FUNDAÇÃO UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL

FACULDADE DE CIÊNCIAS FARMACÊUTICAS, ALIMENTOS E NUTRIÇÃO

DJACELI SAMPAIO DE OLIVEIRA DEMBOGURSKI

POTENCIAL ANTI-INFLAMATÓRIO, ANTIMICROBIANO E ANTIBIOFILME DAS FOLHAS DE *MICONIA ALBICANS* (MELASTOMATACEAE)

CAMPO GRANDE – MS

2023

DJACELI SAMPAIO DE OLIVEIRA DEMBOGURSKI

POTENCIAL ANTI-INFLAMATÓRIO, ANTIMICROBIANO E ANTIBIOFILME DAS FOLHAS DE *MICONIA ALBICANS* (MELASTOMATACEAE)

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biotecnologia e Biodiversidade – Rede Pró-Centro Oeste como pré-requisito para obtenção do título de Doutor.

Área de concentração: Desenvolvimento de produtos, processos e serviços biotecnológicos.

Orientadora: Profa. Dra. Denise Brentan da Silva

CAMPO GRANDE – MS

2023

FOLHA DE APROVAÇÃO

Djaceli Sampaio de Oliveira Dembogurski

Potencial anti-inflamatório, antimicrobiano e antibiofilme das folhas de *Miconia albicans* (Melastomataceae)

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biotecnologia e Biodiversidade – Rede Pró-Centro Oeste como pré-requisito para obtenção do título de Doutor.

Área de concentração: Desenvolvimento de produtos, processos e serviços biotecnológicos.

Aprovada em: 24/03/2023

Banca Examinadora

Orientadora: Profª Drª. Denise Brentan da Silva

Profª Drª. Ana Camila Micheletti (Interno)

Profª Drª. Lillian May Grespan Estodutto da Silva (Externo)

Profª Drª. Thalita Bachelli Riul (Interno)

Prof. Dr. Saulo Euclides da Silva Filho (Interno)

Profª Drª. Nadla Soares Cassemiro (Interno) (Suplente)

Este trabalho é dedicado:

A minha avó Maria (*in memoriam*), foi a primeira pessoa a me mostrar que os remédios estão nas plantas, sem conhecimento científico algum, tinha uma imensa sabedoria sobre o uso de plantas medicinais e o cuidado com as mesmas.

AGRADECIMENTOS

A profa. Dra. Denise Brentan da Silva pela orientação e ensinamentos. Obrigada por acreditar em mim e em meu trabalho, é admirável ver seu entusiasmo com a ciência e em ensinar, bem como seu talento e sabedoria em encorajar seus alunos a seguirem em frente e não desistir. Agradeço pelo apoio e por tornar este doutorado possível.

Aos meus pais Dilza e Juseval que sempre me incentivaram a seguir meus estudos e são meus exemplos de trabalho, valores e dedicação.

Ao meu amado marido Alexangelo, pela força e companheirismo, por estar ao meu lado nessa longa caminhada cheia de altos e baixos, enxugando minhas lágrimas e me reerguendo, seus gestos de apoio e aprovação foram essenciais para conclusão desse trabalho.

A minha irmã Joyce Sampaio de Oliveira Fagundes, por sua generosidade e influência inestimável que agrega em minha vida, obrigada pelos meus sobrinhos Maitê e Theo, que me trazem alegria e são parte do meu esforço para que eu seja um exemplo a eles.

A Ma. Iluska Senna Bonfá, pela sua enorme paciência e talento em ensinar, obrigada pela parceria em meus ensaios e pela amizade que criamos. A prof. Dra. Mônica Cristina Toffoli Kadri por disponibilizar seu laboratório, considerações e toda ajuda necessária para que uma importante parte deste trabalho fosse desenvolvido.

A Ma. Amanda Galdi Boaretto, por seu talento em ensinar, dentro do laboratório e em campo, sou muito grata pela parceria dessa exímia bióloga.

A Dra. Nadla Cassemiro Soares, por toda ajuda com as análises processadas durante esse trabalho, convivência e amizade no laboratório.

A profa. Dra. Luciane Candeloro e ao prof. Dr. Eduardo Benedetti Parisotto, pela parceria e processamento de amostras para conclusão desse trabalho.

Ao prof. Dr. Flávio Macedo Alves, por auxiliar nas coletas e na identificação correta da espécie usada nessa tese.

Ao Me. André Nunes da Silva, por me acompanhar em todas as coletas de planta, pelo grande auxílio no processamento dos dados e parceria nesse trabalho, principalmente pela amizade e convivência no decorrer desses anos.

Ao prof. Dr. Alexandre José de Macedo e ao Me. Rodrigo Campos Silva do Laboratório de Biofilmes e Diversidade Microbiana, da Universidade Federal do Rio Grande do Sul, pela parceria, suporte e desenvolvimento de experimentos.

A meus sogros Albano (*in memoriam*) e Sônia pelo incentivo e apoio, sou grata por fazer parte da família de vocês.

A todos os colegas do LaPNEM (Laboratório de Produtos Naturais e Espectrometria de Massa), aos que fazem parte e que fizeram parte desse grupo de pesquisa, obrigada pela convivência, amizade, risadas, almoços e happy hour, levarei vocês para sempre em minha memória: Augusto, Arthur, Bruno, Carol, Fernanda, Guilherme, Izadora, Karen, Katyuce, Luana, Maikeli, Mariana, Samara e Vanessa.

Agradeço a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), órgão financiador da bolsa de estudos concedida, a Fundação Universidade Federal de Mato Grosso do Sul e a Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição pelo suporte e integração fornecidos.

“Não é o crítico que importa; nem aquele que aponta onde foi que o homem tropeçou ou como o autor das façanhas poderia ter feito melhor.

O crédito pertence ao homem que está por inteiro na arena da vida, cujo rosto está manchado de poeira, suor e sangue; que luta bravamente; que erra, que decepciona, porque não há esforço sem erros e decepções; mas que, na verdade, se empenha em seus feitos; que conhece o entusiasmo, as grandes paixões; que se entrega a uma causa digna; que, na melhor das hipóteses, conhece no final o triunfo da grande conquista e que, na pior, se fracassar, ao menos fracassa ousando grandemente. ”

Theodore Roosevelt

DEMBOGURSKI, Djaceli Sampaio de Oliveira. Potencial anti-inflamatório, antimicrobiano e antibiofilme das folhas de *Miconia albicans* (Melastomataceae) (Tese). Doutorado em Biotecnologia e Biodiversidade, Universidade Federal de Mato Grosso do Sul; 2023.

RESUMO

Miconia albicans (Melastomataceae) é conhecida popularmente pelo nome canela de velho e suas folhas são utilizadas em forma de chá para tratar principalmente inflamações e dores articulares. No entanto, escassas evidências científicas avaliam o potencial anti-inflamatório *in vivo* correlacionado ao perfil químico e a variação sazonal de seus constituintes. Com isso, foi obtido um extrato aquoso das folhas de *M. albicans* (MAAE), similar ao utilizado pela população, e o mesmo foi submetido a ensaios de inflamação, analgesia e toxicidade. MAAE exibiu na dose de 256 mg/kg, uma inibição do efeito edematogênico nos animais tratados, assim como uma redução da migração leucocitária, diante a resposta inflamatória aguda. As contorções abdominais induzidas por ácido acético foram reduzidas no tratamento com MAAE no teste de nocicepção, bem como o tempo de lambida de pata, mostrando um potencial nociceptivo. MAAE não provocou efeitos toxicológicos nos ensaios de toxicidade aguda e de doses repetidas. Os animais não apresentaram alterações quando analisados os parâmetros bioquímicos, histológicos e hematológicos. A atividade antimicrobiana e antibiofilme de MAAE e 4 frações foram avaliadas frente as bactérias *Staphylococcus aureus* e *Pseudomonas aeruginosa*, onde todas as amostras submetidas não apresentaram atividade eficaz contra as cepas patogênicas. A análise fitoquímica feita pela técnica de CLAE-DAD-EM, possibilitou anotar vinte e quatro compostos a partir do MAAE, como megastigmanos, flavonoides, elagitaninos e triterpenos, destacando que essas classes de metabólitos são reconhecidas por seu potencial anti-inflamatório. Na investigação sazonal, 4 indivíduos da mesma espécie foram monitorados durante 10 meses, dessa forma, observou-se que as estações seca e chuvosa interferem na produção de metabólitos secundários da planta. Sendo observado que as classes de flavonoides e megastigmanos ocorrem com maior intensidade no período de seca, enquanto os taninos ocorrem mais intensamente em estação chuvosa. Neste trabalho, a classe dos megastigmanos foram identificados pela primeira vez no gênero *Miconia*, assim como não haviam informações na literatura inferindo a variação sazonal relacionada a esses compostos.

Palavras-chave: *Miconia albicans*, inflamação, toxicidade, megastigmanos, sazonalidade.

DEMBOGURSKI, Djaceli Sampaio de Oliveira. Anti-inflammatory, antimicrobial and antibiofilm potencial of *Miconia albicans* (Melastomataceae) leaves (Thesis). Doctorate in Biotechnology and Biodiversity, Federal University of Mato Grosso do Sul; 2023

ABSTRACT

The *Miconia albicans* (Melastomataceae) is popularly known as canela-de-velho, and its leaves are used as tea to treat inflammation and joint pain. However, few scientific evidence evaluates the *in vivo* anti-inflammatory potential correlated to the chemical profile and seasonal interference of the compounds. In this sense, was developed a *M. albicans* aqueous extract (MAAE), similar to use by the population, and used to inflammation, analgesia, and toxicity assays. It revealed that the MAAE at a dose of 256 mg/kg, inhibits the edematogenic effect in treated animals, as well as reduces the level of leukocyte migration, against the acute inflammatory response. The acetic acid-induced abdominal writhings were decreased with MAAE treatment, as well as the paw licking time response was reduced, showing nociceptive potential. MAAE showed no toxicological effects, as evaluated in acute and repeated-dose toxicities. The animals showed no alterations when the biochemical, histological and hematological parameters were analyzed. The antimicrobial and antibiofilm activities of MAAE and 4 fractions were evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria, all samples submitted did not show effective activity against the pathogenic strains. The phytochemical analysis by the CLAE-DAD-EM technique, made it possible to identify 24 compounds from the MAAE, such as megastigmanes, flavonoids, ellagitannins and triterpenes, highlighted that these classes of metabolites are recognized for their anti-inflammatory potential. In the seasonal investigation, 4 individuals of the same species were monitored for 10 months, this way, it was observed, that the dry and rainy seasons interfere in the production of secondary metabolites of the plant. Was observed the classes of flavonoids and megastigmanes occur with greater intensity in the dry season, while tannins occur more intensely during the rainy season. In this work, the class of megastigmanes was identified for the first time in the genus *Miconia*, and there was not information about the season variation related to these compounds.

Keywords: *Miconia albicans*, inflammation, toxicity, megastigmanes, seasonality.

LISTA DE ABREVIATURAS

AcOEt	Acetato de etila
AU	Ácido ursólico
AO	Ácido oleanólico
AINEs	Anti-inflamatórios não-esteroidais
BuOH	Butanol
CLAE	Cromatografia líquida de alta eficiência
COX	Ciclo-oxigenase
DAD	Detector de arranjos de diodos
DCM	Diclorometano
DMSO	Dimetilsulfóxido
DO	Densidade ótica
EM	Espectrometria de massas
EM/EM	Espectrometria de massas *tandem*
EtOH	Etanol
Hx	Hexano
IES	Ionização por eletrospray
CI_{50}	Concentração Inibitória média
IL-1	Interleucina 1
i.p.	Intraperitoneal
i.pl.	Intraplantar
LOX	Lipo-oxigenase
MAAE	*Miconia albicans* aqueous extract (extrato aquoso de *Miconia albicans*)
MeOH	Metanol
CIM	Concentração Inibitória Mínima
m/z	Relação massa/carga
MN	Mononuclear
NO	Nitric Oxide (Óxido nítrico)
PMN	Polimorfonuclear
TNF	Fator de necrose tumoral
UV	Espectro de ultravioleta

SUMÁRIO

1. INTRODUÇÃO

A base para medicina tradicional e moderna sempre foram as produtos naturais, em que muitas plantas foram utilizadas como matéria-prima vegetal e tornaram-se fonte de estudos no desenvolvimento e busca de novos fármacos (SERPELONI *et al*., 2011). Neste sentido, encontramos no vasto cerrado brasileiro uma rica flora com inúmeras espécies a serem estudadas, muitas delas com aplicações etnofarmacológicas. Explorando esse bioma selecionamos a espécie *Miconia albicans*, a qual pertence a um gênero da grande família Melastomataceae, que possui diversas espécies com potencial uso terapêutico, assim promoveremos uma ampliação dos conhecimentos sobre a biodiversidade do cerrado brasileiro.

Miconia é o maior gênero em número de espécies da família Melastomataceae, ocorre em toda América do Sul e Central e possui um grande apelo comercial e econômico pelo fato de suas espécies serem ornamentais (FERREIRA *et al.,* 2013). A espécie *Miconia albicans* é uma das mais populares do gênero, conhecida por canela-de-velho. É muito difundida na medicina popular para tratamento de diversas doenças, como por exemplo, inflamações, infecções, artrose e dores articulares (TARAWNEH *et al.*, 2014; SERPELONI *et al.,* 2008; RODRIGUES *et al.*, 2011).

A droga vegetal de *M. albicans* é comercializada em forma de folhas secas, cápsulas, pomada e tintura. No entanto, em 2017 a Agência Nacional de Vigilância Sanitária (ANVISA) divulgou a primeira resolução proibindo a comercialização de produtos contendo *M. albicans* de algumas empresas, por não possuirem registro, notificação ou cadastro junto ao órgão (DOU 35/17). Em informe Resolução-RE nº 1.417, de 1º de junho de 2018, a ANVISA determinou como medida de interesse sanitário, em todo o território nacional, a proibição da fabricação, distribuição, comercialização e uso do produto canela de velho como medicamento, bem como a divulgação em qualquer mídia, até que o produto tenha registro no órgão. Atualizado em, 04 de julho de 2022, ainda fica proibido a distribuição de produtos à base de *M. albicans*, por não possuir registro na agência. Ressaltando assim, a ausência de dados que comprovem a segurança e eficácia dessa planta medicinal.

É conveniente ressaltar que muitas dessas espécies vegetais do gênero *Miconia* são utilizadas pela população e não possuem descrição na literatura quanto à composição química,

eficácia, segurança, perfil farmacológico e toxicológico. Esses apontamentos estimulam o desenvolvimento desta pesquisa, tanto no sentido da busca pela validação de ação terapêutica e toxicidade que envolvem o uso quanto a caracterização química e variações sazonais que interfiram no teor dos seus constituintes. Portanto, o objetivo desse estudo é explorar a aplicação de técnicas metabolômicas e ensaios *in vivo*, no intuito de corroborar o conhecimento popular sobre a espécie vegetal *M. albicans.*

2. REVISÃO BIBLIOGRÁFICA

2.1. Etnofarmacologia

A biodiversidade brasileira é uma fonte extremamente importante para a pesquisa de novos medicamentos e alimentos. As plantas são fontes valiosas de estudos e desenvolvimento de produtos no Brasil, gerando grande destaque e oportunidades na pesquisa científica e tecnológica (FERNANDES *et al*., 2019; CARVALHO *et al*., 2018). O Brasil possui uma das floras mais ricas do mundo, abrangendo seis biomas distintos. No estado de Mato Grosso do Sul encontram-se dois biomas, o cerrado e o Pantanal. O cerrado é considerado um dos maiores *hotspots* mundiais em biodiversidade, além de ser considerado a maior savana tropical do mundo contendo 11.627 espécies nativas e 4.000 endêmicas (GARCEZ *et al*., 2016; MACEDO *et al*., 2018).

