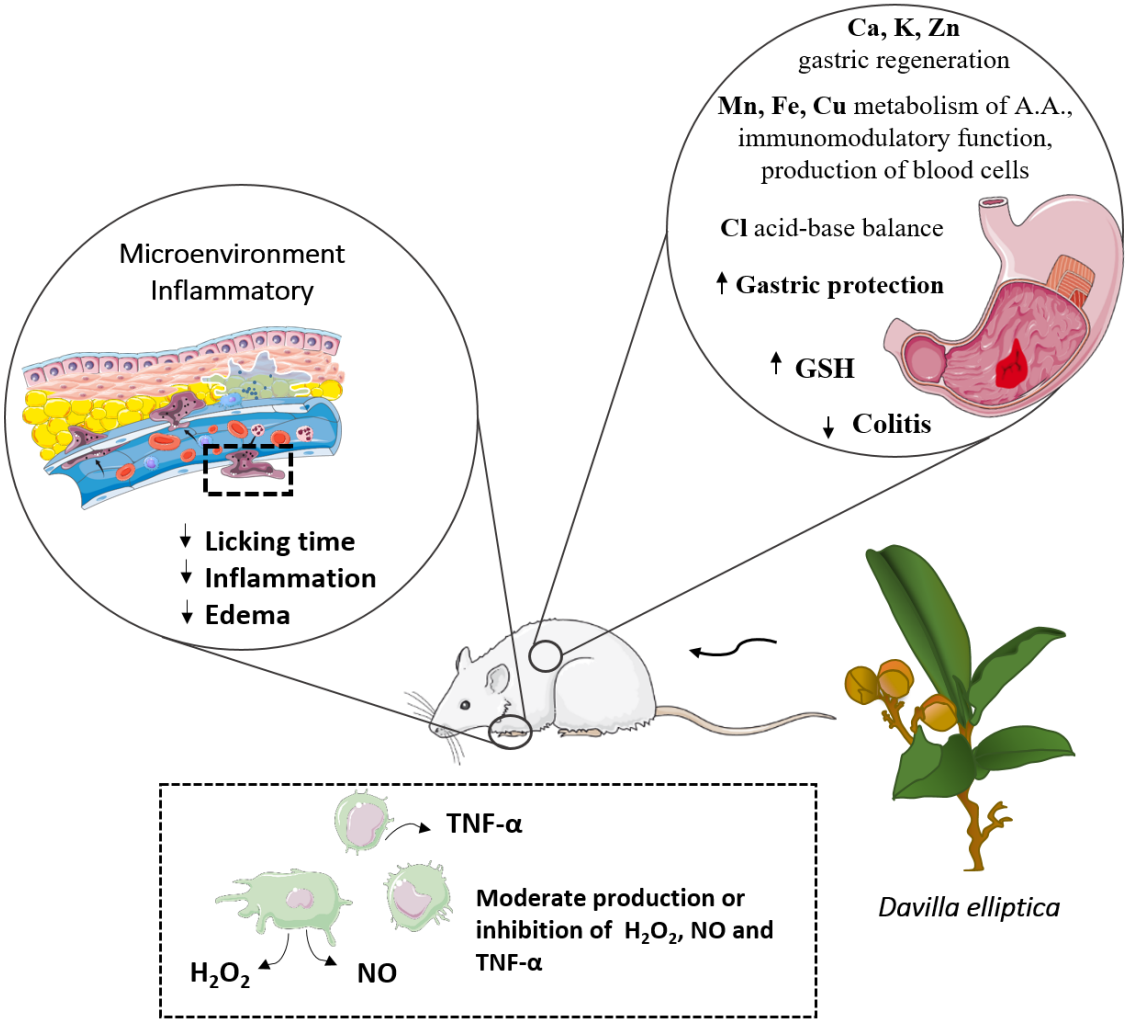


Figure 4. *Davilla elliptica* extract and compounds effects on inflammation, gastritis, colitis and oxidative stress.

Despite the different methodological settings (dosages, fractions, extracts), and experimental models used in the reported studies that aimed to evaluate the *D. elliptica* effects on inflammatory process and stress oxidative-associated conditions, it is possible to assess the protective and modulation role of inflammatory cytokines, as well as oxygen reactive species generating enzymes. However, studies aimed to investigate further the signaling pathways modulated by *D. elliptica* in inflammatory reactions are necessary. Additionally, the literature findings regarding the *D. elliptica* extract oral administration seem to be not effective to regulate inflammation as compared to the local application of the extract that was more

effective to inhibit inflammatory mediators recruitment, such as leucocytes and macrophages, and consequently, the cytokines response.

14. Other biological activities

Growing evidence has demonstrated promising molecules obtained from medicinal plants used in the treatment of neoplasms via diverse different action mechanisms. In this sense, flavonoids isolated from *D. elliptica* were tested on murine mammary tumor cells lineage LM2. In the MTT test for antitumoral activity evaluation, the following IC50 values were obtained: from 31.5 ± 297 to 203.1 ± 5.9 μg/mL. The compounds quercetin, quercetin-3-O-galactopyranoside, quercetin-3-O-arabinopyranoside, Myricetin-3-O-β-galactopyranoside, myricetin, quercetin-3-O-α-L-rhamnopyranoside, and Myricetin-3-O- L-rhamnopyranoside displayed antitumoral effect, being more pronounced in the last two compounds (Carli et al., 2009).

The antimutagen effect is attributed to the *D. elliptica* chloroform extract. The micronucleus test in peripheral blood cells from male and female mice submitted to the acute treatment with cyclophosphamide (50 mg/Kg) was performed. The fraction demonstrated a significant reduction of cells with micronucleus, being 41.56%, 51.86% and 42.9% for the 111 mg/mL, 83.25 mg/mL and 55 mg/mL, respectively (Lira, 2007).

The quercetin, quercetin-3-O-α-L-rhamnopyranoside, myricetin, and myricetin-3-O-α-L-rhamnopyranoside flavonoids phytoestrogenic potential (E-Screen test) was investigated for the *D. elliptica* isolates by evaluating the MCF-7 and adenocarcinoma cells proliferation in different dosages, where no proliferative activity was detected. However, new studies need to be performed for more consistent conclusions. This may be justified by the absence of activity in the control with the steroid 17-β-estradiol hormone, being necessary to review a more effective dosage for proliferation effects (Biso, 2008).

15. Toxicity

According to the data retrieval, only a few specific studies that evaluated the *D. elliptica* toxicity were published in the literature. However, different dosages and its actions were evidenced for the investigation of the extracts and fractions bioactive effects in in vitro and in vitro experiments, where alterations and toxic effects were observed.

Mutagenicity evaluation by the Ames test (Maron and Ames, 1983), performed by Biso and cols (Biso et al., 2010), observed different actions of the *D. elliptica* leaves fractions on *Salmonella typhimurium* strains with and without metabolization. In the trial performed without metabolization, the ethanolic and chloroform extracts showed evidence of

mutagenicity in TA97a lineage (12.53 mg/plate), while toxicity signs were observed in TA102 in the 8.35 and 12.53 mg/plate concentrations. In the presence of metabolization, the ethanolic, methanolic and chloroform fractions displayed genotoxic activity in TA98 (0.65-4.18 mg/plate), as verified by Lira in another study (Lira, 2007), while evidence of toxicity was observed for the aqueous fraction in TA79a and ethyl acetate TA98. For the T100 cells strain, the genotoxicity was confirmed (Biso, 2008). In another study that used the Ames model to evaluate the chloroform fraction antimutagen effect, a significant reduction in the TA95a lineage in the concentration of 1.04-8.35 mg/plate treated with the indirect mutagen4-nitro-o-phenylenediamine, disagreeing with the toxicity evidence reported by Biso and cols in the same cells lineage and extracts concentration (Biso, 2008). However, according to Borstel et al. (1998), a few compounds that have antimutagenic activity may as well present themselves as mutagenic compounds, according to the experimental conditions (Von Borstel and Higgins, 1998).

To further investigate the *D. elliptica* extracts and fractions, Biso and contributors evaluated different isolated molecules in the pUC9.1 plasmid. The authors verified that the compounds quercetin, myricetin, quercetin-3-O-α-L-rhamnopyranoside, myricetin-3-O-α-L-rhamnopyranoside, and gallic acid did not induce breaks for any of the tested concentrations that were pre-defined according to the studies substances solubility. However, the addition of copper sulfate II resulted in the emergence of open circular DNA and linear DNA bands (Biso, 2008). On the other hand, Walcélio and contributors in a study with the chloroform fraction did not identify toxicity for quercetin-3-O-α-L-rhamnopyranoside, myricetin-3-O-α-L-aminopyranoside, and gallic acid in the dosages of 0.45 and 0.9 mg/mL, while the quercetin (0.9 mg/mL) presented plasmid DNA breaks, similarly to the tin chloride (200μL/mL). In this study, it was demonstrated an antimutagenic activity of the following compounds: galic acid (0.45 and 0.9 mg/mL) and myricetin (0.45 mg/mL) as compared to the tin chloride effects on plasmid DNA (Lira, 2007).

In the cytotoxicity test using MCF-7 cells lineages, it was verified cytotoxicity that after 24 hours of incubation with the quercetin (100 μM) and myricetin (200 μM). For the same dosages, it was verified an inhibition of the cell division for the quercetin-3-O-α-L-rhamnopyranoside and myricetin-3-O-α-L-rhamnopyranoside. Forty-eight hours incubation with quercetin (100 e 200 μM), myricetin (100 μM), and myricetin-3-O-α-L-rhamnopyranoside (200 μM) resulted in reduced cell viability. Cells observed after 72 hours of incubation with the reported compounds, evidenced cytotoxicity for quercetin, quercetin-3-

O-α-L-rhamnopyranoside, myricetin and mirycetin-3-O-α-L-rhamnopyranoside in both dosages, except with quercetin-3-O-α-L-rhamnopyranoside at 100 μM (Biso, 2008)

In the ex vivo evaluation of peritoneal macrophage modulation by methanolic and ethanolic extracts, both extracts exhibited the cytotoxicity index 50 (IC50) higher than the 300 μg/mL dose; however, the dose ranges tested were not shown (Carlos et al., 2005). In vivo experiments with male Swiss mice treated with methanolic extract in single dosage (5000 mg) did not demonstrate behavioral alterations over 14 days, which presents absence of toxicity according to Loomis and Hayes (1996) (Kushima, 2006; Loomis and Hayes, 1996). Martins et al., on the other hand, pointed to alterations on spermatogenesis. Mice were treated with a flavonoid-rich fraction and *D. elliptica* hydroalcoholic extract for 42 days in the 100, 200 and 400 mg/Kg dosages by gavage. Both fractions demonstrated in the histological analysis a decrease of the total tubule length, decreased tubule and epithelial volume, where more pronounced effects were observed in the flavonoid-rich fractions (200 and 400 mg/Kg), but also in the hydroalcoholic extract in the 400 mg/Kg dosage. Moreover, the SOD activity (superoxide dismutase), the Leydig nuclei size and amount of connective tissue were increased in animals treated with a 400 mg/Kg dosage of the flavonoid-rich fraction. In the hydroalcoholic fraction test, increased GST (glutathione s-transferase) activity and decreased body weight and sperm motility was observed (Martins, 2018).

Although isolate effects were observed in the *D. elliptica* toxicity evaluations, a gap in the scientific literature is still present, since no dose response curves and different long-term vegetative parts were seen. Systemic effects should be explored to evaluate tissue damage in target organs (liver, kidneys, spleen, heart, and lung), as well as biochemical parameters to certify its safe use by the population.

16. Perspectives

The studies about the medicinal properties of *D. elliptica* have evidenced important bioactive activities such as anti-inflammatory, antimicrobial, gastroprotective, antioxidant and anti-tumoral. Also, molluscicide and allelopathic activities of hydroalcoholic extracts have been reported, although some studies will require additional validation using statistical parameters. Phytochemical studies have shown a diversity of secondary compounds such as triterpenes, steroids, catechins, flavonoids, saponins, phenolic acid derivatives, coumarins, condensed tannins, and tannins, as well as the presence of molecules such as gallic acid, myricetin, and derivatives isolated from this species. However, studies that evaluated the *D. elliptica* biological effects/mechanisms of action under different aspects and in different

vegetative parts, such as fruits, flowers, and seeds are scarce in the literature. These recent findings open perspectives for the investigation of new pharmacological properties of bioactive compounds present in *D. elliptica* and their use as a potential therapeutic agent.

Author's contributions

JNS, VHDG, and SHSS conceived the study. JNS, VHDG, AFP, and VM wrote the manuscript. VHDG performed graphic designer present in figures. SHSS and DFL provided a critical revision of the paper. All authors read and have approved the final version of the paper.

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Conflict of interest

All authors do not have conflicts of interest.

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5.2 PRODUTO 2

Effects of oral treatment with leaf and extract of *Davilla elliptica* on diet-induced obese mice

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ABSTRACT

Ethnopharmacological relevance: Davilla elliptica A. St.-Hil, popularly known as "*lixeirinha*", is a shrub belonging to the Dilleniaceae family that occurs naturally in the Brazilian Savannah (Cerrado). In popular medicine, *D. elliptica* has been used to treat gastritis, ulcers and diarrhea. Research studies have proven evidences of its gastroprotective effect, as well as, its benefits as anti-nociceptive, anti-inflammatory, antioxidant. Howerever there are no studies that have tested the potential effects of *D. elliptica* on metabolic parameters and obesity.

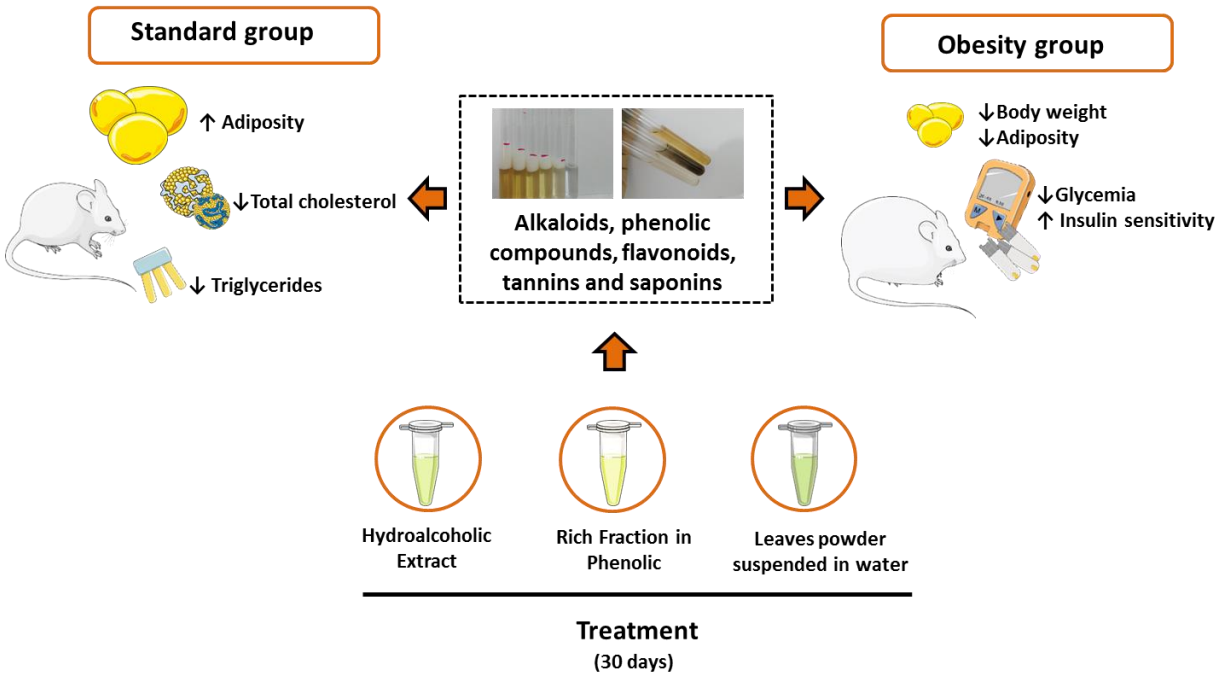
Aims: This study aimed to evaluate the effect of *Davilla elliptica* leaf extract in the metabolic parameters of in diet-induced obese mice, as well as to carry out the phytochemical characterization of the species.

Materials and methods: Leaves of *D. elliptica* were used to obtain an hydroalcoholic extract and phytochemical assays were performed. Animal experimentation was carried out using male Swiss mice divided into eight groups: ST (Standard), ST plus EA (fraction ethyl acetate), ST plus HE (hydroalcoholic extract), ST plus PL (leaf powder), HFD (obese control), HFD plus EA (fraction ethyl acetate), HFD plus HE (hydroalcoholic extract), HFD plus PL (leaf powder) and treated for four weeks. Insulin sensitivity and glucose tolerance tests were performed one week prior to sacrifice. Plasma analyzes of triglyceride, total cholesterol and glycemia levels were measured by ELISA kit.

Results: A significant reduction of adiposity and body weight between obese groups was verified. Statistical differences in the biochemical analyzes, triglycerides and total cholesterol were observed in the animals of the standard group treated with fraction of *D. elliptica*, and surprisingly, the adiposity in this group was increased.

Conclusions: Oral treatment with *D. elliptica* reduce adiposity and body weight, improving plasma levels of triglycerides and total cholesterol. However, further analyzes are needed to investigate which molecular mechanisms are involved with the modulation of metabolism upon treatment of mice with this extract.

Graph abstract:



Key words: Sambaibinha; Adiposity; Flavonoids; Brazilian Savanna; Diet- induced.

Abbreviators:

WHO (World Health Organization); HFD (High- fat diet); ST (Standart); EA (Ethyl acetate); HE (Hydroalcoholic extract) PL (leaft powder); UCP1 (Uncoupling protein 1); SIRT1 (Sirtuin); PGC-1α (Peroxisome proliferator- activated receptor –gamma coactivator 1 alpha); BAT (brown adipose tissue).

1. INTRODUCTION

Obesity is defined as a metabolic disorder characterized by abnormal body fat accumulation and imbalance between consumption and energy expenditure (Despres and Lemieux, 2006; Hill et al., 2000; Lee et al., 2017). According to data from the World Health Organization (WHO), obesity has almost tripled since 1975, and by 2016, more than 1.9 billion adults were overweight, from which over 650 million were obese. The majority of the world's population lives in countries where overweight and obesity is the leading cause of death (World Health Organization and WHO, 2018).