O uso medicinal de plantas é um hábito brasileiro comum, sendo praticado por uma boa parte da população incluindo, principalmente, aqueles com acesso limitado a serviços de saúde e medicamentos, o que leva ao consumo de plantas como alternativa para o alívio de sintomas e tratamento de doenças. Diversas espécies nativas possuem valor socioeconômico e medicinal sobretudo para populações que estão em contato direto com elas, incluindo comunidades rurais e indígenas, o que as tornam fontes primárias de conhecimento tradicional e uso medicinal dessas espécies. O reconhecimento dessas práticas populares que existem há séculos é um importante instrumento que auxilia na descoberta de novos fármacos, moléculas bioativas e alvos terapêuticos (CECHINEL-ZANCHETT *et al*., 2017; GASPAROTTO-JUNIOR *et al.,* 2019; HAKALA *et al*., 2015; TROJAN-RODRIGUEZ *et al*., 2012).

O conhecimento que mescla farmacologia, botânica, antropologia e farmacognosia é denominado etnofarmacologia, no qual etno significa cultura ou grupo e tem como origem o conhecimento popular. Esta prática está cada vez mais se tornando um grande foco de interesse científico e estimulando novas pesquisas, o que é embasado por exemplos de sucesso, como o desenvolvimento de ácido acetilsalicílico inspirado em *Salix alba,* morfina de *Papaver somniferum* e outros (ERHABOR *et al*., 2020; YEUNG *et al*., 2020).

Uma abordagem de investigação de novos fármacos, a partir de espécies vegetais inexploradas, pode-se iniciar de uma visão geral de conhecimentos populares, centrados em aspectos socioculturais de uso local e tradicional. Com base nessas informações primárias é dado início a busca de embasamento científico para validar o conhecimento popular (MOURA *et al*., 2019). Nesse cenário, começa-se a pesquisa farmacológica empregando tecnologias para destrinchar minuciosamente a composição química do material vegetal, bem como a execução de ensaios *in vivo* nas investigações terapêuticas e de toxicidade (TOLOUEI *et al*., 2019; TABACH *et al.,* 2017).

Estudos etnofarmacológicos estão sendo realizados por diversos grupos de pesquisa de inúmeras áreas. Tais trabalhos, no domínio da etnofarmacologia, necessitam mimetizar em seu desenho experimental, ou seja, ser o mais próximo possível do modo de utilização tradicional, para que os resultados possam ser validados e devolvidos a comunidade. Em consequência, mais estudos farmacológicos podem ser conduzidos a partir dos resultados iniciais para esclarecer, por exemplo, o mecanismo de ação, realizar o controle de qualidade e determinar sua aplicação clínica com maior propriedade (HE *et al.,* 2020; WANG *et al*., 2020; EZENYI *et al*., 2020).

Embora haja um grande aumento de informações nas bases científicas sobre a utilização de plantas medicinais, o fundamento que envolve sua dose terapêutica e toxicidade ainda é diminuto. Por isso, ainda há grande necessidade de investigações sobre a flora brasileira, incluindo do cerrado, visando a descoberta de substâncias bioativas, aplicações biotecnológicas, nutracêuticos e produtos para prevenção de doenças (RIBEIRO-NETO *et al*., 2020; MOURA *et al.,* 2019).

2.2. Família Melastomataceae

A família Melastomataceae, ordem Myrtales, possui 166 gêneros e aproximadamente 4.600 espécies, apresentando ampla distribuição por toda região tropical e subtropical, porém com maior incidência na América do Sul (GOLDENBERG *et al.,* 2012). Algumas espécies pertencentes aos gêneros *Melastoma, Medinilla* e *Osbeckia* são utilizadas há muito tempo como plantas medicinais

para o tratamento de diarreia, doenças de pele, hemorragias e como adstringente. Na medicina tradicional chinesa, as folhas de *Melastoma dodecadrum* são utilizadas no tratamento de hemorroidas, dermatites, picada de cobra, escabiose, bem como alternativa para se evitar o aborto, enquanto que suas raízes são aplicadas no tratamento de diarreia, dores abdominais e pós-parto (ISHII *et al*., 1999). Em Chiapas, México, as pessoas mastigam e comem as folhas de *Arthrostema ciliatum* para tratar as causas de vômito, uma antiga tradição Maia que perdura até a atualidade, e em outras partes da América Central a decocção de toda a planta é utilizada como diurético e laxante (ALMEDA, 1993; BARUCH *et al*., 2000).

As espécies dessa família destacam-se por produzirem flores e frutos ao longo de todo o ano e apresentam características vegetativas e reprodutivas diversas, podendo ser facilmente reconhecidas entre dicotiledôneas pelas suas folhas, uma vez que possuem venação acrodrômica característica (RIBEIRO *et al.*, 2016; SERNA, MARTINEZ, 2015). Em levantamento da biodiversidade brasileira, a família Melastomataceae é identificada como o quinto maior grupo de Angiospermas do Brasil ocorrendo, principalmente, na Mata Atlântica e Cerrado, e contribuindo de forma significativa em termo de espécies endêmicas do país (ZAPPI *et al*., 2015).

A partir de levantamentos bibliográficos aplicando o termo Melastomataceae na plataforma *SciFinder*, do banco de dados *Chemical Abstracts,* foram encontrados 688 resultados (incluindo 33 patentes). Verificando os resultados por ano de publicação, foi analisado o período entre 1970 e 2023, sendo possível constatar o aumento progressivo de publicações, principalmente após o ano 2003, o que demonstra o crescente interesse pelas espécies da família Melastomataceae (**Figura 1**). O maior número de publicações ocorreu no ano de 2016 com 53 resultados e dentre esses, cinco trabalhos são patentes. Quando filtrados por conceitos os resultados segregam-se em: *Plant extracts* (128 resultados), *Brazil* (70), *Pharmaceutical natural products* (68), *Miconia* (23), *Anti-inflammatory agents* (38) e *Tradicional Medicine* (14).

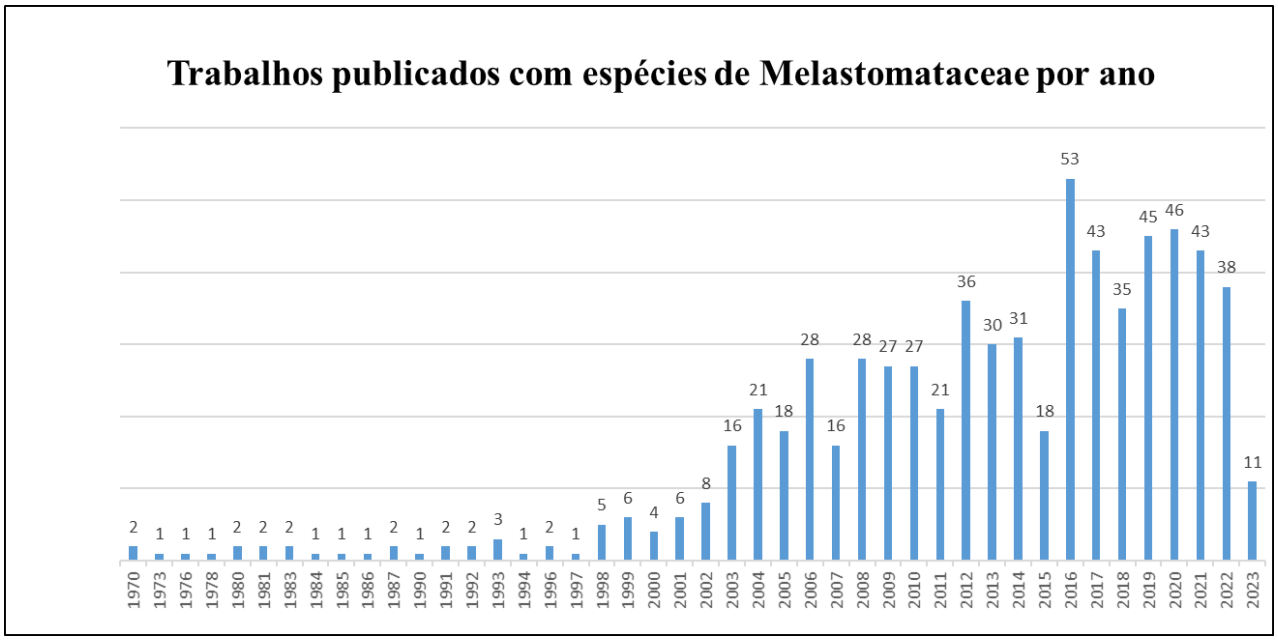


Figura 1. Gráfico anual de publicações de trabalhos referentes a espécies da família Melastomataceae desde o ano 1970 a 2023. Fonte: CHEMICAL ABSTRACTS, 2023.

Dados químicos da família Melastomataceae, em geral, ainda são escassos, porém dentre os metabólitos secundários já descritos encontram-se flavonoides, terpenoides, triterpenos, taninos, antraquinonas, lignanas, quinonas e cumarinas. Esses são os principais compostos que têm sido relacionados às suas propriedades biológicas como antioxidante, hepatoprotetora, anti-inflamatória, antinociceptiva, antidiarreica, antibacteriana, antitumoral, antitripanossoma e antimalária (MOURA *et al*., 2013; MURUGAN, PARIMELAZHAGAN, 2013; GORDON *et al.*, 2011; GILBERT *et al.*, 2014; CUNHA *et al.*, 2003).

Os flavonoides kaempferol, apigenina, luteolina, quercetina e seus derivados, principalmente os glicosilados, foram frequentemente identificados em grande parte dos gêneros da família Melastomataceae (BONFIM-PATRICIO *et al.*, 2000; SERNA, MARTINEZ, 2015). Mimura e colaboradores (2004) analisaram oito espécies do gênero *Huberia* (*H. carvalhoi, H. espirito-santensis, H. minor, H. glazioviana, H. nettoana, H. ovalifolia and H. semiserrata* e *H. consimilis*) e relataram esses flavonoides em todas as espécies, além de também publicarem dados morfológicos.

A espécie *Dissotis perkinsiae* é utilizada como planta medicinal no tratamento de febre, doenças infecciosas e reumatismo pela população de Camarões (África do Sul). Nadjateu e colaboradores (2014), desenvolveram um extrato etanólico com as folhas de *D. perkinsiae*, e avaliaram a capacidade antioxidante pelo ensaio de DPPH, apresentando uma CI_{50} de 130,66 µg/mL. Bem como foi avaliado o potencial antibacteriano frente as cepas de *Enterococcus faecalis* (CIM 0,04 mg/mL), *Escherichia coli* (CIM 0,08 mg/mL) e *Sthaphylococcus aureus* (CIM 0,08 mg/mL) pelo método de microdiluição em série. Esses dados antibacterianos corroboram com a utilização popular de *D. perkinsiae* para o tratamento de infecções.

A espécie *Bellucia dichotoma*, nativa da região amazônica, é utilizada pela população regional, em forma de chá das cascas, para tratar os efeitos hemorrágicos locais induzidos pelo veneno de serpentes. Moura e colaboradores (2013) avaliaram o extrato aquoso da casca de *B. dichotoma* frente ao veneno de *Bothrops atrox,* na concentração de 1:5 (veneno:extrato; 10 µg:50 µg), por via intraplantar (i.pl.), e observaram inibição total da atividade hemorrágica e edematogênica provocadas pelo veneno. Posteriormente, o extrato aquoso de *B. dichotoma* foi investigado frente ao veneno de *Bothrops jararaca,* em concentração de 1:48 (veneno:extrato; 10 µg:480 µg), via intradérmica (i.d.) na região dorsal, ao qual inibiu completamente a hemorragia induzida pelo veneno (MOURA *et al.,* 2015). Tendo em vista os trabalhos anteriores, Moura e colaboradores (2017) analisaram espécimes de *B. dichotoma*, coletadas em diferentes regiões de Manaus (AM) e Santarém (PA), estas foram utilizadas para a obtenção de extratos aquosos, sendo avaliados *in vivo* contra o veneno de *B. atrox* em doses baseadas no uso popular (48,3; 145 e 289,8 mg/kg, por via oral). Este estudo, relevou que os extratos inibiram 100% da atividade coagulante induzida pelo veneno de *B. atrox* em todas as doses avaliadas. Indicando que a espécie *B. dichotoma,* coletada em duas diferentes regiões possuem composição química similar, ambas contendo propriedades anti-hemorrágicas.

As espécies do gênero *Tibouchina* são encontradas em bordas e clareiras de florestas, sendo amplamente utilizadas para fins de reflorestamento, marcadores de poluição e ornamentação urbana devido a beleza de suas flores **Figura 2** (ZAMPIERI *et al*., 2013). É um gênero com caracterização química escassa, no entanto, Rezende e colaboradores (2019) analisaram 11 espécies do gênero *Tibouchina* e descreveram a identificação de flavonoides, triterpenos,

esteroides e taninos a partir das folhas dessas espécies. Além desses compostos, antocianinas e proantocianidinas foram identificadas a partir de suas flores.

Figura 2: *Tibouchina sellowiana*, nome popular: quaresmeira, manacá, manacá-da-serra. Fonte: Flora digital da Universidade Federal do Rio Grande do Sul – UFRGS, fotógrafo: Marcio Verdi.

2.3. Gênero *Miconia*

Miconia é o maior gênero da família Melastomataceae, possuindo 1.191 espécies, as quais encontram-se distribuídas desde o sul do México até a Argentina e ocorrendo em toda América Central e América do Sul. O Brasil abrange 276 espécies de *Miconia*, sendo que 121 espécies desse gênero são endêmicas e 250 espécies são consideradas ornamentais e por esse motivo geram grande interesse econômico na sua comercialização (RODRIGUES *et al.*, 2011; FERREIRA *et al.*, 2013).

As principais características morfológicas que fazem uma espécie ser inclusa no gênero *Miconia* são: panículas terminais, ramos adicionais axilares, inflorescências piramidais, glomeruladas ou escorpioides, lacínias do cálice triangulares, anteras curtas e lineares, como ilustrado na **Figura 3** (REZENDE *et al.*, 2013; GOLDENBERG *et al.*, 2020).