Pharmacotherapy may alternatively be used as an adjunct in the treatment of obesity and lifestyle modifications. Medications used in the treatment of obesity have side effects; in this sense, natural compounds and medicinal plants have been suggested as a therapeutic method (Cordido et al., 2014). Studies have shown that endemic species of the Brazilian Savanna seem to exert positive effects on obesity by improving oral glucose tolerance, insulin sensitivity and inflammatory responses in adipose tissue and liver (Oliveira et al., 2014).

Davilla elliptica (Dilleniaceae) A. St.-Hil, also popularly known as “lixeirinha”, “sambaibinha”, “muricizinho”, “cipó-caboclo” e “cipó-de-carijó”(Soares et al., 2005; Rodrigues et al., 2002; Rodrigues et al., 2001) is a native species of the Brazilian Savanna, occurring in the Pandeiros river basin, and presenting shrub habits and branched sub-bushes (Jácome et al., 2010). This plant presents in its chemical composition a wide diversity of secondary metabolism substances, such as flavonoids, saponins, steroids, tannins, coumarins and triterpenoids. These bioactive compounds have anti-inflammatory, antioxidant, antitumoral, anti-nociceptive, anti-microbial, anti-mutagenic and gastroprotective activities (Carlos et al., 2002).

The molecular mechanisms through which *D. elliptica* act are still unknown due to lack of studies addressing. Therefore, the present study aims to evaluate the therapeutic potential of *D. elliptica* over metabolic parameters of from diet- induced obese mice.

2. MATERIALS AND METHODS

2.1 Plant material

For the achievement of the study, *Davilla elliptica* St.-Hil leaves, collected between July and August 2018 in the municipality of Bonito de Minas - Minas Gerias, Brazil (15°13'31.37 "S and 44°55'1.52" W) (Figure 1), were used. Herbarium specimens of *D. elliptica* may be found in the Herbarium of the State University of Montes Claros, under registry No. 332.

Figure 1. Location map of the study area. Source: Google Earth.

2.2 Extract

2.2.1 Preparation of Plant Material

Leaves of *D. elliptica* were harvested, washed thoroughly in current water and dried in a drying stove with air circulation (New Ethics) under heating and air circulation at 38°C (±2). Subsequently, the plant material was pulverized in a Willey-type mill, packed in paper containers and kept under refrigeration (5°C) for preparation of the extracts (Rotta et al., 2008).

2.2.2 Maceration

The pulverized samples of *D. elliptica* leaves were conditioned in the proportion of 10 mL of absolute ethanol for each gram of plant powder. The mixture was stored for one week,

and then it was filtered and placed in an drying stove at 35 ±2 °C. After drying the solvent, the samples were stored in the refrigerator at 10 C.

2.2.3 Extract partitioning

For the fractionation of the flavonoid extract, the samples obtained in the previous procedure were resuspended in a mixture of ethanol: water (7:3), in the ratio of 3 g of extract to 250 mL of 70% ethanol. At the first wash of the mixture, 200 mL of hexane PA were added three times. A volume of 200 mL ethyl acetate was added three times to the first wash residue. Partitions containing the compounds of interest were brought to the stove under air circulation at 38 °C until the solvents dried (Andreo and Jorge, 2006).

2.2 Qualitative chemical screening

Using the dry leaves, qualitative tests were performed to detect the content of tannins, saponins, flavonoids, alkaloids and phenolic compounds: 10% neutral lead acetate and 2% iron chloride for tannins, 2% iron chloride and Shinoda reactions for flavonoids, Mayer's Reagents, Bouchadart, Bertrand and Dragendorf for alkaloids, persistent foam test for saponins. The positive result is interpreted by the formation of greenish color (Marcela et al., 2015).

2.4 Animals

The experiment was conducted with forty *SWISS* male mice (six weeks old), from the State University of Montes Claros animal. The animals were housed in cages under the 12: 12h light-dark cycle (lights from 7 a.m. to 7 p.m.) at 25.0 ± 2.0°C. After a 7-day adjustment period, the mice were randomly divided into eight groups (n = 5 per treatment), followed by induction of obesity for 3 months. After this period, the animals were fed the following diets for 4 weeks: Standard Diet (ST); ST plus EA (fraction of ethyl acetate); ST plus HE (hydroalcoholic extract) ; ST plus PL (leaf powder); High fat diet (HFD); HFD plus EA (fraction of ethyl acetate); HFD plus HE (hydroalcoholic extract) ; HFD plus PL (leaf powder)(0.26 mg/Kg/body weight) (Azevedo et al., 2015, Campos et al., 2013). Food and water were offered *ad libitum*. This study was approved by the Ethics Committee on Animal Experimentation and Welfare of the State University of Montes Claros, Brazil (process No. 164/2018).

2.5 Diets

The standard diet (Purina - Labina ®) used for regular maintenance of mice was composed of 50.3% carbohydrate, 41.9% protein and 7.8% fat, representing a total of 2.18 kcal per 1g of diet. The high-fat diet was composed of 36.59% carbohydrate, 12.88% protein and 50.53% fat, presenting a total of 5.1 kcal per 1 g of diet. All components of the high fat-diet were purchased from Rhoster LTDA (São Paulo, Brazil).

2.6 Tests of glucose tolerance and insulin sensitivity

For the glucose tolerance test, D-glucose (2 mg/g body weight) was injected intraperitoneally into mice fasted over a period of 12 hours. Glucose levels of the tail blood samples were monitored at 0, 15, 30, 60 and 120 min. An insulin sensitivity test was performed on mice in the fed state after intraperitoneal injection of insulin (0.75 units/kg body weight). Tail blood samples were obtained at 0, 15, 30 and 60 min after injection. Glucose levels were assessed on an Accu-Check glycometer (Roche Diagnostics®, Indianapolis, USA).

2.7 Measures of body weight, food intake, tissue collection and plasma parameters.

Food intake was measured twice a week throughout the treatment to ensure food efficiency (food intake/body weight). At the end of the experiment, the animals were fasted overnight (12h) and euthanized by decapitation. Samples were collected, weighed, stored immediately in liquid nitrogen and stored at -80°C for further analysis.

2.8 Blood measurement determination

Serum was obtained after centrifugation (3200 rpm for 10 min). Total serum cholesterol, triglycerides and glucose were evaluated using enzyme kits (Wiener® Argentina).

2.9 Statistical analyzes

All data were analyzed by Graph Pad Prism software (version 5.0®, San Diego, USA) and subjected to specific tests with a statistical confidence of 95% ($p<0.05$). Data are expressed as the mean ± error of the mean (SEM). The statistical significance of differences in mean values between the groups of mice was assessed by one-way ANOVA or two-way ANOVA and Tukey's post-test.

3. RESULTS

3.1 Phytochemical screening, flavonoid quantification from *Davilla elliptica* leaves.

The qualitative evaluation of secondary metabolites: tannins, alkaloids, flavonoids, saponins and phenolic compounds of *D. elliptica* leaves are described in Table 1. The results were positive for saponin heterosides and hydrolyzable tannins, as well as the alkaloids and flavonoids present in the leaves in hydroalcoholic extract.

Tabela 1. Phytochemical screening of extract of *Davilla elliptica*

Class	Test	Leaf
Tannins	Ferric chloride	+++
	Copper acetate	+++
	Neutral lead acetate	++
Flavonoids	Iron chloride	++
	Sodium hydroxide	+
Alkaloids	Mayer reagente	++
	Bouchadart reagente	+
	Bertrand reagente	+++
	Dragendorff reagente	++
Phenolic compounds	Iron chloride	+
	Sodium hydroxide	++

(-) Negative, (+) Weak positive, (++) Moderate positive, (+++) Strong positive.

Saponin		
H_2O	EX	
5	-	-
4	1	+
3	2	+
2	3	+
1	4	++
-	5	++

(-) Negative, (+) Weak positive, (++) Moderate positive, (+++) Strong positive.

3.2 Treatment with *D. elliptica* improved metabolic parameters in HFD-fed mice.

Adiposity was significantly decreased in the HFD plus EA group mean ± standard error (0.0470±0.0078) compared to the obese control ($p<0.01$); differences between treated

groups were also observed for HFD plus EA (0.0470±0.0078) vs. HFD plus HE (0.0767±0.0017) p<0.01, and HFD plus EA (0.0470±0.0078) vs. HFD plus PL (0.0806±0.0074) p<0.05. As observed the body weight gain was lower in the groups HFD plus EA (48.10±0.3390) and HFD plus PL (52.68±0.4536), when compared to the obese control group HFD (57.01±0,7890), p<0.01. Differences between treatments could also be observed for HFD plus EA (48.10±0.3390) vs. HFD plus HE (55.79±1.055), HFD plus EA (48.10±0.3390) vs. HFD plus PL (52.68±0.4536), and HFD plus HE (55.79±1.055) vs. HFD plus PL (52.68±0.4536) (Figure 1. A-B)

Interestingly, adiposity was significantly increased in ST plus EA (0.0372±0.0049) when compared to the ST control group (0.0196±0.0036) (p<0.05). Differences in ST plus EA (0.1857± 0.0192) vs. ST plus HE (0.1183± 0.0238) were observed, the ST plus PL group (0.0174±0.0032) had a reduced adiposity when compared to the ST plus EA group (0.1857±0.0192). There were no significant differences in body weight gain in treated ST groups (Figure 1 C-D).

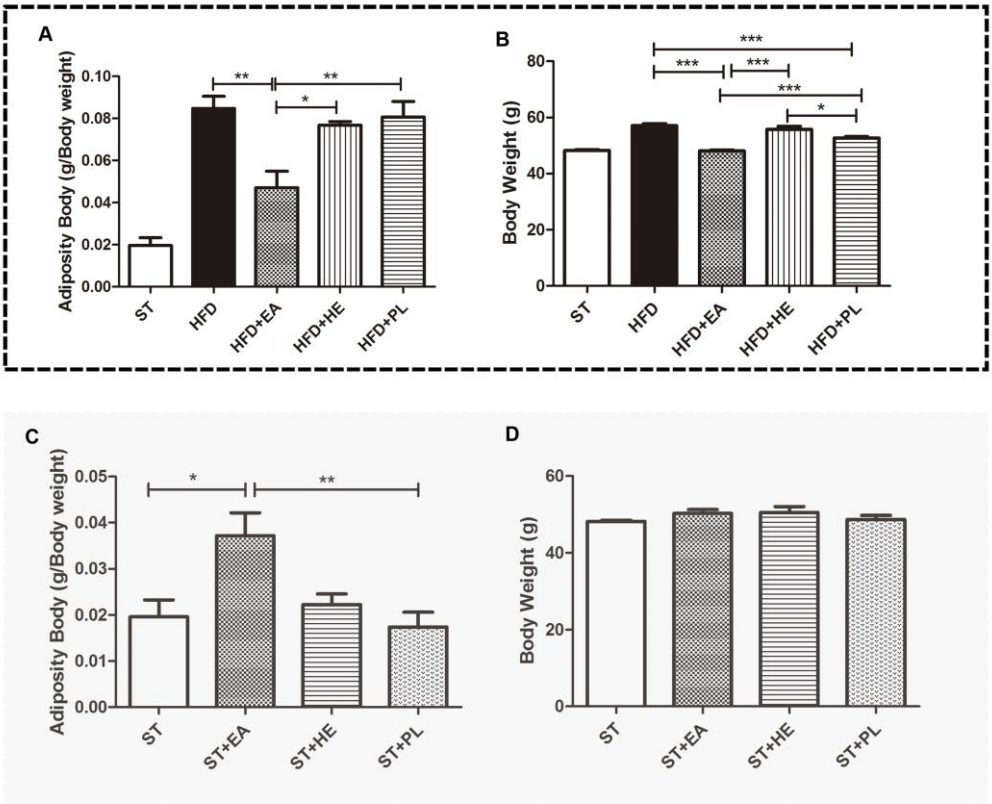


Figure 1. Effects of *D. elliptica* about adiposity and body weight of animals in treatment with high- fat diet and standard diet. (A, C) Sum of all white adipose tissues: epididimal, mesenteric and retroperitoneal. (B, D) Mean weight of the animals during the treatment period 30 days. Values shown are mean ± standard error (SEM) (n = 05). Significant differences,

using one-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$). ST: Standard (Control), HFD: High- fat diet (Control), EA: Fraction ethyl acetate, HE: hydroalcoholic extract, and PL: leaf powder.

3.3 Biochemical parameters

Significant differences were observed in the total cholesterol levels of the animals treated with standard ST diet (285.3± 32.80) vs. ST plus EA (155.2± 5.886) $p<0.01$, ST (285.3± 32.80) vs. ST plus HE (128.8±11.67) $p<0.001$, and ST (285.3± 32.80) vs. ST plus PL (145.7± 10.91) $p<0.001$. Levels of triglycerides were significantly decreased between ST groups (442.7± 54.91) vs. ST plus EA (237.6± 47.83) $p<0.05$, and ST (442.7± 54.91) vs. ST plus PL (162.7±22.53) $p<0.0$. (Figure 2 D-F). No statistical differences were found in plasma glucose, triglyceride and total cholesterol levels between treated obese groups (Figure 2A-C).

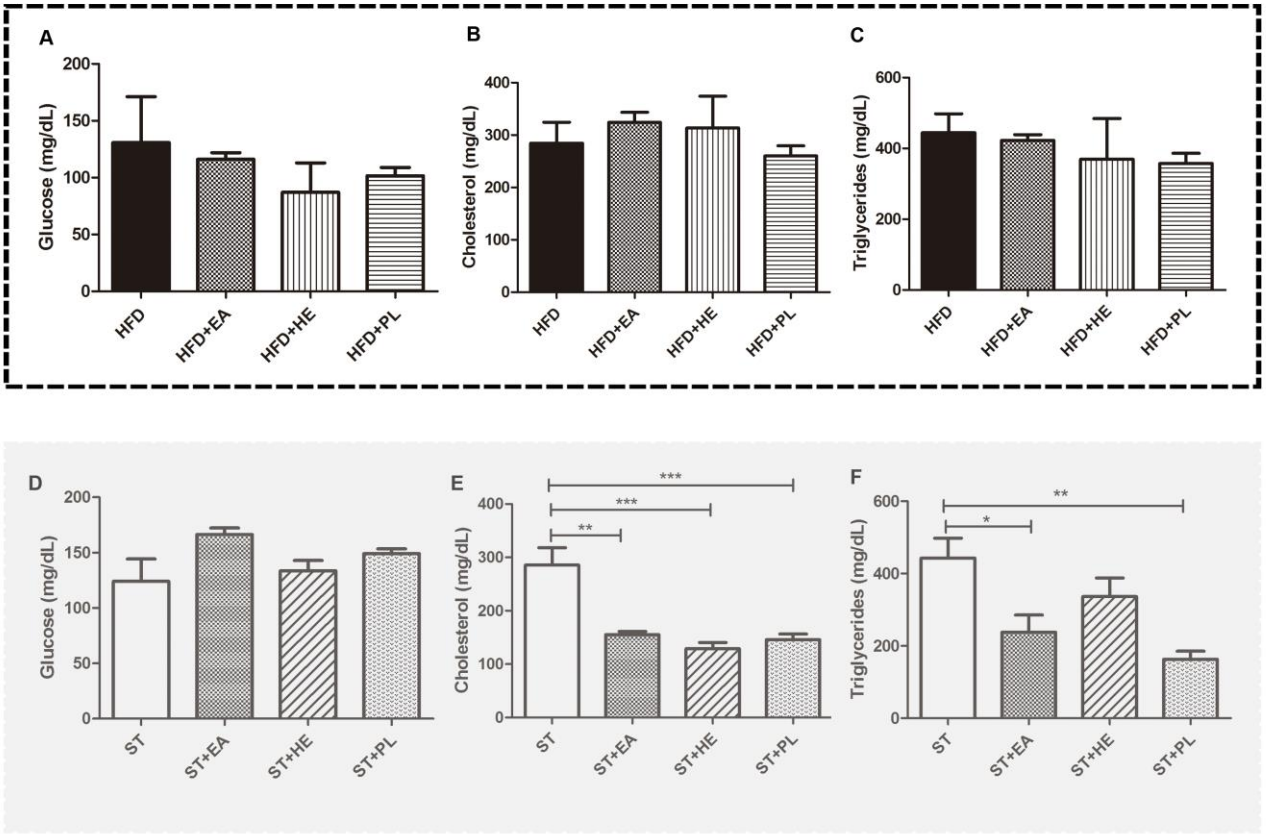


Figure 2. Effects of oral administration of hydroalcoholic extract, ethyl acetate fraction and leaf on total cholesterol, triglycerides and high density lipoprotein levels in mice fed standard or high-fat diet. Values shown are mean ± SEM (n = 05). Significant differences, using one-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p<0.05$); ** ($p<0.01$); ***

(p<0.001). ST: Standard (Control), HFD: High- fat diet (Control), EA: Fraction ethyl acetate, HE: hydroalcoholic extract, and PL: leaf powder.

3.4 Insulin sensitivity and glucose tolerance tests

In mice fed a high-fat diet, the insulin sensitivity test demonstrated a decrease in blood glucose levels of group obese treated com fraction of the ethyl acetate compared to HFD (554.9±89.39) vs HFD plus EA (316.3±17.68) (p <0.05) (Figure 3 A-B). This result was accompanied by the glucose tolerance test that demonstrated a reduction in glucose levels in the HFD plus EA group when compared to the control group obese HFD (1507±241.9), HFD plus EA (897.0±48.35) (p <0.05) (Figure 3C-D). These results indicate that the ethyl acetate fraction prevented the increase of glycemia even when consuming a high- fat diet. For the groups treated with standard diet the results of the test of glucose tolerance and insulin sensitivity did not present statistical difference (Figure 4 A-D).