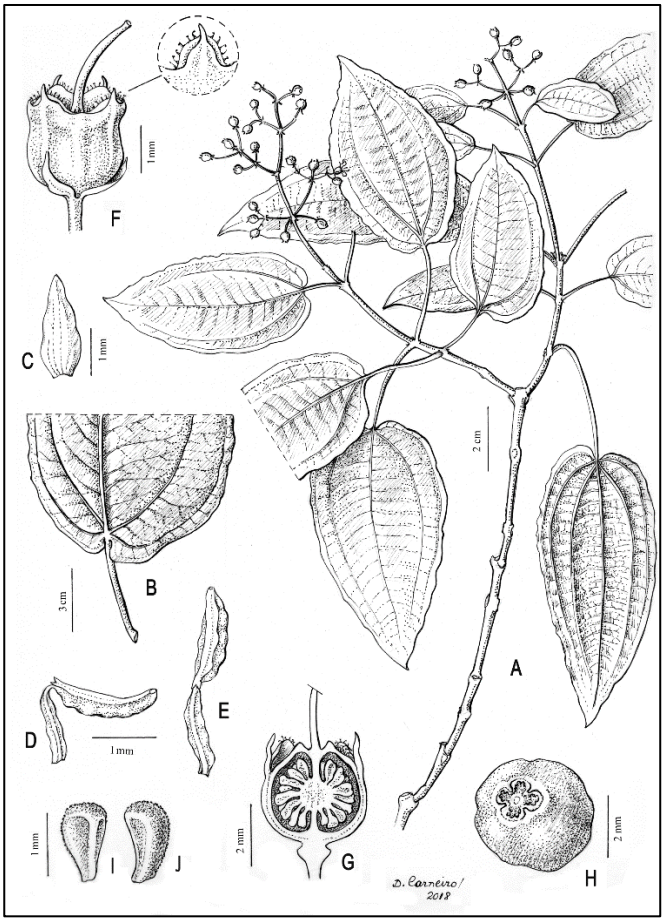


Figura 3: Ilustração com as principais características morfológicas do gênero *Miconia*. (A) ramo fértil, (B) base foliar, (C) pétala, (D) estame vista lateral, (E) estame vista dorsal, (F) flor vista abaxial, (G) flor vista longitudinal, (H) fruta, (I) e (J) sementes. Fonte: GOLDENBERG *et al*, 2020, autor: Diana Carneiro.

O uso tradicional de diversas espécies do gênero *Miconia* é descrito na literatura brasileira (SILVA *et al*., 2022), o que destaca a elevada importância dessas espécies para estudos etnofarmacológicos e também como fontes de compostos bioativos a partir de nossa vasta biodiversidade nacional. Apesar do gênero *Miconia* ser composto por um elevado número de espécies, apenas uma pequena parcela dessas foram estudadas em relação às suas propriedades químicas ou biológicas, sendo que a maior parte dos trabalhos publicados focam em morfologia e taxonomia. Entretanto, é crescente o interesse etnofarmacológico por espécies deste gênero, pois são amplamente utilizadas na medicina tradicional (MILANEZ *et al.*, 2017; GATIS-CARRAZZONI *et al*., 2019).

Diversas espécies de *Miconia* são encontradas em todo território brasileiro, como pode ser observado nos registros de coletas levantados no banco de dados SpeciesLink (**Figura 4**).

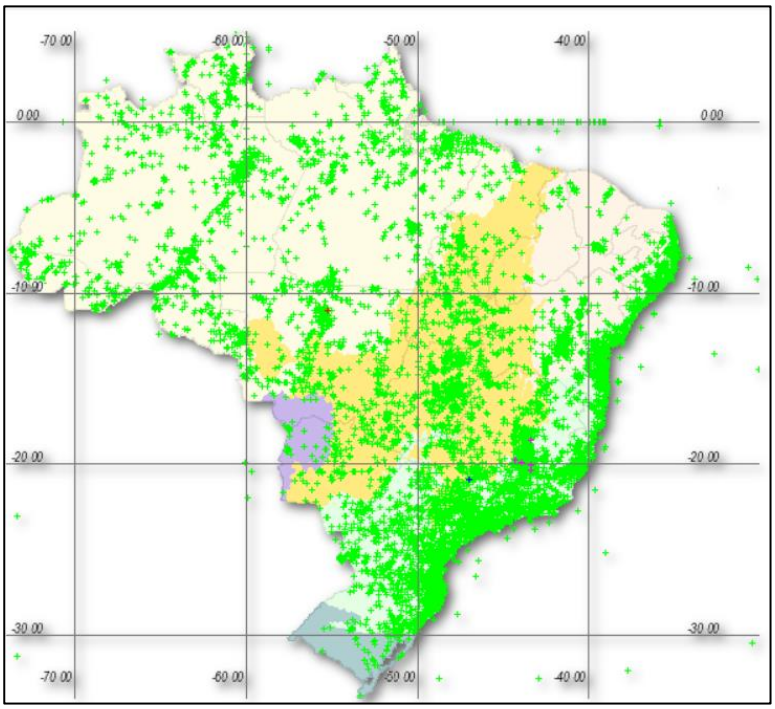


Figura 4: Mapa de registros de coletas de espécies do gênero *Miconia* no Brasil. Os pontos verdes indicam o registro de coleta e depósito de exemplares com as coordenadas fornecidas. Fonte: SPECIESLINK, 2020.

Uma pesquisa etnobotânica na região do Alto Rio Grande, MG, levantou as plantas medicinais de grande interesse na região a partir de conhecimento de raizeiros, bem como a ocorrência dessas. Entre as 167 espécies investigadas, *Miconia rubiginosa* foi citada como uma importante espécie utilizada no tratamento de infecções de garganta (RODRIGUES, CARVALHO, 2001). Em 2018, Boscolo e Valle fizeram um levantamento das plantas medicinais mais utilizadas pela população de Quissamã, RJ, utilizando informações de moradores locais. Neste trabalho, foi relatado o uso das folhas de *Miconia cinnamomifolia*, em forma de chá para o tratamento de resfriados e febre.

Os compostos químicos de maior ocorrência no gênero *Miconia* encontram-se sumariados na **Tabela 1.** Dentre estes componentes de maior ocorrência encontram-se flavonoides, triterpenos, taninos hidrolisáveis e esteroides.

Tabela 1: Principais substâncias encontradas em espécies do gênero *Miconia*.

Espécie vegetal	**Composto químico (classe)**	**Referência**
Miconia affinis	Ácido arjunólico (triterpeno)	GULDBRANDSEN *et al*., 2015
Miconia albicans	Ácido ursólico (triterpeno) Ácido oleanólico (triterpeno) β-amirina (triterpeno) α-amirina (triterpeno) Lupeol (triterpeno) β-sitosterol (esteroide) Campesterol (esteroide) Estigmasterol (esteroide) Rutina (flavonoide) Quercetina 3-*O*-β-glicosídeo (flavonoide) Castalagina (tanino hidrolisável) Vescalagina (tanino hidrolisável) Ácido gálico (ácido fenólico) Ácido elágico (ácido fenólico) Miricetina *O*-hexosídeo (flavonoide)	SERNA *et al*., 2015 PIERONI *et al.*, 2011 VIEGAS *et al*., 2017; 2019 QUINTANS-JUNIOR *et al*., 2020 CREVELIN *et al*., 2006
Miconia cabucu	Quercetina-3-*O*-α-L-ramnopiranosil-(2→1)-*O*-β-*D*-xilopiranosideo (flavonoide) Quercetina-3-*O*-*α*-*L*-ramnopiranosideo (flavonoide) Miricetina-3-*O*-*α*-*L*-ramnopiranosideo (flavonoide) Quercetina-3-*O*-*β*-*D*-glucopiranosideo (flavonoide) Kaempferol-3-*O*-β-*D*-(6-coumaroil)-glucopiranosideo (flavonoide)	RODRIGUES *et al*., 2007
Miconia chamissois	Mateucinol (flavonoide)	SILVA *et al*., 2020 GIMENEZ *et al*., 2020
Miconia fallax	Ácido ursólico (triterpeno) Ácido oleanólico (triterpeno) Acetato de α-amirina (triterpeno) Acetato de β-amirina (triterpeno) Lupeol (triterpeno) β-aitosterol (esteroide) Estigmasterol (esteroide)	CUNHA *et al.*, 2008; 2010 CREVELIN *et al*., 2006
Miconia ferruginata	5,6,7-trihidroxi-4-metoxiflavona (flavonoide) 5,7,4-trihidroxi-6,8-dimetilflavona (flavonoide) 5-hidroxi-7,4-dimetoxi-8-metilflavona (flavonoide) Ácido ursólico (triterpeno) Ácido oleanólico (triterpeno) β-sitosterol (esteroide) Estigmasterol (esteroide)	CUNHA *et al*., 2006
Miconia ligustroides	Ácido ursólico (triterpeno) Ácido oleanólico (triterpeno) β-amirina (triterpeno) α-amirina (triterpeno) Estigmasterol (esteroide)	CUNHA *et al.*, 2008; 2010 CREVELIN *et al*., 2006

	β-sitosterol (esteroide)	
Miconia myriantha	Mattucinol-7-*O*-β-*D*-glucopyranoside (flavonoide) Matucinol-7-*O*-[4,6-di-*O*-galoil]-β-*D*-glucopiranosideo (flavonoide)	LI *et al*., 2002
Miconia pepericarpa	Lupeol (triterpeno) β-amirina (triterpeno) α-amirina (triterpeno) Friedelina (triterpeno) β-amirina (triterpeno) α-amirina (triterpeno) Campesterol (esteroide) Estigmasterol (esteroide) β-Sitosterol (esteroide)	CREVELIN *et al*., 2006
Miconia prasina	Miconioside C (flavonoide) Matteucinol (flavonoide)	TARAWNEH *et al*., 2014
Miconia rubiginosa	Lupeol (triterpeno) β-Sitosterol (esteroide) Quercetina (flavonoide) Kaempferol (flavonoide) Ácido gálico (ácido fenólico) Epicatequina (flavonoide) Casuarina (tanino hidrolisável) Friedelina (triterpeno) Acetato de α-amirina (triterpeno) Acetato de β-amirina (triterpeno)	CUNHA *et al*., 2006 RODRIGUES *et al*., 2011 CREVELIN *et al*., 2006
Miconia sellowiana	Lupeol (triterpeno) β-Amirina (triterpeno) α-Amirina (triterpeno) Campesterol (esteroide) Estigmasterol (esteroide) β-Sitosterol (esteroide)	CREVELIN *et al*., 2006
Miconia trailii	Matteucinol (flavonoide) Ácido bartogênico (triterpeno) Ácido arjunólico (triterpeno) Ácido miriantico (triterpeno) Stigmast-4-ene-3,6-dione (esteroide)	ZHANG *et al*., 2003

2.3.1. Atividade anti-inflamatória e nociceptiva de espécies de *Miconia*

Miconia minutiflora é popularmente utilizada para tratar inflamações e dores reumáticas. Das folhas dessa espécie foi obtido um extrato metanólico e avaliado quanto as propriedades anti-inflamatória, antinociceptiva e pesquisa de toxicidade aguda. O extrato mostrou inibir 70% da migração leucocitária na dose de 100 mg/kg, em modelo de bolsa de ar subcutânea produzida por ar estéril com indução de inflamação por carragenina, em ratos Wistar. No ensaio de pleurisia induzido por carragenina, este extrato também revelou uma redução de 62% na migração de células polimorfonucleares da cavidade pleural na dose de 100 mg/kg. Este extrato também provocou uma

redução na produção dos níveis de citocinas TNF e IL-1β, além disso foi observada nocicepção para o extrato metanólico com uma redução de 58,9% das contorções abdominais induzidas por ácido acético. Desta maneira, este estudo confirmou as atividades anti-inflamatória e antinociceptiva do extrato metanólico de *M. minutiflora*, correlacionado com a presença de taninos hidrolisáveis e compostos fenólicos do perfil químico (GATIS-CARRAZZONI *et al*., 2019).

O efeito analgésico do extrato de *Miconia rubiginosa* foi avaliado pelos ensaios de contorção abdominal induzida por ácido acético e placa quente, utilizando camundongos Swiss. Foram produzidos diferentes extratos, obtidos a partir da extração com os solventes hexano, diclorometano e etanol. O extrato hexânico inibiu mais de 90% das contorções abdominais na dose de 200 mg/kg. No ensaio de placa quente, os extratos hexânico e etanólico, exibiram efeito analgésico significativo, com inibição de 65% da reação motora sobre a placa aquecida. Empregando métodos de RMN e cromatografia a gás, puderam ser identificados os seguintes compostos, α-amirina, β-amirina, β-sitosterol e lupeol. Tais metabólitos secundários foram estabelecidos como responsáveis pelas propriedades analgésicas de *M. rubiginosa* (SPESSOTO *et al.*, 2003).

Desta maneira, os estudos encontrados na literatura demonstram que espécies do gênero *Miconia* são promissoras para a determinação e busca de compostos anti-inflamatórios e analgésicos, assim como ainda são pouco exploradas frente a diversidade botânica presente.

2.3.2. Outras atividades biológicas de espécies de *Miconia*

2.3.2.1. Atividade antiparasitária

O extrato etanólico de *Miconia langsdorffii* revelou atividade contra *Schistosoma mansoni*, exibindo 100% de mortalidade em 120 h na concentração de 100 µg/mL. Esse extrato foi submetido a fracionamento cromatográfico usando Celite e Norit (3:1; v/v; 60 g). A fração obtida com hexano e acetato de etila 50:50 (v/v) apresentou atividade esquistossomicida a 100 µg/mL com 100% de mortalidade em 24 h. Subsequentemente, foram isolados da fração bioativa, os triterpenos ácido ursólico (AU) e ácido oleanólico (AO), porém as substâncias isoladas não apresentaram atividade contra *S. mansoni* (CUNHA *et al*., 2012).

O extrato etanólico das folhas de *Miconia willdenowii* também exibiu atividade contra *S. mansoni*, eliminando 65% dos platelmintos a uma concentração de 200 µg/mL, o que representou um efeito similar ao observado para o medicamento praziquantel a 2 µg/mL. Essa propriedade antiparasitária foi correlacionada com o metabólito primina, que após isolado apresentou CI_{50} de 7,08 µg/mL (VIEGAS *et al*., 2017). O extrato de *M. willdenowii foi pos*teriormente avaliado contra diferentes agentes, revelando também atividade contra promastigota de *Leishmania amazonensis* (inibição de 99% a uma concentração de 80 µg/mL), bactéria *Staphylococcus aureus* (CI_{90} = 250 µg/mL) e fungos *Candida glabrata* (CI_{50} = 125 µg/mL) e *Candida parapsilosis* (CI_{50} = 125 µg/mL) (VIEGAS *et al*., 2019).

2.3.2.2. Atividade antitumoral

O extrato etanólico das partes aéreas de *Miconia fallax* foi avaliado contra células de adenocarcinoma uterino humano – HeLa e subsequentemente uma mistura dos triterpenos ácidos ursólico e oleanólico foi obtida e também avaliada com relação ao seu potencial citotóxico. Essa mistura de triterpenos se mostrou mais efetiva nas avaliações citotóxicas do que os extratos, sendo que uma maior atividade foi observada na concentração de 45 µg/mL (CUNHA *et al*., 2008).