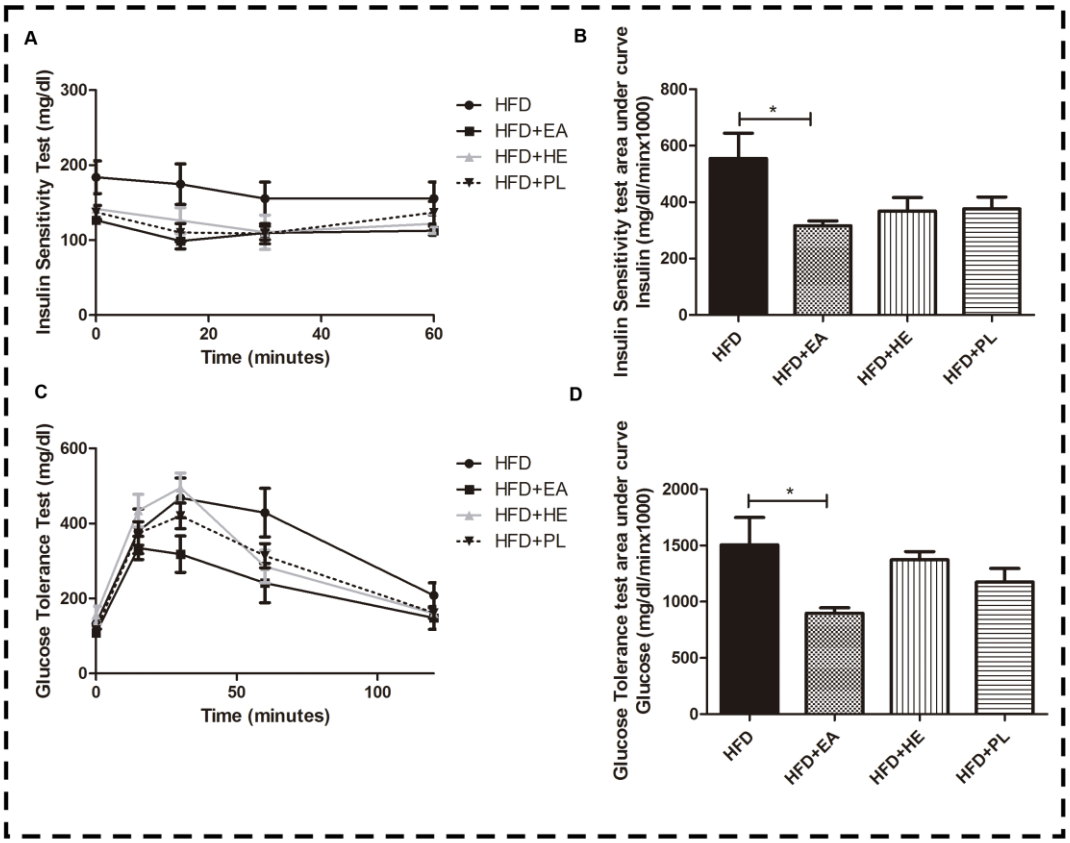


Figure 3. Effects of oral administration of hydroalcoholic extract, ethyl acetate fraction and leaf powder of *D. elliptica* on inulin sensitivity, glucose tolerance levels in mice fed a high fat- diet. (A, B) Intraperitoneal Insulin Sensitivity Test (IPIST) and area under the curve

(mg/dL) (C, D) Intraperitoneal glucose tolerance test (IPGTT) and IPGTT glucose area under the curve. Significant differences, using two-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$). IPIST: Intraperitoneal Insulin Sensitivity Test, IPGTT: Intraperitoneal Glucose Tolerance test, HFD: High- fat diet (Control), EA: fraction ethyl acetate, HE: hydroalcoholic extract and PL: leaf powder.

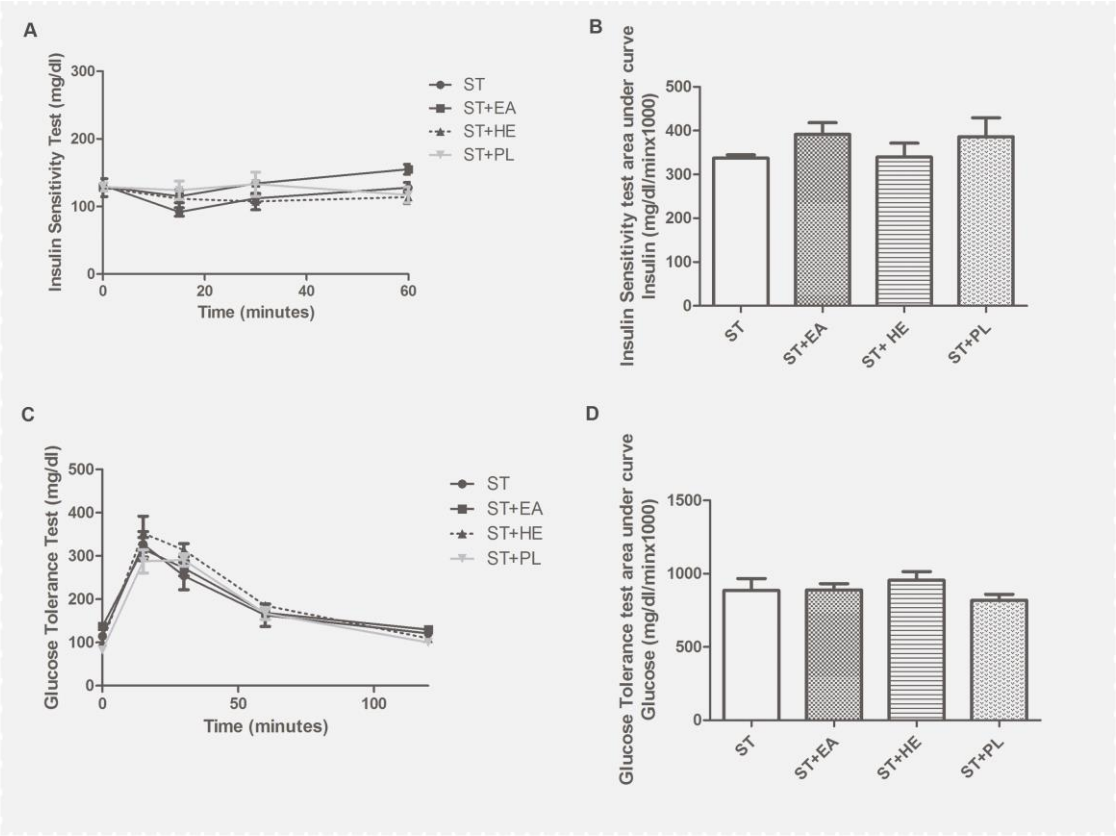


Figure 4. Effects of oral administration of hydroalcoholic extract, ethyl acetate fraction and leaf powder of *D. elliptica* on inulin sensitivity, glucose tolerance and fasting glucose levels in mice fed a standard diet. (A, B) Intraperitoneal Insulin Sensitivity Test (IPIST) and IPIST insulin area under the curve (mg/dL) (C, D) Intraperitoneal glucose tolerance test (IPGTT) and IPGTT glucose area under the curve. Significant differences, using two-way ANOVA and Tukey's post-test, are indicated by asterisks * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$). IPIST: Intraperitoneal Insulin Sensitivity Test, IPGTT: Intraperitoneal Glucose Tolerance test, ST: Standard, EA: fraction ethyl acetate, HE: hydroalcoholic extract and PL: leaf powder.

4. DISCUSSION

Our results indicate that the leaves of *D. elliptica* acts on the treatment of obesity, reducing adiposity and body weight, increasing insulin sensitivity of obese mice induced by diet, also improving plasma levels of triglycerides and total cholesterol of mice fed with a diet standard.

4.1 Compounds present in the leaves of *D. elliptica*

The compound classes of flavonoids, tannins and saponins were also described previously, in a phytochemical study with leaves and bark of *D. elliptica* (Michelin et al., 2005). The same results were elucidated by Guaraldo et al. (2001), with studies made from the stems of *D. rugosa*. Therefore, it is possible to infer the presence of these compounds in the different structures of the plants, and also that the species of the genus have similar compounds, differing only in tannin and flavonoid content (Guaraldo et al., 2001; Jácome et al. 2010). In a previous study with the leaves of *D. elliptica*, the results were positive for flavonoids, triterpenoids, steroids, gallic acid and catechins (Carlos et al., 2000; Soares et al., 2005).

Flavonoids, the products of secondary plant metabolism, exhibit antioxidant activity as well as effects on cardiovascular diseases. In a context of inflammation, some of the mechanisms of action of flavonoids are responsible for modulating the gene expression of cytokines (Panche et al. 2016). Our study evaluates the effect of the ethyl acetate fraction, rich in flavonoids on obesity.

4.2 Metabolic parameters

As we observed the increase in body composition and adiposity between ST groups vs. ST plus EA, we hypothesized that the effects of the polyphenols present in the ethyl acetate fraction are directly dependent on the amount and uptake of the polyphenols from the diet, through the gastrointestinal tract.

We suggest that the effects of *D. elliptica* observed in the treatment of obesity are due to the interaction of compounds present in larger quantities in the leaves of the species under study and that possibly the synergism between these compounds, quercetin, myricetin and its ramifications, are responsible for a mechanism not yet described in the literature.

Studies with myricetin have shown a decrease in body weight gain in obese animals, but differences in daily intake have not been observed (Chang et al., 2012), resembling the observations made in our study. In contrast, animals treated with quercetin showed no

difference in body weight gain or body composition. These findings corroborate in part with our study when we observed body weight effects in the ST group (Kuipers et al., 2018).