A partir de *Miconia fallax*, foram isolados os triterpenos, AU e A. Esses compostos isolados foram utilizados no tratamento de animais para a determinação de genotoxicidade, da seguinte forma: AU (80 mg/kg), AO (80 mg/kg), mistura de AU + AO (80 mg/kg) e mistura de AU + AO + doxorubicina (DXR 90 mg/kg). Foi empregado o teste de micronúcleo usando sangue periférico e células de medula óssea de camundongos Balb/c. A mistura de AU + AO + DXR apresentou redução de 78,4% na frequência de eritrócitos policromáticos micronucleados no sangue periférico e também de 46,8% na medula óssea na dose de 80 mg/kg. Esse resultado mostra que essa mistura com triterpenos advindos da planta *M. fallax*, potencializa o efeito nos micronúcleos, em comparação com DXR administrado isoladamente. Ainda demonstra um efeito protetor frente a genotoxicidade induzida por DXR, que é um fármaco quimioterápico amplamente utilizado no tratamento antitumoral (RESENDE *et al.*, 2006).

A ação quimiopreventiva dos triterpenos, AU e AO, isolados a partir da espécie *Miconia fallax* também foi avaliada, tanto dos triterpenos isolados quanto em combinação, frente a lesões pré-neoplásicas de cólon, sendo determinada a frequência de foco de criptas aberrantes induzidas por 1,2-dimetilhidrazina em ratos Wistar. Os resultados revelaram que nos animais tratados com a mistura de AU+AO (25 mg/kg) a média do número de criptas aberrantes foi 1,5 ± 0,3, comparado com AO isolado (25 mg/kg) o resultado observado foi 1,4 ± 0,3, e para AU isolado (25 mg/kg) 1,4 ± 0,4. Revelou-se que as amostram reduziram significativamente a frequência do número de criptas aberrantes comparado aos grupos não tratados, nesse sentido não se configura o efeito de sinergismo, pois as substâncias isoladas apresentam valor semelhante ao da mistura (FURTADO *et al.,* 2008).

As folhas de *Miconia chamissois* foram coletadas e utilizadas para produzir um extrato hidroetanólico. A partir do extrato bruto foi feita uma partição líquido-líquido com clorofórmio e depois um fracionamento em coluna com sílica gel (60G; 0,05-0,020 mm; 170-270 mesh). Usando a fração de clorofórmio como material de partida foi possível isolar o composto matteucinol, por coluna cromatográfica de sílica gel flash. O teste inicial foi o índice de seletividade (IS) que deve ter o valor menor que 2, o extrato bruto de *M. chamissois* apresentou IS de 5,77, enquanto a fração clorofórmica apresentou IS de 1,60. Assim os testes seguiram-se com matteucinol isolado, em 26,57 µg/mL apresentou atividade antitumoral para glioblastoma, reduzindo o perímetro do tumor e promovendo morte celular através de apoptose das células tumorais. Além disso, matteucinol (28 µg/mL) também revelou efeito sinérgico com temozolomida (9,71 µg/mL) contra células tumorais de glioblastoma (GAMG e U251MG) com CI_{50} de 0,401 µg/mL (SILVA *et al.,* 2020).

2.3.2.3. Atividade antimicrobiana

O extrato aquoso das folhas de *Miconia latecrenata* foi testado frente a cepas de *Staphylococcus aureus* e *Escherichia coli.* A cepa de *S. aureus* se mostrou mais susceptível ao extrato (42,5 µg/mL) apresentando inibição de 61,7 ± 5,1%. No entanto, para a cepa de *E. coli* a atividade bacteriana ocorreu em concentrações elevadas de extrato (1500 µg/mL) para inibir 38.8 ± 4,6%. Foi feita a desreplicação do extrato por UPLC-DAD-ESI-MS/MS, para identificar os

constituintes químicos responsáveis pela atividade bacteriana, ao qual foi correlacionada com os elevados teores de taninos e compostos fenólicos presente no extrato (GONTIJO *et al.*, 2019).

Na linha de avaliação do potencial antimicrobiano, o extrato etanólico de *Miconia rubiginosa* foi testado contra as cepas de *E. coli, Klebsiella oxytoca* e *Klebsiella pneumonie,* utilizando a técnica de microdiluição em placa de 96 poços. O extrato foi testado em concentrações entre 0,3 mg/mL e 2 mg/mL, onde exibiu CIM de 2 mg/mL para todas as cepas avaliadas. Enquanto, o controle imipenem apresentou CIM de 0,001 mg/mL. Pode-se concluir que mesmo a combinação de substâncias do extrato bruto rica em triterpenos (AU e AO), este agiu de forma diminuta se comparado ao controle, frente a essas cepas de bactérias resistentes. Embora seja válida a investigação da espécie vegetal relacionada a diferentes bactérias patogênicas (QUEIROZ *et al*., 2011).

O extrato etanólico das raízes de *Miconia pilgeriana* apresentou atividade contra o fungo *Cryptococcus neoformans.* A partir deste extrato foi isolado o triterpeno ácido arjunólico que mostrou atividade contra *C. neoformans* com CI_{50} de 20 µg/mL, e também apresentou uma moderada atividade frente a enzima ácido graxo sintase (CI_{50} 27,5 µg/mL) (LI *et al*., 2002).

As partes aéreas da espécie *Miconia ligustroides* foram extraídas com diclorometano, e a partir desse extrato foram isolados AU e AO. Esse extrato diclorometanico de *M. ligustroides* exibiu atividade contra *Bacillus cereus* com CIM de 625 µg/mL, enquanto o AO e AU apresentaram MIC de 80 e 20 µg/mL, respectivamente. Esses triterpenos também revelaram atividade antibacteriana contra *Streptococcus pneumoniae* com CIM de 80 e 50 µg/mL, respectivamente (CUNHA *et al*., 2010).

2.4. *Miconia albicans* (Sw.) Steud.

A espécie *Miconia albicans* (Sw.) Steud. (**Figura 5)**, conhecida popularmente por canela-de-velho, tem o uso disseminado como planta medicinal e possui diversas indicações, como no tratamento de artrite, artrose, inflamações, dores reumáticas, depurativo, e infecções (TARAWNEH *et al.*, 2014; SERPELONI *et al.*, 2008; RODRIGUES *et al.*, 2011).

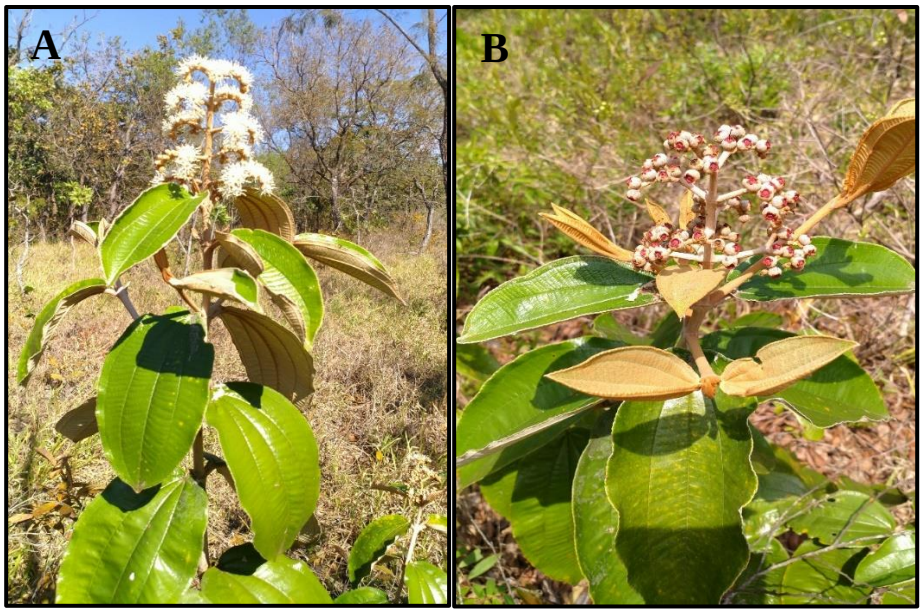


Figura 5: Foto da espécie *Miconia albicans*, com inflorescências (**A**) e frutos imaturos (**B**). Fonte: Própria.

A partir de pesquisas em diferentes bancos de dados realizadas em 2023 e utilizando a palavra-chave *Miconia albicans*, foram obtidos os seguintes resultados em relação ao número de publicações no *Chemical Abstract/SciFinder* (65), *ScienceDirect* (142), *Google Scholar* (3.690), *Scopus* (105) e *PubMed* (166). Na principal base de dados, o *Chemical Abstract*, foram encontradas 65 referências relacionadas a palavra-chave, destacando o ano de 2018 em que houve o maior número de publicações (12) **Figura 6**.

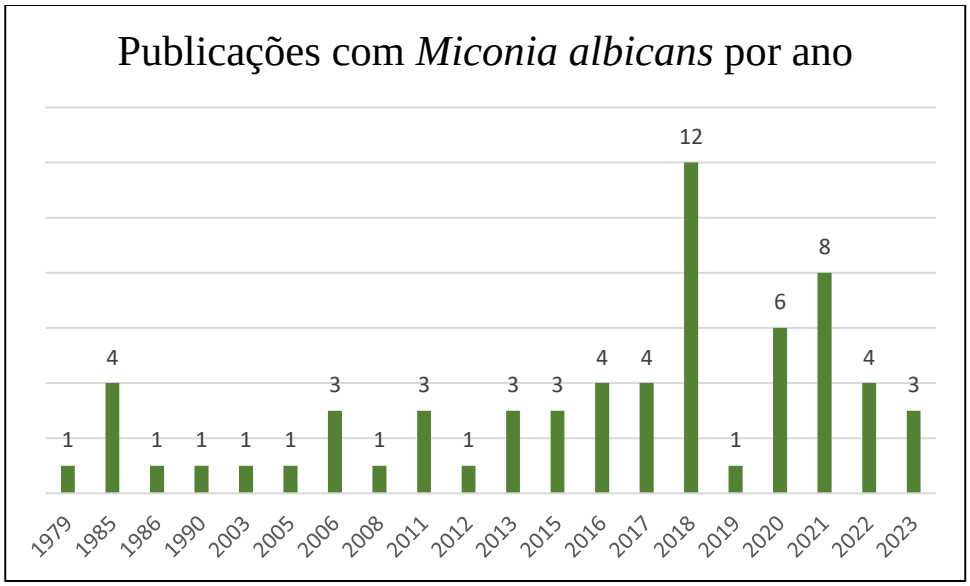


Figura 6: Gráfico anual de publicações de trabalhos referentes a *Miconia albicans* no período de 1979 a 2023. Fonte: CHEMICAL ABSTRACTS, 2023.

Dentre os resultados obtidos, foi observado que 11 trabalhos descrevem o perfil químico da planta, em 16 publicações investiga-se diferentes atividades biológicas, e apenas em 9 trabalhos são correlacionados a atividade biológica com os compostos presentes no perfil químico da amostra. Notando-se que o maior número de trabalhos (29), concentram-se em aspectos ecológicos e de metabolismo vegetal que envolvem essa espécie.

A partir da espécie *M. albicans*, diversos compostos químicos já foram identificados e/ou isolados, sendo que os mais recorrentes são metabólitos como flavonoides, taninos, esteroides e triterpenos, como ilustrado na **Figura 7**. Além disso, alguns estudos tem demonstrado variações químicas em *M. albicans* dependo da localização geográfica onde o indivíduo é coletado e estação do ano, além de variações intra e interpopulacionais (GIMENEZ *et al.*, 2020).

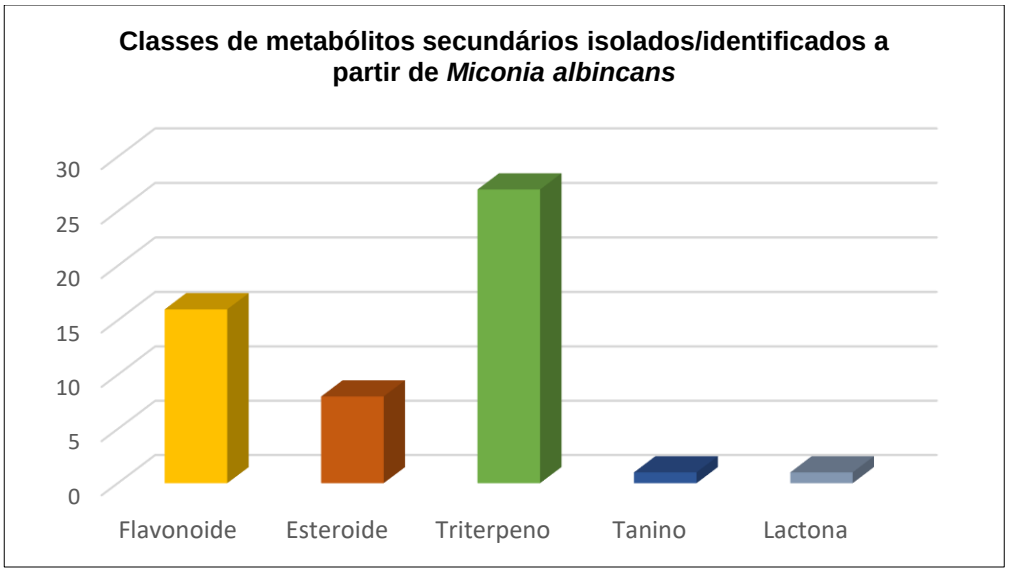


Figura 7: Gráfico de ocorrência das principais classes de metabólitos secundários já descritos em *Miconia albicans*. Fonte: CHEMICAL ABSTRACTS, 2023.

Lima e colaboradores (2018) analisaram a espécie *M. albicans* coletada na área de cerrado do Parque Nacional da Chapada dos Veadeiros, em Goiás. As folhas recolhidas foram submetidas a extração com acetato de etila, e a partir do extrato foram preparadas frações por HPLC em escala preparativa. As frações foram utilizadas como material de partida para o isolamento de cinco flavonoides e oito triterpenos, por HPLC em escala analítica. Tanto o extrato bruto, quanto as substâncias isoladas, foram avaliadas diante as propriedades antidiabéticas frente a inibição da proteína tirosina fosfatase 1B, que atua na desativação do receptor de insulina e consequentemente é um regulador negativo do sinal de transdução de insulina. O extrato apresentou CI_{50} de 4,92 µg/mL, enquanto as substâncias isoladas revelaram valores de CI_{50} entre 0,46 e 3,21 µM. O ácido 3-*O*-*cis*-*p*-cumaroil maslínico foi o constituinte mais potente, exibindo uma ação inibitória da proteína tirosina fostatase 1B com CI_{50} de 0,46 ± 0,07 µM.

Lima e colaboradores (2020) investigaram a espécie *M. albicans* coletada no Parque Nacional da Serra de Itabaiana, que comtempla os biomas de Mata Atlântica e Caatinga, em Sergipe. O extrato hidroetanólico das folhas de *M. albicans* foi avaliado em ensaios de inflamação e dor, utilizando modelos de edema de pata induzido por carragenina. Os resultados mostraram que o tratamento com o extrato hidroalcoólico de *M. albicans*, na dose 25 mg/Kg, reduziu em 29% a formação de edema, enquanto o controle indometacina (10 mg/kg) apresentou redução de 25% do edema. Além disso, o tratamento com esse extrato provocou a diminuição dos níveis de duas

citocinas envolvidas no processo inflamatório, o TNF e a IL1-β. Esse perfil anti-inflamatório do extrato de *M. albicans* tem sido relacionado ao seu elevado potencial antioxidante devido a presença de compostos fenólicos e flavonoides (LIMA *et al.*, 2020).