It is assumed that bioactive compounds are present in crude extracts at lower concentrations, acting through a combination of additive and synergistic effects (Kim et al., 2017; Seeram et al., 2004). Similar results were also reported by Kim et al. (2017) in an *in vivo* study using methanolic extract of *Limonium tetragonum* shoot, in which the presence of myricetin-3-O-β-D-galactopyranoside compound reduced significantly obese mouse adiposity. On the other hand, differences between food consumption were not reported (Kim et al., 2017).

Supposedly, adiposity is intrinsically related to the activation of brown adipose tissue (BAT). According to studies by Hu et al. (2018), myricetin, a dietary flavonoid present in leaves of *D. elliptica*, is responsible for activating BAT by increasing the expression of thermogenic proteins such as UCP1, SIRT1 and PGC1α, which appear to be responsible for modulating mitochondrial biogenesis (Hu et al., 2018) and may be a mechanism through which *D. elliptica* acts.

4.3 Plasma analyzes

We hypothesize that the effects observed in the reduction of triglyceride levels and total cholesterol in ST group animals are due to the interaction between the compounds myricetin and quercetin. These compounds are present in large quantities in *D. elliptica* leaves, as demonstrated in studies (Biso et al., 2010; Rodrigues et al., 2008), being responsible for altering the process of fatty acid uptake by adipocytes, a mechanism not yet elucidated.

Attributed to the effect of quercetin, studies have shown a reduction in plasma levels of triglycerides and fatty acids, associated with an increased flow of fatty acids, derived from triglycerides in relation to subcutaneous adipose tissue, which during this process undergoes a differentiation through the “browning” process (Kuipers et al., 2018). The mechanism behind these effects and the ability of quercetin to induce browning of adipocytes still need to be elucidated.

In vitro studies demonstrated that *L. tetragonum* extract inhibited fat accumulation during differentiation of 3T3-L1 pre-adipocytes in a dose-dependent manner, at a concentration of 6.25-50 μg/mL. This finding demonstrates that both the compound quercetin and myricetin may act on the browning process, reducing plasma levels of triglycerides and total cholesterol (Kim et al., 2017).

Quercetin seems to be a potent modulator of plasma triglyceride levels, and studies in humans and animal models suggest that quercetin is a possible new agent on metabolic parameters (Hoek-van den Hil et al., 2013; Kuipers et al. Sahebkar, 2017).

4.4 Glucose intolerance and insulin sensitivity

In previous studies, the use of quercetin, a compound isolated from *Phyllanthus emblica* fruit methanolic extracts, promoted a highly effective antihyperglycemic response in streptomycin-induced diabetic rats. Elevated plasma glucose levels decreased markedly after oral administration of the compound (Srinivasan et al., 2018). We did not observe the effects of glucose on obese mice induced by diet and treated with extract, fraction and leaf of *D. elliptica* although quercetin is one of the constituents of this extract. Possibly, the presence of other bioactive compounds in small concentrations may interact with the molecules already described and promote these results.

Other findings confirm the isolated action of compounds on glucose. Plasma glucose levels were also significantly decreased in myricetin-treated *db/db* mice (Hu et al., 2018).

The compounds myricetin and quercetin present in the aqueous extract of *Chrysobalanus icaco* leaves at the concentration of 0.35 mg/mL significantly reduced the blood glucose levels of animals treated with an ST diet when compared to the HFD groups (White et al., 2016). Hence, the aqueous extract of *Chrysobalanus icaco* significantly normalized glucose tolerance.

Shao et al. (2013) demonstrated in their studies that the presence of flavonoids such as quercetin modulates glycemic homeostasis against inflammation in animal models, inducing glucose intolerance and attenuating insulin sensitivity (Shao et al., 2013).

A Chinese medicinal formula prepared with *Gynostemma pentaphyllum* and consumed as tea reduced serum glucose, lowering glucose intolerance in diet-induced obese mice, so that tea screening confirmed the presence of quercetin and its glycosides (Guruvaiah et al., 2018).

Theoretically, some flavonoids may act by suppressing the intestinal lipid dietal adsorption, inhibiting pancreatic lipase (Seyedan et al., 2015), or these compounds may act on the browning process of white adipose tissue, which is considered a potential therapeutic target for treatment of obesity and metabolic diseases.

Supposedly, the synergistic effects of *D. elliptica* treatment for anti-obesity treatment may act through more than one mechanism or signaling pathway. Also, a hypothesis worth

considering is that the treatment with this plant may lead to increased thermogenesis through elevated UCP1 gene expression (Fu et al., 2016).

5. CONCLUSION

In conclusion, the findings of the present study indicate the *Davilla ellpitica* may be a therapeutic alternative in treatment of obesity decreasing adiposity by improving plasma levels of total cholesterol, triglycerides and increasing insulin sensitivity. However, new studies need to be performed to elucidate which pathways *D. elliptica* acts on the obesity process.

Author's contributions

JNS and SHSS conceived the study. JNS, wrote the manuscript. VHDG performed graphic designer present in figures. SHSS and VMC provided a critical revision of the paper. All authors read and have approved the final version of the paper.

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Conflict of interest

All authors do not have conflicts of interest.

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6 CONCLUSÕES

Com base no levantamento científico realizado através da revisão de literatura, é possível concluir que a *D. elliptica* exerce efeitos benéficos sobre inflamação, modulando genes associados a esse processo, como TNF-α, COX-1. Além disso, apresenta efeitos gastroprotetores, aumentando a biodisponibilidade da glutationa de modo a atuar como sistema de defesa antioxidante.

Fundamentado nas investigações experimentais realizadas até o momento, conclui-se que a *D. elliptica* reduz adiposidade em animais obesos induzidos por dieta e melhora parâmetros bioquímicos, triglicérides e colesterol total, em animais alimentados com dieta padrão, exercendo possíveis efeitos sobre vias moleculares ainda não elucidadas.

Em conjunto, esses achados apontam a *D. elliptica*, como possível agente antiobesidade, novos estudos serão necessários para explanar o mecanismo molecular envolvido nos efeitos observados, permitindo que a *D. elliptica,* futuramente, possa ser utilizada como um medicamento para o tratamento de doenças e abra a perspectiva de desenvolvimento de novos medicamentos.

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ANEXO

ANEXO A – Parecer do Comitê de Ética e Pesquisa

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
COMISSÃO DE ÉTICA EM EXPERIMENTAÇÃO
E BEM-ESTAR ANIMAL

PARECER CONSUBSTANCIADO

Montes Claros, 06 de setembro de 2018.

Processo N. º **164**
Título do Projeto: **Efeitos do extrato hidroalcoólico da folha de *Davilla elliptica* (Dilleniaceae) A. St.-Hil sobre parâmetros metabólicos de camundongos obesos induzidos por dieta**
Coordenador: **Dr. Sérgio Henrique de Souza Santos**

Histórico
A Organização Mundial de Saúde (OMS) aponta a obesidade como um dos mais importantes problemas de saúde pública.O potencial dos produtos naturais pode fornecer uma excelente estratégia na redução do ganho de peso. Os produtos naturais, incluindo extratos brutos e compostos isolados, induzem à redução do peso corporal e previnem a obesidade induzida pela dieta e doenças relacionadas. O Cerrado apresenta um importante bioma com espécies com potencial fitoterápico como a *Davilla elliptica*, descrita na literatura por seus efeitos anti-inflamatórios, antinociceptivo, antimicrobiano, gastroprotetor, gastro-hepatoprotetores, ação imunomoduladora e antitumoral. É válido o estudo do potencial terapêutico dessa planta dentro do contexto da obesidade. O presente projeto objetiva avaliar o potencial do extrato de folhas da *Davilla elliptica* na modulação da disfunção metabólica e inflamatória induzida em camundongos submetidos a uma dieta hiperlipídica.

Mérito
O experimento será realizado no biotério da UNIMONTES. Serão utilizados ao todo 64 camundongos, machos (entre 5-7 semanas) da linhagem *Swiss*, provenientes do Biotério do Centro de Ciências Biológicas e da Saúde (CCBS/ Unimontes).Durante as 8 primeiras semanas do experimento, os animais serão divididos em 2 grupos: dieta controle e dieta hiperlipídica. Após este período, todos os animais serão aleatoriamente separados em 8 grupos, contendo 8 animais/gaiola, Os grupos (G1 e G2) receberão as dietas padrão e hiperlipídica, respectivamente. O grupo (G3 e G6) receberá o extrato hidroalcoólico na dose de 5000 mg/kg [34] de peso corporal. Na mesma dose será administrada a fração alcaloidica (G4 e G7) e o pó das folhas pulverizadas (G5 e G8) da *D. elliptica*. Todos os animais serão tratados por um período de 30 dias. Ao final do tratamento, todos os animais, após jejum de 12 horas, serão eutanasiados por decapitação.

Parecer
Comissão de Ética em Experimentação e Bem-Estar Animal da Unimontes analisou o processo **164 e** entende que o mesmo está dentro das normas da Comissão. Sendo assim, somos pela **APROVAÇÃO** do projeto de pesquisa.