2.5. Inflamação

O processo inflamatório é uma resposta imune a algum estímulo lesivo, danos celulares e teciduais, que podem ser ocasionados por diversos agentes nocivos como por exemplo: bactérias, anticorpos e lesões. Esse processo tem como objetivo restaurar o tecido lesado e a homeostasia através da ativação de componentes envolvidos na promoção e resolução do processo (GAO *et al*., 2020; NEWMAN, ZLOZA, 2017).

Inicialmente, ocorre a inflamação aguda no local da lesão ou infecção que envolve diversos eventos como, o aumento do fluxo sanguíneo, a permeabilidade celular, a liberação e acúmulo de células sanguíneas (neutrófilos, macrófagos, mastócitos, linfócitos, plaquetas, células dendríticas, células endoteliais e fibroblastos). Os eventos celulares e moleculares apresentam as principais características de resposta fisiológica como dor, calor, edema, rubor e perda de função de tecidos comprometidos (ALESSANDRI *et al.*, 2013, DUFFIN *et al*., 2010).

Em seguida, os macrófagos residentes do tecido e os recrutados, trabalham na reparação tecidual, no qual estão envolvidos mediadores anti-inflamatórios, como as lipoxinas que removem as células mortas e iniciam a restauração tecidual. No entanto, se essas intervenções não forem suficientes para eliminação do patógeno é iniciado um novo recrutamento de neutrófilos, macrófagos e linfócitos T, se ainda assim as medidas não forem capazes de reparar o dano, o processo inflamatório crônico será iniciado (LUAN, HORNG, 2021). Logo a fase crônica, tem longa duração e está correlacionada com infiltrados de células mononucleares, proliferação de vasos sanguíneos, degeneração e fibrose do tecido (WANG, LIN, 2008).

O recrutamento de células inflamatórias para os locais de lesão envolve interações entre vários tipos de mediadores, como algumas citocinas que executam ação essencial no processo inflamatório, mais especificamente o fator de necrose tumoral (TNF), interleucinas (IL), interferons (IFN), fator estimulador de colônias (CSF) e fator de transformação de crescimento (TGF-β). O TNF aumenta a permeabilidade vascular, induz a expressão de moléculas de adesão celular e vascular, como também, influencia na produção de citocinas mediadoras fundamentais

para resposta biológica ao lipopolissacarídeo bacteriano (LPS). Essas citocinas são secretadas por monócitos, macrófagos, adipócitos e fatores de crescimento como as IL-6 e IL-8, que induzem a expressão gênica e síntese protéica em uma gama de células para mediar e promover a inflamação (MURATA, 2018; COUSSENS, WERB, 2002).

2.6. Tratamento medicamentoso da inflamação

Um fármaco com atividade anti-inflamatória tem a finalidade de agir em alguma etapa do processo inflamatório e, com isso, inibir a inflamação. A principal classe de anti-inflamatórios é dos não esteroides, mais conhecidos como AINEs, anti-inflamatórios não esteroidais. No entanto, com os avanços nas pesquisas foram desenvolvidos fármacos com especificidade e complexidade que atuam de modo demarcado na cascata inflamatória (SILVA *et al*., 2022).

O grupo dos AINEs é quimicamente heterogêneo de ácidos orgânicos e compartilham basicamente as mesmas ações terapêuticas e efeitos adversos. Podem ser subdivididos em inibidores não seletivos da ciclo-oxigenase 2 (COX-2) e os inibidores seletivos da COX-2, ambos agindo de forma anti-inflamatória, analgésica e antipirética (VIEIRA *et al*., 2022). Esses agentes atuam desde a inibição da biossíntese de prostaglandinas a redução da produção de radicais superóxidos, induzindo à apoptose, diminuindo o óxido nítrico sintetase e modificando a atividade de linfócitos alterando as funções da membrana celular (PSOMAS, 2020).

Os anti-inflamatórios são a classe de medicamentos mais utilizados no mundo, só no Brasil fatura-se com eles 2,5 bilhões de reais anualmente, sendo a nimesulida o medicamento desse grupo mais prescrito e consumido no país, a venda desses no mercado farmacêutico atual, de janeiro até o mês de maio de 2022, já movimentou mais de R$ 41 milhões (SIDUSFARMA, 2022).

Os AINEs, são os principais medicamentos que provocam toxicidade gastrointestinal em seus usuários, essa classe também pode causar, devido a seu uso prolongado, fatores de risco como, doenças cardiovasculares principalmente em pacientes com mais de 60 anos (MCEVOY, CARR, PIRMOHAMED, 2021). Nos EUA, mais de 16,5 mil pessoas morrem a cada ano em decorrência da ingestão de anti-inflamatórios que causam sangramento gastrointestinal (WOLFE *et al*., 1999).

Desse modo, destaca-se ainda a necessidade de desenvolvimento de novos fármacos para o tratamento de inflamação com reduzidos efeitos adversos. Neste contexto, a vasta diversidade química de produtos naturais a partir da flora brasileira é um ponto promissor para essa busca e

desenvolvimento de potenciais candidatos a fármacos a serem utilizados para o tratamento de infecções, inflamações e outras doenças.

O medicamento fitoterápico Acheflan®, por exemplo, é um anti-inflamatório para via tópica que foi desenvolvido e produzido totalmente no Brasil. Este desenvolvimento foi realizado a partir de um projeto conduzido em parceria entre cinco universidades brasileiras e, portanto, é o primeiro medicamento 100% nacional. O Acheflan® é produzido a partir do óleo essencial de *Cordia verbanaceae* (erva baleeira) e seus principais metabólitos ativos são α-humuleno, alloaromadendreno e *trans*-cariofileno (MORAIS *et al*., 2022).

2.7. Biofilmes bacterianos

Os biofilmes bacterianos são considerados um grande problema de saúde pública e ocorrem com bactérias que são capazes de se aderir a uma superfície e formarem uma rede polissacarídica que protege e imobiliza a colônia, como um sistema de sobrevivência, e essa comunidade microbiana pode conter uma ou inúmeras espécies de bactérias diferentes. Os biofilmes podem estar presentes em diversas superfícies, tanto bióticas (células e tecidos animais e vegetais) quanto abióticas (plásticos e metais) (TRENTIN *et al.,* 2013; BARROS *et al*., 2023).

A formação do biofilme envolve várias etapas, se iniciando pela adesão das bactérias colonizadoras a uma superfície e que ocorre em duas fases (adesão reversível e irreversível). Posteriormente a aderência, inicia-se o processo de *quorum sensing* (*QS*), onde ocorre uma ampliação da densidade populacional devido a células sinalizadoras especializadas que são liberadas no meio extracelular, agregando novas células e contribuindo para a formação da colônia, assim são reunidos nutrientes para o ambiente, suporte para crescimento e maturação celular, e manutenção do biofilme (**Figura 8**) (SCHNEIDER *et al*., 2020, BOROWSKI *et al*., 2020).

O biofilme maduro se transforma em um sistema com uma área densa, canais onde circularam nutrientes, gases, ácidos e resíduos metabólicos. Essa matriz protege as bactérias contra a ação de antibióticos, desinfetantes, raios ultravioleta e mudanças ambientais. No entanto, com a superpopulação o meio se torna desfavorável, ocorrendo então o desprendimento celular, liberando então esses microrganismos virulentos no meio externo que poderão colonizar outras superfícies caracterizando um novo ciclo de contaminação (**Figura 8**) (BOROWSKI *et al*., 2018, REIS *et al*., 2020).

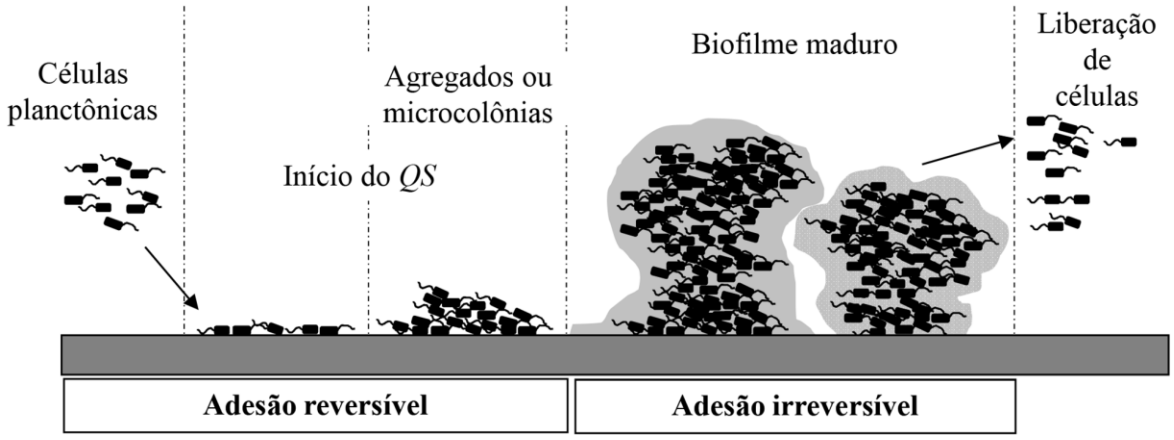


Figura 8: Mecanismo de formação dos biofilmes. Fonte: TRENTIN *et al*., 2013.

Presume-se que mais de 80% das infecções microbianas que ocorrem na clínica médica estão relacionadas aos biofilmes, o que representa uma grave ameaça à medicina moderna, representando o aumento na incidência de mortes e o alto custo relacionado a tratamentos consecutivos, uma vez que ainda não temos eficientes medicamentos para o combate de bactérias em biofilmes (CAMPOS-SILVA *et al*., 2019). Diante disso, a indústria farmacêutica procura desenvolver novos medicamentos que sejam capazes de modular a formação do biofilme e/ou erradicar o biofilme já formado.

3. OBJETIVOS

O presente trabalho tem como objetivo explorar os aspectos químicos e biológicos das folhas de *Miconia albicans*, propondo:

- Determinar o perfil químico da espécie utilizando a técnica de CLAE-DAD-EM;
- Avaliar as propriedades anti-inflamatória, anti-hiperalgésica, toxicidade aguda e de doses repetidas do extrato aquoso de *M. albicans*;
- Avaliar a atividade antibacteriana e antibiofilme *in vitro* frente às bactérias *Staphylococcus aureus* e *Pseudomonas aeruginosa*;
- Avaliar a capacidade antioxidante e os teores de taninos e fenóis totais;
- Correlacionar os compostos ativos com as propriedades biológicas;
- Realizar monitoramento mensal da espécie, afim de verificar interferência da variação sazonal nos metabólitos secundários.

4. CAPÍTULO 1 – Infusion from *Miconia albicans* (Melastomataceae) leaves exhibits anti-inflammatory and anti-hyperalgesic activities without toxicity

Manuscrito publicado no periódico **Journal of Ethnopharmacology.**

Journal of Ethnopharmacology 308 (2023) 116251

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Infusion from *Miconia albicans* (Melastomataceae) leaves exhibits anti-inflammatory and anti-hyperalgesic activities without toxicity

Djaceli Sampaio de Oliveira Dembogurski [a], Iluska Senna Bonfá [b], Luciane Candeloro [c], Eduardo Benedetti Parisotto [d], Mônica Cristina Toffoli Kadri [b], Denise Brentan Silva [a,*]

[a] *Laboratory of Natural Products and Mass Spectrometry (LaPNEM), Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil*
[b] *Laboratory of Pharmacology and Inflammation, FACFAN/ Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil*
[c] *Laboratory of Histology, Biosciences Institute (INBIO), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil*
[d] *Laboratory of Hematology, Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil*

ARTICLE INFO

Handling Editor: dr. P Fernandes

Keywords:
Megastigmane
Melastomataceae
Toxicity
Ellagic acid
Ellagitannins
Inflammation

ABSTRACT

Ethnopharmacological relevance: The leaves of *Miconia albicans* have been extensively used as a traditional medicine to treat inflammation, infection, arthritis, joint pain, and analgesia, which can be purchased easily. Nevertheless, the scientific evidence of chemical profile identification and toxicity investigation is meager.
Aim of the study: This study aimed to determine the chemical profile of *Miconia albicans* aqueous extract (MAAE), to investigate its anti-inflammatory and hyperalgesic effects, and toxicity (acute and repeated-dose oral) *in vivo* studies.
Materials and methods: MAAE was obtained by infusion method and its chemical constituents were analyzed and annotated by LC-DAD-MS. The *in vivo* tests were performed with male and female Swiss mice. Toxicity studies were examined by acute (2000 mg/kg) and repeated-dose oral assays (51.2; 256; 1280 mg/kg); anti-inflammatory evaluation was performed by paw edema and leukocyte migration, and anti-hyperalgesic properties were analyzed by abdominal writhing induced by acetic acid and formalin. The animals were treated by oral means with 51.2, 256, and 1280 mg/kg of MAAE.
Results: Twenty-four compounds were annotated from MAAE by LC-DAD-MS, such as ellagitannins, ellagic acid derivatives, flavan-3-ol, and *O*-glycosylated compounds, including flavonols, triterpenes, and megastigmanes. MAAE induced no significant toxicological effects in the acute and repeated-dose oral assays at lower doses and no histological changes were observed. Hematological and biochemical showed no significant alterations. The oral administration of MAAE 256 mg/kg inhibited the edematogenic effect and reduced the leukocyte migration. In addition, MAAE decreased the abdominal writhings induced by acetic acid and the paw-licking time by formalin assay.
Conclusion: MAAE showed a significant reduction in inflammatory levels and leukocyte migration, revealing anti-hyperalgesic properties. Additionally, MAAE revealed no acute and repeated-doses toxicities.

1. Introduction

Inflammation, a physiological response of organisms to microbial infections and tissue injury, presents signals as pain, swelling, heat, redness, and loss of physiological function when an intense reaction happens. The immune cells (macrophages, monocytes, and neutrophils) are activated in the inflammation, and mediators are synthesized and released, such as chemokines, cytokines, histamine, nitric oxide, prostaglandins, and bradykinin. Inflammation is also related to many several human diseases, such as type 2 diabetes, obesity, cancer, cardiovascular disease, arthritis, celiac disease, inflammatory bowel, and autoimmune diseases (El-Gabalawy et al., 2010; Hotamisligil, 2006).

The drugs normally prescribed to treat pain and inflammation conditions are nonsteroidal anti-inflammatory drugs (NSAIDs), which can act on the cyclooxygenase 1 and 2 enzymes (COX 1 and COX 2), inhibiting the synthesis of thromboxanes and prostaglandins. These non-selective drugs generate gastrointestinal and cardiovascular disorders,

* Corresponding author. Laboratory of Natural Products and Mass Spectrometry, Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Av. Costa e Silva, s/nº, 79070-900, Campo Grande, Mato Grosso do Sul, Brazil.
E-mail address: denise.brentan@ufms.br (D.B. Silva).

https://doi.org/10.1016/j.jep.2023.116251
Received 1 November 2022; Received in revised form 2 February 2023; Accepted 6 February 2023
Available online 13 February 2023

Abbreviations

ALB	albumin
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CHOL	total cholesterol
COX	cyclooxygenase
DPPH	2,2-diphenyl-1-picrydrazyl
GLU	glucose
HCT	hematocrit
HGB	hemoglobin
LOX	lipoxygenase
MAAE	*M. albicans* aqueous extract
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
PLT	platelet count
RBC	erythrocytes
UFMS	Federal University of Mato Grosso do Sul
WBC	leukocytes

even the COX 2 selective drugs have shown side effects, that stimulate the development of new drugs (Bindu et al., 2020; Nunes et al., 2020; Strand, 2007). In this context, plants have been an important source for the search and development of new anti-inflammatory drugs based on phytopharmaceutical compounds or herbal medicines (Castaneda et al., 2019). For example, the herbal medicine Acheflan® produced from *Cordia verbenacea* DC (Boraginaceae) and used to treat inflammation via topic (Ribeiro et al., 2018). The phytoconstituents of medicinal plants have revealed diverse pathways to interfere in the inflammatory process, such as the inhibition of the production of cytokines and lipid mediators, nitric oxide synthesis, the production and secretion of various mediators such as chemokines, arachidonic acid, and reactive oxygen species (Nunes et al., 2020).

The pain is a response to mechanical, chemical, and thermal stimuli, which is also a signal of the inflammation process. For acute inflammation pain, the sensibilization of primary nociceptors happens, and it affects significantly the quality of life, increasing health costs. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been frequently applied in the treatment of inflammation pain, but these treatments can be inefficient in many cases (Singh et al., 2018; Pinho-Ribeiro et al., 2016; Chen et al., 2013). Then, medicinal plants are an interesting alternative to treat pain, since their constituents have been frequently reported as anti-hyperalgesic, such as flavonoids, terpenoids, alkaloids, and phenolic derivatives, besides they can act synergistically (Silva-Correa et al., 2021).

Miconia is the largest genus of the Melastomataceae family and it is founded in South and Central America (Ferreira et al., 2013). *Miconia albicans* (Sw.) Steud., the most popular species from this genus, is popularly known as "canela-de-velho" which means old man's shin (Almeida et al., 2014). Its aqueous extract is widely used in Brazilian folk medicine to treat inflammation, infection, arthritis, and joint pain, while it is used to treat diabetes in Mexico (Lima et al., 2018; Almeida et al., 2014; Tarawneh et al., 2014; Rodrigues et al., 2011; Serpeloni et al., 2008). The leaves of *M. albicans* are easily found to buy in the Brazilian market for medicinal purposes. Their extracts showed several phenolic constituents, including hydrolysable tannins, triterpenes, flavonoids, and ellagic acid and its derivatives (Quintans-Junior et al., 2020; Lima et al., 2018; Crevelin et al., 2006; Vasconcelos et al., 2006). The composition of *M. albicans* is affected by seasonality, in which the levels of flavonols (e.g. rutin and quercetin) are higher in the rainy season, while the triterpenes (e.g. ursolic and oleanolic acids) are lower (Gimenez et al., 2020).

Cytotoxic, antimicrobial, antidiabetic, anti-inflammatory, and antinociceptive properties are reported from extracts of *M. albicans* (Quintans-Junior et al., 2020; Serpeloni et al., 2011; Celotto et al., 2003). The ethanolic extracts from leaves also reduced the number of leukocytes in the pleurisy, the TNF-α and IL-1β levels in the pleural lavage, the IL-6 in the joint knee, as well as reduced the nociceptive and hyperalgesic events. In addition, these extracts only exhibited in their compositions flavonols, some ellagic acid derivatives, and few hydrolysable tannins (Quintans-Junior et al., 2020); these last two were not the same observed here in the infusion from *M. albicans* leaves.

Therefore, here we aimed to evaluate the anti-inflammatory and anti-hyperalgesic effects of the aqueous extract of *M. albicans* (MAAE), to determine its acute and repeated-dose oral toxicities and to characterize its chemical composition.

2. Materials and methods

2.1. Plant material and extraction

Miconia albicans was collected in Campo Grande, Mato Grosso do Sul, Brazil, on May/2018 at the Federal University of Mato Grosso do Sul (UFMS) campus. It was identified by prof. Dr. Flávio Macedo Alves. A voucher was deposited at CGMS herbarium (CGMS 79407) of UFMS. The protocol number of registration in SISGEN is ACD1712.

The leaves of *M. albicans* were dried in a circulation air oven and powdered by a knife mill. Subsequently, the powdered material was extracted by infusion according to RDC 10/2010 (Anvisa, 2010). Thus, 500 mL of boiled ultrapure water was added to 18 g of plant material for 15 min. After this time, it was filtered and the extract was dried by lyophilization to obtain the aqueous extract of *M. albicans* (MAAE) with yield of 18%.

2.2. Liquid chromatography coupled to diode array detector and mass spectrometry (LC-DAD-MS) analyses of MAAE

An UFLC LC-20AD Shimadzu Prominence coupled to a diode array detector and a high-resolution mass spectrometer with electrospray ionization source (MicrOTOF-Q III – Bruker Daltonics, Billerica, MA, USA) was used in the analyses. The chromatography column was a Kinetex C18 column (2.6 μm, 100A, 150 × 2.1 mm, Phenomenex), which was applied a flow rate of 0.3 mL/min, and oven temperature was maintained at 50 °C during the analyses. The mobile phase was composed by acetonitrile (B) and ultrapure water (A) with formic acid 0.1% (v/v) and the following elution gradient profile was applied: 0–2 min - 3% of B, 2–25 min - 3 to 25% of B, 25–40 min - 25 to 80% of B, and 40–43 min 80% of B. MS and MS/MS analyses were acquired in negative and positive ion modes, and N_2 was used as nebulizer gas (4 Bar), collision gas, and dry gas (9 L/min). The capillary voltage was 2.500 and 4.500 kV for negative and positive ion modes, respectively. MAAE was prepared at concentration 6 mg/mL, filtered on PTFE syringe filters (Millex 0.22 μm, Millipore®), and 1 μL was injected into the chromatographic system.

2.3. Total phenolic (TPC) and tannins contents (TTC)

The total phenolic (TPC) and tannin contents (TTC) were determined according to Herald et al. (2012). MAAE solution was prepared at 1 mg/mL in methanol and water (1:1 v/v). MAAE solution was applied to determine TPC and an aliquot was used for TTC that was previously submitted to precipitation of tannins by hide powder (protease substrate, Sigma-Aldrich). For this, the solution with hide powder was stirred for 60 min and TPC from the supernatant was quantified. TTC was determined by the difference of TPC values measured from the extract and after the precipitation of its tannins with hide powder, as described by Santos et al. (2017).

To determine the TPC, 50 μL of MAAE (1 mg/mL) or the standard

(gallic acid, 1 mg/mL) were mixed to 50 μL of ultrapure water in a 96-well plate. Subsequently, they were serially diluted to obtain the solutions of MAAE at 31.25–500 mg/mL and gallic acid at 0.002–1 mg/mL. All the analyses were performed in triplicate. Subsequently, Folin-Ciocalteu reagent (Sigma-Aldrich) (1:1, v/v) was added in the samples, except in controls, mixed softly, and after 6 min 100 μL of Na_2CO_3 75 g/L (Vetec) solution were added in the samples. The plate was shaken and maintained in the dark for 90 min. After this, the optical density was measured at 765 nm on a spectrophotometer (SpectraMax Plus 384). Total phenolic (TPC) and tannin contents (TTC) were calculated in mg of gallic acid/g of dried extract (gallic acid equivalent) by the calibration curve of gallic acid standard. The calibration curve was obtained by regression linear and it was the following: $Y = 0.008X+0.0028$ ($r^2 = 0.9971$).

2.4. DPPH radical scavenging assay

The radical scavenging capacity was performed by DPPH (2,2-diphenyl-1-picrydrazyl, Sigma-Aldrich) method, as described by Herald et al. (2012). In a 96-well plate, 25 μL of MAAE at 1 mg/mL or quercetin standard (400 μg/mL – positive control) were added and they were serially diluted resulting in ten concentrations: MAAE at 0.002–1 mg/mL and quercetin at 0.78–400 μg/mL. The analyses were performed in triplicate for each sample. The stock solution of DPPH was prepared at 150 μM using methanol and water (8:2 v/v), and 200 μL of this solution were added in all samples. After 12 h of incubation at room temperature and protected from light, the optical density was measured at 514 nm by an Elisa reader (SpectraMax Plus 384). The concentration required to reduce 50% of the radical DPPH, expressed as IC_{50}, was calculated by applying the equation from the standard curve and replacing the y value to reduce 50% of the DPPH. The results were calculated by Minitab 16 software.

2.5. Animals

Male and female Swiss mice (18–25 g) were used in the experiments. These animals were obtained from Federal University of Mato Grosso do Sul (UFMS), and they were stayed in collective cages (5 animals/cage) at controlled temperature (22 ± 2 °C), light cycle (12/12 h light/dark), and food and water *ad libidum*. The experiments were performed according to National Institutes of Health Regulations on the Use and Care of Animals for Scientific Purposes, which was approved by the Ethics Committee on Animal Experimentation of the UFMS (protocol 1.033/2019). The animals had free access only to water, 6 h before the experiments. For the anti-inflammatory and anti-hyperalgesic assays, only male Swiss mice were used. All the animals were euthanized by inhalation of carbon dioxide in the chamber.

2.6. In vivo toxicity study

2.6.1. Acute toxicity assay

The study was performed according to the Organization for Economic Co-operation and Development OECD 423 (2002). A single dose of MAAE 2000 mg/kg (p.o.) was administered to eleven Swiss mice (five female and six male animals) and water was applied for negative control (10 mL/kg). After the drug administration, the animals were monitored to detect clinical and toxicity signs in the first 12 h and daily for 14 days. The observations include changes in the locomotion, auditory and corneal reflex, as well as the incidence of salivation, diarrhea, lethargy, tremors, irritability, writhing, or death. Body weight was also monitored.

2.6.2. Repeated-dose oral toxicity study

The four-week dose oral study was examined according to the OECD 407 (2008). MAAE (51.2, 256, and 1280 mg/kg) and water (negative control) were daily administered by via oral in twenty-eight animals (eleven females, seventeen males). Clinical signs of toxicity were scored daily and the body weight was measured weekly.

2.6.3. Biochemical, hematological and histopathological analyses

For both toxicity studies, the animals were anaesthetized with intraperitoneal injection (i.p.) of xylazine (50 mg/mL) and ketamine (2%) at ratio 1:2 (v/v) (50 μL). The blood samples were collected by retro-orbital punction from each animal, centrifuged at 3000 rpm for 10 min, and the serum samples were removed. The biochemical and hematological analyses were performed by standard techniques (Teixeira et al., 2000), and the parameters evaluated were glucose (GLU), albumin (ALB), cholesterol (CHOL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Additionally, the hematological parameters measured were leukocyte (WBC), erythrocyte (RCB), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT). The organs (heart, liver, spleen, pancreas, lung, kidney, stomach, epididymis, uterus, and ovary) were removed, weighted, and stored in formaldehyde (10%) for histopathological analyses (Greaves, 2012; Willard and Tvedten, 2012). The tissue sections (4 mm) were acquired, stained (hematoxylin and eosin), and analyzed by a light microscope.

2.7. In vivo anti-inflammatory evaluation

2.7.1. Carrageenan-induced paw edema

The carrageenan-induced paw edema assay was performed based on the method described by Winter et al. (1962). The animals were randomly distributed into three groups (5 animals per group). Before 1h carrageenan administration, the animals were treated p.o. with water - negative control (vehicle), indomethacin 15 mg/kg - positive control, and MAAE – 256 mg/kg, The animals received an intraplantar injection (i.pl.) in the right hind paw of 40 μL 1% carrageenan (Cg) after the treatments. The same volume of saline was injected to the left hind paw. The edema was evaluated in both paws at 30, 60, 120, and 240 min after the injection of carrageenan by a digital plethysmometer (Insight®). Paw edema was measured by the differences between the volumes of control and stimulated paw. The results were expressed in mL.

2.7.2. Leukocyte influx

Leukocyte influx assay was conducted as described by Souza and Ferreira (1985) with adaptations. The animals were pre-treated p.o. (0.2 mL) according to the following groups: (1) water 10 mL/kg (negative control), (2) indomethacin 15 mg/kg (positive control), (3) MAAE 51.2 mg/kg, (4) MAAE 256 mg/kg, and (5) MAAE 1280 mg/kg. After 1 h of the pre-treatment, 1% carrageenan (0.5 mL) was administered intraperitoneally (i.p.) in the animals. Then, the animals were euthanized after 4 h and the exudates were collected by washing the peritoneal cavity (3 mL, 0.1% heparin in phosphate-buffered saline). For the quantification of leukocyte, the exudate was diluted in Turk's solution (0.2% crystal violet in 30% acetic acid). The exudate was centrifuged (1000 rpm, 5 min) for differential cell counts, and the cells were stained with HEMA3. Cells were classified as mononuclear or polymorphonuclear according to morphological criteria. The results were expressed as the number of cells per mm^3.

2.8. In vivo anti-hyperalgesic potential

2.8.1. Acetic acid-induced abdominal writhings

The anti-hyperalgesic activity was evaluated by the acetic acid injected i.p., and the contraction of abdominal muscles and enlargement of the hind limbs were examined as described previously by Koster (1959). The animals pre-treated by p.o. (0.2 mL) according to the following groups: (1) water 10 mL/kg (negative control), (2) indomethacin 15 mg/kg (positive control), (3)- MAAE 51.2 mg/kg, (4) MAAE 256 mg/kg, and (5) MAAE 1280 mg/kg. After 1 h of the oral

pre-treatment, 0.6% acetic acid was administrated intraperitoneally (i.p.) in the animals (0.1 mL/10g body weight). The number of abdominal writhings was counted between 5 and 30 min after the injection of stimulus. The results were expressed in number of abdominal writhings.

2.8.2. Formalin-induced paw licking response in mice assay

The assays were performed according to Hunskaar and Hole (1987) with modifications. The animals were pre-treated with saline 10 mL/kg (negative control, p.o.), morphine 5 mg/kg (positive control, i.p.), indomethacin 15 mg/kg (p.o.) or MAAE 256 mg/kg (p.o.). After 30 min for morphine and 60 min for another group, the animals received an injection (i.pl.) of 1.2% formalin (40 μL) into the right hind paw. The duration of the licking paw was recorded at first phase (from 0 to 5 min) and the second phase (from 15 to 30 min). The results were expressed in seconds of paw licking.

2.9. Statistical analyses

The results were showed as the mean ± standard error of the mean. Variations between the groups were analyzed by one- or two-way ANOVA followed by the Tukey test, p values less than 0.05 were considered significant. All the analyses were carried out by GraphPad Prism 5 software (GraphPad Software Inc., USA).

3. Results

3.1. Chemical profile of MAAE

The compounds from MAAE were annotated based on their spectral data (UV, MS, and MS/MS) compared to data reported in the literature, besides the injection of authentic standards was performed for some compounds. Thus, twenty-four compounds were annotated from MAAE (Fig. 1 and Table 1).