Prof. Orlando Raphael Lopasso Jr.
Comissão de Ética em Experimentação e Bem-Estar Animal da UNIMONTES

ANEXO B – Autorização de coleta de material vegetal- SISBIO

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Comprovante de registro para coleta de material botânico, fúngico e microbiológico

Número: 66693-1	Data da Emissão: 06/11/2018 16:54:26

Dados do titular

Nome: Sérgio Henrique Sousa Santos	CPF: 055.482.156-71

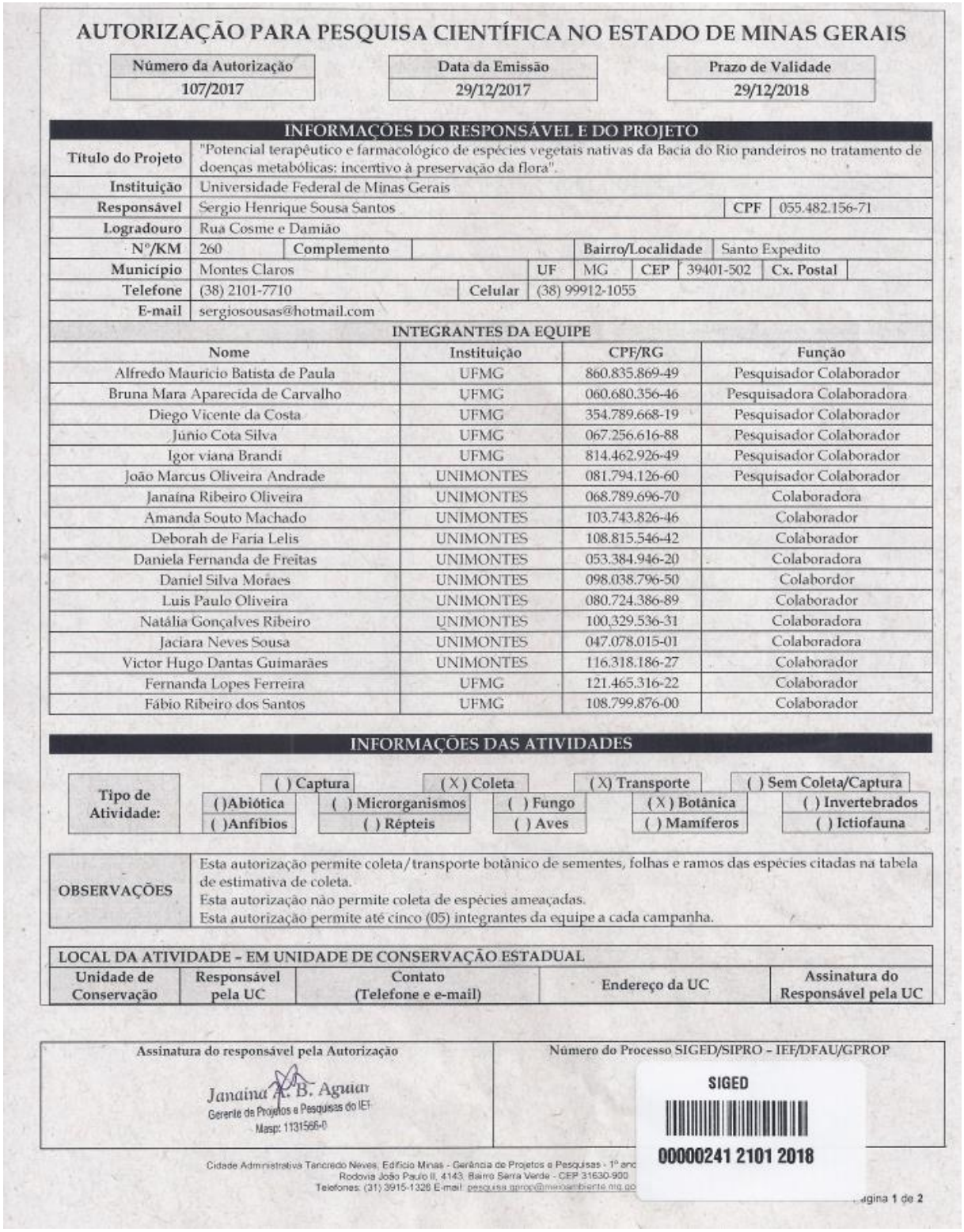
Observações e ressalvas

1	O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
2	Este documento não abrange a coleta de vegetais hidróbios, tendo em vista que o Decreto-Lei nº 221/1967 e o Art. 36 da Lei nº 9.605/1998 estabelecem a necessidade de obtenção de autorização para coleta de vegetais hidróbios para fins científicos..
3	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
4	Esse documento não eximirá o pesquisador da necessidade de obter outras anuências, como: I) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; II) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; III) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; IV) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; V) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outra
5	Este documento não é válido para: a) coleta ou transporte de espécies que constem nas listas oficiais de espécies ameaçadas de extinção; b) recebimento ou envio de material biológico ao exterior; e c) realização de pesquisa em unidade de conservação federal ou em caverna.
6	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.

Táxons autorizados

#	Nível taxonômico	Táxon(s)
1	Espécie	Plantae > Angiospermae > Dicotyledoneae > Lythraceae > Lafoensia > Pacari
2	Espécie	Plantae > Magnoliophyta > Magnoliopsida > Dilleniales > Dilleniaceae > Davilla > Elliptica
3	Espécie	Plantae > Magnoliophyta > Magnoliopsida > Lamiales > Boraginaceae > Cordia > Verbenacea
4	Espécie	Plantae > Magnoliophyta > Magnoliopsida > Asterales > Asteraceae > Lychnophora > Ericoides

ANEXO C – Autorização para pesquisa cientifica do estado de Minas Gerais



AUTORIZAÇÃO PARA PESQUISA CIENTÍFICA NO ESTADO DE MINAS GERAIS

Número da Autorização	Data da Emissão	Prazo de Validade
107/2017	29/12/2017	29/12/2018

INFORMAÇÕES DO RESPONSÁVEL E DO PROJETO

Título do Projeto	"Potencial terapêutico e farmacológico de espécies vegetais nativas da Bacia do Rio pandeiros no tratamento de doenças metabólicas: incentivo à preservação da flora".							
Instituição	Universidade Federal de Minas Gerais							
Responsável	Sergio Henrique Sousa Santos					CPF	055.482.156-71	
Logradouro	Rua Cosme e Damião							
Nº/KM	260	Complemento		Bairro/Localidade	Santo Expedito			
Município	Montes Claros		UF	MG	CEP	39401-502	Cx. Postal	
Telefone	(38) 2101-7710	Celular	(38) 99912-1055					
E-mail	sergiosousas@hotmail.com							

INTEGRANTES DA EQUIPE

Nome	Instituição	CPF/RG	Função
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Bruna Mara Aparecida de Carvalho	UFMG	060.680.356-46	Pesquisadora Colaboradora
Diego Vicente da Costa	UFMG	354.789.668-19	Pesquisador Colaborador
Júnio Cota Silva	UFMG	067.256.616-88	Pesquisador Colaborador
Igor viana Brandi	UFMG	814.462.926-49	Pesquisador Colaborador
João Marcus Oliveira Andrade	UNIMONTES	081.794.126-60	Pesquisador Colaborador
Janaína Ribeiro Oliveira	UNIMONTES	068.789.696-70	Colaboradora
Amanda Souto Machado	UNIMONTES	103.743.826-46	Colaborador
Deborah de Faria Lelis	UNIMONTES	108.815.546-42	Colaborador
Daniela Fernanda de Freitas	UNIMONTES	053.384.946-20	Colaboradora
Daniel Silva Moraes	UNIMONTES	098.038.796-50	Colabordor
Luis Paulo Oliveira	UNIMONTES	080.724.386-89	Colaborador
Natália Gonçalves Ribeiro	UNIMONTES	100.329.536-31	Colaboradora
Jaciara Neves Sousa	UNIMONTES	047.078.015-01	Colaboradora
Victor Hugo Dantas Guimarães	UNIMONTES	116.318.186-27	Colaborador
Fernanda Lopes Ferreira	UFMG	121.465.316-22	Colaborador
Fábio Ribeiro dos Santos	UFMG	108.799.876-00	Colaborador

INFORMAÇÕES DAS ATIVIDADES

Tipo de Atividade:	( ) Captura	(X) Coleta	(X) Transporte	( ) Sem Coleta/Captura
	( )Abiótica	( ) Microrganismos	( ) Fungo	(X) Botânica	( ) Invertebrados
	( )Anfíbios	( ) Répteis	( ) Aves	( ) Mamíferos	( ) Ictiofauna

OBSERVAÇÕES	Esta autorização permite coleta/transporte botânico de sementes, folhas e ramos das espécies citadas na tabela de estimativa de coleta. Esta autorização não permite coleta de espécies ameaçadas. Esta autorização permite até cinco (05) integrantes da equipe a cada campanha.