The peaks **2–4** and **10** revealed deprotonated ions at *m/z* 341.1061, 191.0229, 169.0118, and 289.0709, while **7** showed an intense ion at *m/z* 205.0958 $[M+H]^+$ that were compatible with molecular formula $C_{12}H_{22}O_{11}$, $C_6H_8O_7$, $C_7H_6O_5$, $C_{15}H_{14}O_6$, and $C_{11}H_{12}N_2O_2$, respectively. Compound **1** was putatively annotated as di-*O*-hexoside, additionally the peaks **3–4**, **10,** and **7** were confirmed by injection of authentic standards as citric acid, gallic acid, epicatechin, and the amino acid tryptophan.

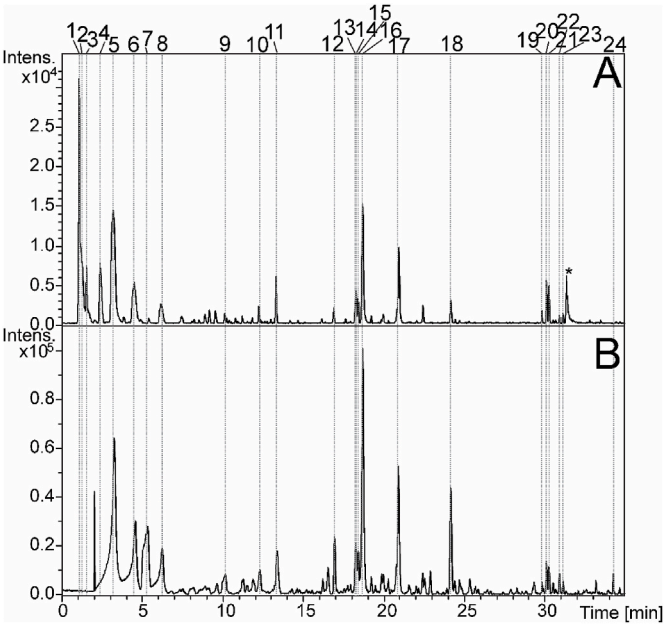


Fig. 1. Base peak chromatogram in (**A**) negative and (**B**) positive ion modes from aqueous extract of *Miconia albicans* leaves (MAAE).

The chromatographic peaks **5/6**, **8,** and **9** showed absorption bands at λ_{max} 280 nm, and deprotonated ions at *m/z* 933.00627, 947.0811, and the double-charged ion *m/z* 602.0252 were observed in the mass spectra. These compounds showed fragmentation patterns of hydrolysable tannins typically of ellagitannins, which were confirmed by the product ion at *m/z* 301 that corresponds to ellagic acid yielded from losses of hexahydroxydiphenyl units (HHDP) with subsequently cyclization reactions on the mass spectrometer (Cerulli et al., 2020). Compound **9** also exhibited the fragment ion at *m/z* 289 relative to procyanidin. Thus, the compounds **5/6**, **8,** and **9** were annotated as NHTP-HHDP-hexose (NHTP: nonahydroxytriphenoyl), *O*-methyl NHTP-HHDP hexoside, and C-procyanidin NHTP-HHDP-hexoside, which revealed spectral data compatible with the data described in the literature (Cerulli et al., 2020; Rasines-Perea et al., 2019).

Peaks **11** and **12** reveal no UV absorption, and their protonated ions at *m/z* 387.1992 and 391.2295 indicated the molecular formula $C_{19}H_{30}O_8$ and $C_{19}H_{34}O_8$. They showed losses of an *O*-hexosyl group yielding the fragment ions *m/z* 207 $[M + H\text{-}162\text{-}H_2O]^+$ and 229 $[M + H\text{-}162]^+$. These fragments are relative to aglycones with thirteen carbons and compatible to megastigmanes (Jo et al., 2020), which were annotated as dihydroxy-megastigmadienone O-hexoside (roseoside) and trihydroxymegastigmanene O-hexoside (Chang et al., 2020; Spínola et al., 2015).

The compounds **13** and **15–17** presented two absorption bands in the UV spectrum at $\lambda_{max} \approx 270$ and 350 nm that are compatible to flavonols. The losses of 162 and 146 *u* indicated the *O*-hexosyl and *O*-deoxyhexosyl substituents, respectively (Silva et al., 2013). In addition, they showed the same fragment ion at *m/z* 303 and other diagnostic ions relative to quercetin as reported by Younis et al. (2020). Thus, they were annotated as *O*-hexosyl quercetin (**13** and **16**), rutin (**15**), and *O*-deoxyhexosyl quercetin (**17**).

The UV spectra of **18** and **22** revealed two absorption bands similar to the ellagic acid chromophore (with $\lambda_{max} \approx$ 250 nm and 370 nm) (Reichert et al., 2018). From their protonated ions *m/z* 345, the fragment ions at m/z 330, 315, and 300 were observed, which are yielded from subsequent losses of radical methyl (15 *u*) (Reichert et al., 2018; Moilanen et al., 2013). Compounds **18** and **22** were annotated as tri--*O*-methyl ellagic acid.

The mass spectra of **19**, **20** and **21** revealed ions of *m/z* 711.3980 $[M\text{-}H + HCOOH]^-$ compatible with the molecular formula of $C_{36}H_{58}O_{11}$. The fragment ions at *m/z* 503 are yielded from losses of 208 *u* (relative to hexosyl + HCOOH), suggesting the aglycone triterpene (Gao et al., 2011). Thus, the compounds were annotated as *O*-glycosylated triterpenes.

3.2. DPPH radical scavenging assay, total phenol (TPC) and tannins contents (TTC)

MAAE was evaluated on DPPH assay and showed IC_{50} of 4.25 μg/mL, while the flavonoid quercetin and rutin (controls) exhibited IC_{50} of 0.17 and 4.37 μg/mL, respectively. The determinations of TPC and TTC from were also performed, and the obtained results from MAAE were 327.40 ± 5.56 and 140.29 ± 8.70 mg of gallic acid/g of dried extract, respectively.

3.3. In vivo toxicity study

3.3.1. Acute toxicity study

All the animals received de single-dose of MAAE 2000 mg/kg and they were monitored daily, twice a day. The mice did not show any behavioral abnormalities or clinical signs of toxicity effects during 14 days of examination. There were no deaths. The body weight of animals after the treatment for 14 days with MAAE 2000 mg/kg and water (control) were evaluated and illustrated in Figs. S1A–B (Supplementary

Table 1
Compounds annotated from *Miconia albicans* aqueous extract (MAAE) by LC-DAD-MS.

Peak	RT (min)	Compound	UV(nm)	Molecular formula	Negative mode (*m/z*)		Positive mode (*m/z*)	
					MS[M-H]$^-$	MS/MS	MS [M+H]$^+$	EM/EM
1	1.1	Unknow	262	–	202.9402	–	–	
2	1.2	di-*O*-hexoside	–	$C_{12}H_{22}O_{11}$	341.1061	–	–	
3	1.5	Citric acid*	–	$C_6H_8O_7$	191.0229	–	–	
4	2.4	Gallic acid*	270	$C_7H_6O_5$	169.0118	–	171.0282	
5	3.2	NHTP-HHDP-hexoside (vescalagin or castalagin isomer)	282	$C_{41}H_{26}O_{26}$	933.0615	467, 421, 301, 275, 257	935.0720	469, 439, 307, 277
6	4.5	NHTP-HHDP-hexoside (vescalagin or castalagin isomer)	280	$C_{41}H_{26}O_{26}$	933.00627	631, 587, 569, 425, 301	935.0731	615, 495, 469, 439, 307, 277
7	5.2	Tryptophan*	272, 280, 288	$C_{11}H_{12}N_2O_2$	203.0839	–	205.0958	188, 170, 159
8	6.2	*O*-methyl NHTP-HHDP-hexoside (*O*-methyl vescalagin or castalagin isomer)	280	$C_{42}H_{28}O_{26}$	947.0811	493, 467, 421, 301, 275, 203	949.0886	–
9	10.0	*C*-procyanidin NHTP-HHDP-hexoside (acutissimin A/B or epiacutissimin A/B)	280	$C_{56}H_{38}O_{31}$	602.0252^{-2}	493, 467, 301, 289, 275, 249	–	–
10	12.3	Epicatechin*	280	$C_{15}H_{14}O_6$	289.0709	163	291.0861	–
11	13.3	Dihydroxy-megastigmadienone O-hexoside (roseoside)	–	$C_{19}H_{30}O_8$	385.1845	205, 153	387.1992	207, 189, 161
12	16.9	Trihydroxymegastigmanene O-hexoside	–	$C_{19}H_{34}O_8$	389.2150	–	391.2295	229, 211, 193, 175
13	18.2	Quercetin *O*-hexoside	270, 356	$C_{21}H_{20}O_{12}$	463.0860	300, 271, 255, 243, 179, 151	465.1003	303
14	18.3	Unknown (galloyl derivative)	280	$C_{26}H_{34}O_{12}$	537.1966	385, 313, 271, 211, 169	539.2107	–
15	18.4	Rutin*	270, 352	$C_{27}H_{30}O_{16}$	609.1455	463, 301, 151	677.1591	465, 303
16	18.7	Quercetin *O*-hexoside	265, 352	$C_{21}H_{20}O_{12}$	463.0868	300, 271, 255, 151	465.1011	303
17	21.0	Quercetin *O*-deoxyhexoside	262, 350	$C_{21}H_{20}O_{11}$	447.0910	300, 271, 255, 163	449.1057	303, 257, 229
18	24.2	Tri-*O*-methyl ellagic acid	244, 368	$C_{17}H_{12}O_8$	343.0442	313, 298	345.0585	330, 315, 300, 285
19	24.2	*O*-hexosyl triterpene	–	$C_{36}H_{58}O_{11}$	711.3971$^{\#}$	503	689.3867^{Na}	487, 469, 451, 187
20	30.2	*O*-hexosyl triterpene	–	$C_{36}H_{58}O_{11}$	711.3984$^{\#}$	503	689.3878^{Na}	505, 487, 469, 451, 439, 405, 261, 215, 187
21	30.3	*O*-hexosyl triterpene	–	$C_{36}H_{58}O_{11}$	711.3980$^{\#}$	503	689.3856^{Na}	505, 487, 469, 451, 439, 405, 261, 215, 187
22	30.9	Tri-*O*-methyl ellagic acid	250, 372	$C_{17}H_{12}O_8$	343.0448	–	345.0593	330, 315, 300, 285
23	31.2	*O*-hexosyl triterpene	–	$C_{36}H_{58}O_{10}$	695.4032	487	–	–
24	34.3	Unknow	–	$C_{21}H_{33}N_3O_3$	–	–	376.2590	302, 292, 275, 218, 209

$^{-2}$: [M-2H]$^{-2}$; $^{\#}$: [M-H + HCOOH]$^-$; RT: retention time; HHDP: hexahydroxydiphenoyl; NHTP, nonahydroxytriphenoyl; *: confirmed by injection of authentic standard.

Data). The changes in body weight were not significant with MAAE pretreatment compared with the control groups.

3.3.2. Repeated-dose oral study

The animals did not show clinical alterations, no changes in general behavior until the twenty-eight days of the experiment. After twenty-one days of assay, some female and male mice shown agitation and vocal fremitus, those animals were given 1280 mg/kg oral administration. In addition, the effects on body weight by MAAE pretreatment (51.2, 256, and 1280 mg/kg) for twenty-eight days were also evaluated and the data are shown in Figs. S1C–D (Supplementary Data). The body weight of pretreated and control groups did not reveal statically differences.

3.3.3. Organ weights and histopathology analyses

The organ weights of all mice pretreated with water and MAAE (2000 mg/kg) were measured and summarized in Table 2. All the organs exhibited normal color and visual aspect. The liver of males showed higher weight (1.546 ± 0.124) in comparison with a control group (1.272 ± 0.050), while the stomach of females decreased weight (0.508 ± 0.034) when compared to the control (0.708 ± 0.140). The other organs did not show statistical changes compared to controls.

The organ weights of mice pre-treated with different oral repeated-doses were also measured and illustrated in Table 3. The stomach

Table 2
Weight of organs from acute toxicity evaluation of MAAE.

Organ	Female		Male	
	Water 10 mL/kg	MAAE 2000 mg/kg	Water 10 mL/kg	MAAE 2000 mg/kg
Kidney	0.175 ± 0.014	0.174 ± 0.009	0.255 ± 0.005	0.234 ± 0.016
Liver	1.260 ± 0.062	1.184 ± 0.038	1.272 ± 0.050	1.546 ± 0.124**
Stomach	0.708 ± 0.140	0.508 ± 0.034**	0.414 ± 0.022	0.485 ± 0.119
Heart	0.140 ± 0.022	0.147 ± 0.013	0.175 ± 0.016	0.154 ± 0.015
Lung	0.216 ± 0.015	0.180 ± 0.004	0.224 ± 0.004	0.210 ± 0.012
Spleen	0.118 ± 0.003	0.113 ± 0.008	0.128 ± 0.008	0.1054 ± 0.006
Pancreas	0.087 ± 0.005	0.097 ± 0.002	0.164 ± 0.027	0.1621 ± 0.059
Reproductive	0.288 ± 0.037	0.227 ± 0.008	0.217 ± 0.017	0.160 ± 0.022

MAAE = *Miconia albicans* aqueous extract. All the animals received water (control group) or MAAE by single-dose oral. The organs weight of females and males were registered in the end of experiment, on the fourteenth day. The results were expressed with mean ± EPM. ANOVA and Bonferroni test (n = 4/group). **p < 0.01, when compared with control.

Table 3
Weight of organs from animals treated with repeated-dose oral of MAAE.

Organ	Female				Male			
	Water 10 mL/kg	MAAE 51.2 mg/kg	MAAE 256 mg/kg	MAAE 1280 mg/kg	Water 10 mL/kg	MAAE 51.2 mg/kg	MAAE 256 mg/kg	MAAE 1280 mg/kg
kidney	0.175 ± 0.009	0.186 ± 0.029	0.168 ± 0.014	0.133 ± 0.012	0.255 ± 0.005	0.258 ± 0.009	0.203 ± 0.036	0.248 ± 0.011$^{\alpha}$
Liver	1.260 ± 0.043	1.224 ± 0.183	1.168 ± 0.079	1.164 ± 0.083	1.272 ± 0.050	1.529 ± 0.079	1.471 ± 0.106	1.680 ± 0.090*$^{\alpha}$
Stomach	0.708 ± 0.099	0.478 ± 0.016	0.569 ± 0.096	1.140 ± 0.055**	0.414 ± 0.022	0.588 ± 0.040	0.649 ± 0.075	1.078 ± 0.133***
Heart	0.134 ± 0.015	0.133 ± 0.013	0.167 ± 0.017	0.126 ± 0.014	0.175 ± 0.016	0.203 ± 0.028	0.158 ± 0.007	0.189 ± 0.024
Lung	0.216 ± 0.011	0.205 ± 0.013	0.214 ± 0.017	0.236 ± 0.013	0.224 ± 0.004	0.224 ± 0.010	0.248 ± 0.018	0.264 ± 0.011
Spleen	0.118 ± 0.002	0.110 ± 0.013	0.117 ± 0.012	0.132 ± 0.009	0.128 ± 0.008	0.113 ± 0.006	0.104 ± 0.009	0.126 ± 0.017
Pancreas	0.087 ± 0.003	0.112 ± 0.028	0.150 ± 0.049	0.115 ± 0.019	0.163 ± 0.027	0.079 ± 0.011*	0.114 ± 0.006	0.130 ± 0.007
Reproductive	0.288 ± 0.026	0.193 ± 0.045	0.163 ± 0.032	0.238 ± 0.064	0.217 ± 0.017	0.163 ± 0.011	0.164 ± 0.020	0.180 ± 0.008

MAAE = *Miconia albicans* aqueous extract. All the animals received water (control group) or MAAE in different repeated-dose oral. The organs weight of females and males were registered at the end of experiment, on the twenty-eighth day. The results were expressed with mean ± EPM. ANOVA and Bonferroni test. *p < 0.05, **p < 0.01, ***p < 0.001, when compared with control. $^{\alpha}$ $p < 0.05$, when compared with MAAE 1280 mg/kg female.

weight of male (1.078 ± 0.133) and female (1.140 ± 0.055) animals after the treatment with MAAE 1280 mg/kg exhibited an increase when compared with the control group treated with water (female 0.708 ± 0.099, male 0.414 ± 0.022). The liver weight of males treated with MAAE 1280 mg/kg was raised 1.680 ± 0.090 compared to the control group 1.272 ± 0.050, in the other hand, the liver weight of females declined in the same dose (1.164 ± 0.083) when compared to the control group (1.260 ± 0.043).