LOCAL DA ATIVIDADE - EM UNIDADE DE CONSERVAÇÃO ESTADUAL

Unidade de Conservação	Responsável pela UC	Contato (Telefone e e-mail)	Endereço da UC	Assinatura do Responsável pela UC

Assinatura do responsável pela Autorização	Número do Processo SIGED/SIPRO - IEF/DFAU/GPROP
Janaina A. B. Aguiar Gerente de Projetos e Pesquisas do IEF Masp: 1131566-0	SIGED 00000241 2101 2018

Cidade Administrativa Tancredo Neves, Edifício Minas - Gerência de Projetos e Pesquisas - 1º and
Rodovia João Paulo II, 4143, Bairro Serra Verde - CEP 31630-900
Telefones: (31) 3915-1328 E-mail: pesquisa.gprop@meioambiente.mg.go

...agina 1 de 2

Refúgio de Vida Silvestre Estadual do Rio Pandeiros	Neilton Viana Neves	(38) 3621-0100 neilton.viana@meioambiente.mg.gov.br	BR 479, Vila da CEMIG, S/nº Distrito de Pandeiros. CEP: 39.490-000	
APA Estadual do Rio Pandeiros	Altenfelder Martins da Fonseca	(38) 3625-6222 (38) 3625-6205 altenfelder.fonseca@meioambiente.mg.gov.br	Rua Joaquim Borges Monteiro nº 180 – Bonito de Minas CEP: 39.490-000	

Esta autorização será válida apenas com a autenticação do responsável pela(s) Unidade(s) de Conservação.

LOCAL DA ATIVIDADE – FORA DE UNIDADE DE CONSERVAÇÃO ESTADUAL (apenas para material botânico)	
Município(s)	Não se aplica

TRANSPORTE – DESTINO DO MATERIAL COLETADO	
Instituição(ões)	ICA - UFMG
Endereço(s)	Montes Claros - MG

Outras Observações e Ressalvas:

1. Esta autorização não exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena, da unidade de conservação federal, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação estadual cujo processo de regularização fundiária encontra-se em curso;
2. O pesquisador titular deverá contatar a administração dessa unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade de conservação, quando for o caso;
3. O Instituto Estadual de Florestas não se responsabiliza por qualquer dano a equipamentos, acidentes ou lesões físicas ou psíquicas, estando ainda, o pesquisador responsável e sua equipe ciente da vulnerabilidade da área de realização da pesquisa;
4. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
5. O titular da autorização e os membros de sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos e empregar esforços de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condições *in situ*, quando for o caso;
6. Esta autorização não permite captura/coleta/transporte:
   - para fins comerciais, industriais ou esportivos;
   - para realização de atividades integrantes do processo de licenciamento ambiental de empreendimentos, conforme resolução do CONAMA de nº 237 de 19/12/97, salvo quando especificado;
   - de espécies ameaçadas de extinção em lista oficial federal, salvo quando constante de projeto específico autorizado pelo SISBIO;
   - de espécies ameaçadas de extinção em lista oficial estadual, salvo quando constante de projeto específico autorizado pelo IEF;
   - de fauna e flora em áreas de domínio privado, sem o consentimento expresso ou tácito do proprietário nos termos do Código Civil;
7. Esta autorização não permite transporte interestadual e internacional de material biológico;
8. Esta autorização não dispensa o cumprimento da legislação que dispõe sobre o acesso ao patrimônio genético, sobre a proteção e o acesso ao conhecimento tradicional associado e sobre a repartição de benefícios para conservação e uso sustentável da biodiversidade. Veja maiores informações em www.mma.gov.br;
9. O titular desta autorização, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização suspensa ou revogada pelo IEF e o material biológico coletado apreendido nos termos da legislação em vigor;
10. O responsável poderá, durante a validade desta autorização e conforme Termo de Compromisso firmado, solicitar à Gerência de Projeto e Pesquisas do IEF Renovação, Cancelamento ou Conclusão, conforme instruções no site do IEF (http://www.ief.mg.gov.br/biodiversidade/pesquisa-cientifica);
11. Esta autorização é válida somente sem emendas ou rasuras e exclusivamente no estado de Minas Gerais;
12. O pesquisador deverá estar sempre acompanhado desta autorização para apresentá-la às autoridades, quando solicitado.

Assinatura do responsável pela Autorização	Número do Processo SIGED/SIPRO – IEF/DFAU/GPROP
Janaina A. B. Aguiar Gerente de Projetos e Pesquisas do IEF Masc: 1131566-0	

ANEXO D- Normas para publicação no periódico *Journal of Ethnopharmacology*

JOURNAL OF ETHNOPHARMACOLOGY

GUIDE FOR AUTHORS

INTRODUCTION

The Journal of Ethnopharmacology is dedicated to the exchange of information and understandings about people's use of plants, fungi, animals, microorganisms and minerals and their biological and pharmacological effects based on the principles established through international conventions. Early people, confronted with illness and disease, discovered a wealth of useful therapeutic agents in the plant and animal kingdoms. The empirical knowledge of these medicinal substances and their toxic potential was passed on by oral tradition and sometimes recorded in herbals and other texts on materia medica. Many valuable drugs of today (e.g., atropine, ephedrine, tubocurarine, digoxin, reserpine) came into use through the study of indigenous remedies. Chemists continue to use plant-derived drugs (e.g., morphine, taxol, physostigmine, quinidine, emetine) as prototypes in their attempts to develop more effective and less toxic medicinals.

Please note that figures and tables should be embedded in the text as close as possible to where they are initially cited. It is also mandatory to upload separate graphic and table files as these will be required if your manuscript is accepted for publication.

Classification of your paper

Please note that upon submitting your article you will have to select **at least one classification and at least three of the given keywords**. You can preview the list of classifications and keywords (here). This information is needed by the Editors to more quickly process your article. In addition to this, you can submit free keywords as described below under "Keywords".

The "rules of 5"
The Editors and Editorial Board have developed the "Rules of 5" for publishing in JEP. We have produced five clear criteria that each author needs to think about before submitting a manuscript and setting the whole process of editing and reviewing at work. Click here.

For more details on how to write a world class paper, please visit our Pharmacology Author Resources page.

Authors are encouraged to submit video material or animation sequences to support and enhance your scientific research. For more information please see the paragraph on video data below.

Types of paper
The Journal of Ethnopharmacology will accept the following contributions:

1. Original research articles - whose length is not limited and should include Title, Abstract, Methods and Materials, Results, Discussion, Conclusions, Acknowledgements and

References. As a guideline, a full length paper normally occupies no more than 10 printed pages of the journal, including tables and illustrations.
2. Short Communications - whose average length is not more than 4 pages in print (approx. 2000-2300 words, including abstract and references). A maximum of 2 illustrations (figures or tables) is allowed. See paragraph below for description and format.
3. Letters to the Editors.
4. Reviews - Authors intending to write review articles should consult and send an outline to the Reviews Editor (see inside front cover for contact information) before preparing their manuscripts. The organization and subdivision of review articles can be arranged at the author's discretion. Authors should keep in mind that a good review sets the trend and direction of future research on the subject matter being reviewed. Tables, figures and references are to be arranged in the same way as research articles in the journal. Reviews on topics that address cutting-edge problems are particularly welcome. Outlines for potential reviews need to include: A detailed abstract using the structure provided in the guidelines An annotated table of contents A short CV of the lead author
5. Book reviews - Books for review should be sent to the Reviews Editor.
6. Commentaries - invited, peer-reviewed, critical discussion about crucial aspects of the field but most importantly methodological and conceptual-theoretical developments in the field and should also provide a standard, for example, for pharmacological methods to be used in papers in the Journal of Ethnopharmacology. The scientific dialogue differs greatly in the social / cultural and natural sciences, the discussions about the common foundations of the field are ongoing and the papers published should contribute to a transdisciplinary and multidisciplinary discussion. The length should be a maximum of 2-3 printed pages or 2500 words. Please contact the Reviews Editor j.ethnopharmacol@pharmacy.ac.uk with an outline.
7. Conference announcements and news.

Submission checklist
Please click here to download the Submission **Checklist.** This is a mandatory file during submission. Upload the completed checklist and choose the file type as "Checklist".

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
• E-mail address
• Full postal address

All necessary files have been uploaded:
Manuscript:
• Include keywords
• All figures (include relevant captions)
• All tables (including titles, description, footnotes)
• Ensure all figure and table citations in the text match the files provided
• Indicate clearly if color should be used for any figures in print
Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
• Manuscript has been 'spell checked' and 'grammar checked'
• All references mentioned in the Reference List are cited in the text, and vice versa
• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• Relevant declarations of interest have been made
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN
Ethics in publishing
Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Policy and ethics
In the covering letter, the author must also declare that the study was performed according to the international, national and institutional rules considering animal experiments, clinical studies and biodiversity rights. See below for further information. The ethnopharmacological importance of the study must also be explained in the cover letter.

Animal and clinical studies - Investigations using experimental animals must state in the Methods section that the research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/EEC) or the US guidelines (NIH publication #85-23, revised in 1985). Investigations with human subjects must state in the Methods section that the research followed guidelines of the Declaration of Helsinki and Tokyo for humans, and was approved by the institutional human experimentation committee or equivalent, and that informed consent was obtained. The Editors will reject papers if there is any doubt about the suitability of the animal or human procedures used.

Biodiversity rights - Each country has its own rights on its biodiversity. Consequently for studying plants one needs to follow the international, national and institutional rules concerning the biodiversity rights.

Author contributions
For each author the contribution to the publication should be mentioned.

Declaration of interest
All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted.

2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification
Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyrightholder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

Preprints
Please note that preprints can be shared anywhere at any time, in line with Elsevier's sharing policy. Sharing your preprints e.g. on a preprint server will not count as prior publication (see 'Multiple, redundant or concurrent publication' for more information).

Use of inclusive language
Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Changes to authorship
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