All the treatment groups of animals from the acute toxicity and repeated-dose oral assays did not show morphological changes in the tissues analyzed (liver, spleen, heart, uterus, testicle, stomach, kidney, and lung), and they did not present anomalies such as necrosis, apoptosis, inflammation, degeneration or hemorrhage (Figs. S2–S6, Supplementary Data).

3.3.4. Hematological and biochemical analyses

Hematological and biochemical examination was conducted using whole blood or plasma samples of mice, the results were presented in Tables S1–S4. The parameter values of treated groups, both studies, shown no alterations ($p < 0.05$; $p < 0.01$) within the range of control group.

The hematological parameters measured after the treatment with MAAE in acute toxicity (Table S1, Supplementary Data) and different repeated-dose oral assays (Table S2) were leukocytes (WBC), erythrocytes (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), HGB concentration, and platelet count (PLT). These measured hematological parameters in acute toxicity did not reveal changes for the MAAE treatment at a dose 2000 mg/kg in relation to the control for female and male mice.

The hematological parameters from repeated-dose oral evaluations of MAAE (1280, 256, and 51.2 mg/kg) were not changed when compared to the controls. The PLT increased after the MAAE treatment at higher doses 256 and 1280 mg/kg for male (m) and female (f) (MAAE-256 mg/kg: 1065 ± 74 (f) and 1439 ± 58.8 (m); MAAE-1280 mg/kg: 1569 ± 97.62 (f) and 1628 ± 45.73 (m); control:963.30 ± 114.70 (f), 1180 ± 27.55 (m) x 10^3/μL).

The biochemical parameters evaluated from acute toxicity (Table S3) and repeated-dose oral assays (Table S4) were glucose (GLU), albumin (ALB), total cholesterol (CHOL), alanine (ALT) aminotransferase, and aspartate aminotransferase (AST). The ALT increased in male mice treated with MAEE (2000 mg/kg) from acute toxicity assay in relation to control.

From the repeated-dose oral experiment, the MAAE (51.2, 256, and 1280 mg/mL) induced an increase of GLU, while CHOL was increased in males at dose 1280 mg/kg the male mice. AST was not changed in the animal groups compared to the control and ALT was decreased.

3.4. In vivo anti-inflammatory evaluation

3.4.1. Effect of MAAE in carrageenan-induced paw edema

The effect of pretreatment with MAAE in carrageenan-induced paw edema is shown in Table 4. After 60 min, the oral administration of MAAE at a dose 256 mg/kg inhibited 43% (0.031 ± 0.003) similarly the inhibition showed by the control indomethacin of 44% (0.030 ± 0.004). In addition, the inhibition percentage of carrageenan-induced paw edema was 55% (0.016 ± 0.004) for MAAE at 120 min, which was statistically similar to the inhibition of 67% (0.012 ± 0.004) for indomethacin.

3.4.2. Effect of MAAE on the leukocyte influx induced by carrageenan

The results of pretreatment with MAAE on leukocyte influx are shown in Fig. 2. The leukocyte influx increased into the abdominal cavity after 4h of the injection of carrageenan (4238 ± 526 cells/mm^3), compared to saline control (2100 ± 178 cells/mm^3). The anti-inflammatory drug indomethacin reduced the leukocytes to 1438 ± 201 cells/mm^3 while MAAE at 256 mg/kg reduced to 1488 ± 334 cells/mm^3, representing reductions of 66 and 65%, respectively. In addition, MAAE at 51.2 and 1280 mg/kg reduced the total cells by 36 and 45% (Fig. 2A).

The influx of polymorphonuclear cells (PMN) was decreased from the pretreatment with MAAE 51.2, 256, and 1280 mg/kg, resulting in reductions of 42% (1448 ± 137.9 cells/mm^3), 78% (555 ± 211.2 cells/mm^3) and 66% (844 ± 124.4 cells/mm^3), respectively. While the drug indomethacin presented a reduction of 84% (396 ± 123.8 cells/mm^3) of PMN (Fig. 2B).

The mononuclear cells (MN) showed a reduction of 46% (933 ± 137 cells/mm^3) for MAAE at 256 mg/kg, while indomethacin decreased by 40% (1042 ± 138 cells/mm^3), and they were not revealed statistical differences. The MAAE at doses 51.2 and 1280 mg/kg also revealed reductions to 1269 ± 87 (27%) and 1489 ± 44 cells/mm^3 (14%) (Fig. 2C).

3.4.3. Effect of MAAE on abdominal writhings induced by acetic acid

The results of the abdominal writhing assay are plotted in Fig. 3. The control group with water raised writhings (99.6 ± 7 writhing episodes), in contrast the positive control group pretreated with indomethacin (15 mg/kg) reduced the abdominal writhings by 38% (61.6 ± 2.9 writhing episodes). The MAAE doses (51.2, 256, and 1280 mg/kg) exhibited a gradual reduction in writhings directly proportional to the dosage increase, as follows 19% (80.8 ± 3.4 writhing episodes), 33% (66.4 ± 3.6 writhing episodes) and 42% (57.6 ± 7.6 writhing episodes), respectively.

3.4.4. Effect of MAAE formalin-induced paw licking response in mice

The results from the formalin-induced paw licking assay were illustrated in Fig. 4. The animals pretreated with water (10 mL/kg, control)

Table 4
Effect of pre-treatment with MAAE in carrageenan-induced paw edema.

Group	Conc.	Paw edema (mL) and **(%)** inhibition							
		30 min		60 min		120 min		240 min	
Water	10 mL/kg	0.022 ± 0.003	–	0.054 ± 0.005	–	0.036 ± 0.004	–	0.021 ± 0.002	–
Indomethacin	15 mg/kg	0.026 ± 0.005	–	0.030 ± 0.004***	44.4%	0.012 ± 0.004***	66.6%	0.016 ± 0.002	23.8%
MAAE	256 mg/kg	0.031 ± 0.003	–	0.031 ± 0.003***	42.6%	0.016 ± 0.004**	55.5%	0.029 ± 0.005	–

The animals (n = 5/group) were pretreated with vehicle (10 mL/kg, water/negative control), indomethacin (15 mg/kg, 0.2 mL, positive control) or MAAE (256 mg/kg), p.o. (0.2 mL/animal), 60 min before the stimulus. The edema was evaluated at 30, 60, 120, and 240 min after the intraplantar injection (i.pl.) of carrageenan 1%. Results were expressed with mean ± EPM. ANOVA of two ways, followed by the Tukey test. $^{**}p < 0.001$; $^{***}p < 0.001$ (compared as the group pretreated with water).

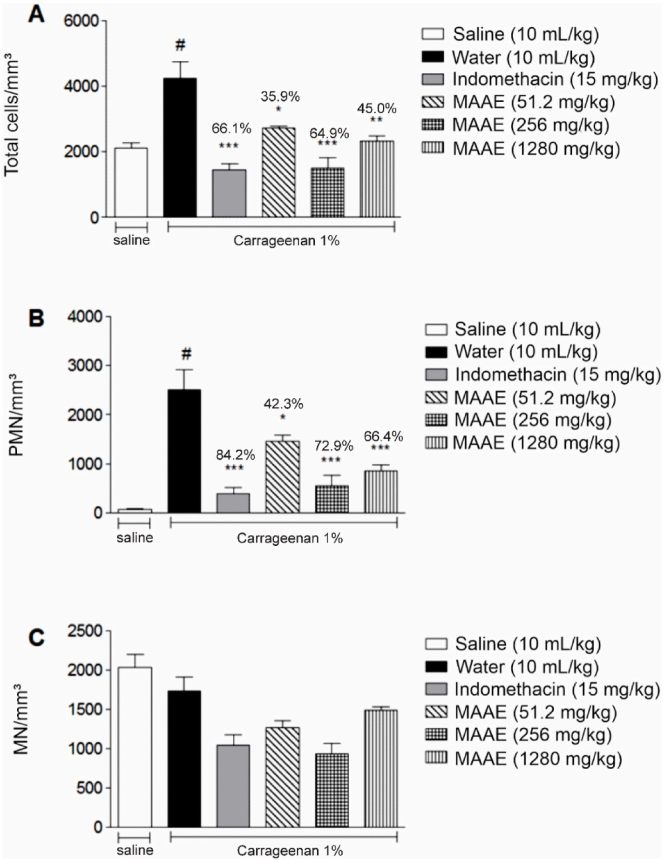


Fig. 2. Effect of pretreatment with *Miconia albicans* aqueous extract (MAAE) on leukocyte influx into the peritoneal cavity in mice. The groups were 0.9% sterile saline solution (saline); water (vehicle), indomethacin (positive control), or MAAE, p.o., 0.2 mL, were administered 60 min before injection of stimulus (Carrageenan 1%, i.p., 0.5 mL). Evaluation of leukocytes total **(A)**, polymorphonuclear cells (PMN, **B**) and mononuclear cells (MN, **C**) was performed after 4h of the stimulus. The results were expressed as the mean ± S.E.M. (n = 4). Data were analyzed employing ANOVA followed by the Tukey test. $\#p < 0.05$ when compared to the saline group; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ when compared to the Water group (vehicle).

showed 43.2 ± 1.9 s in phase I in the paw licking time and in phase II 259.8 ± 22.5 s. The animals pretreated with morphine (5 mg/kg) exhibited reductions at both phases; in phase I and II reduced 93% (3.1 ± 0.8 s) and 96% (10.9 ± 10.9 s) of paw licking, respectively. In addition, the control group pretreated with indomethacin (15 mg/kg) indicate reduction only in phase II by 34% (172.7 ± 14.9 s), while MAAE at dose 256 mg/kg reduced the paw licking time by 23% (200.9 ± 11.1 s) only in phase II.

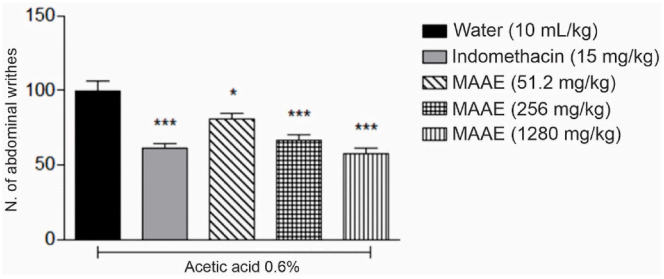


Fig. 3. Effect of pretreatment with *Miconia albicans* aqueous extract (MAAE) on acetic acid-induced writhing response in mice. Water (vehicle), indomethacin (positive control), or MAAE was administered (p.o., 0.2 mL), 60 min before injection of stimulus (acetic acid 0.6%, 0.5 mL, i.p.) and the number of writhes were recorded during 30 min. The results were expressed as the mean ± S.E.M. (n = 4). Data were analyzed employing ANOVA followed by the Tukey Test. $^{*}p < 0.05$ and $^{***}p < 0.001$ when compared to the water group (vehicle).

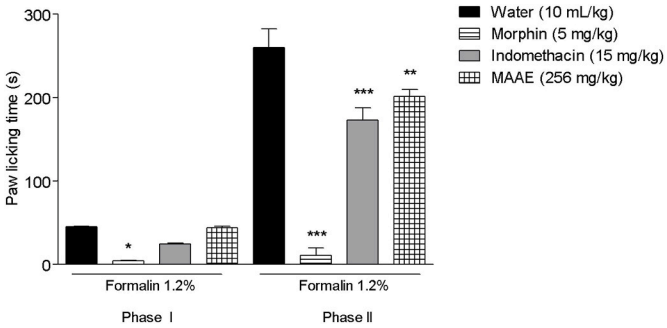


Fig. 4. Effect of pretreatment with *Miconia albicans* aqueous extract (MAAE) on formalin-induced paw licking time. Water (vehicle), morphin (positive control, phase I), indomethacin (positive control, phase II), or MAAE was administered (p.o., 0.2 mL), 60 min before injection of stimulus (Formalin acetic acid 1.2%, 40 μL, i.pl.). The paw licking time was evaluated in **Phase I** (0–5 min) and **Phase II** (15–30 min) after injection of stimulus in the hind paw of mice. The results were expressed as the mean ± S.E.M. (n = 5). Data were analyzed employing ANOVA followed by the Tukey Test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ when compared to the water group (vehicle).

4. Discussion

Inflammation is important response of human organism, but it provokes damages when this process uncontrolled. Nowadays, diverse studies have been performed from natural products as source for new anti-inflammatories with fewer side effects compared to steroidal and non-steroidal anti-inflammatory drugs, since they show diverse side effects such as renal toxicity, gastric intolerance, and cardiovascular problems (Bindu et al., 2020; Nunes et al., 2020; Strand, 2007). In this context, the aqueous extract of *M. albicans* (MAAE) demonstrated anti-inflammatory and anti-hyperalgesic potential, corroborating with some analyses described by Quintans-Junior and coworkers (2020) and

attesting its application in the popular medicine by the same extraction methodology used by population. *M. albicans* is a species widely used in traditional medicine to treat inflammation and arthritis. It can be bought easily on local markets and drug stores of herbals (Lima et al., 2018; Almeida et al., 2014).

The pretreatment with MAAE showed a relevant anti-inflammatory property, reducing paw edema after 60 min. The carrageenan-induced paw edema assay has been an essential process in the development of new nonsteroidal anti-inflammatory drugs by cyclooxygenase (COX) inhibition. This biphasic assay is marked for the first (30–60 min) and the second phase (after 60 min). The mediators histamine, bradykinin, and serotonin are released in the first phase, while in the second phase is observed an increase of prostaglandins and nitric oxide (Wu et al., 2021; Sengar et al., 2015). Thus, MAAE could promote COX inhibition, reducing prostaglandins.

Flavonols, compounds annotated from MAAE, are polyphenols widely found vegetables, fruits, and herbs with antioxidant and anti-inflammatory properties (Beecher, 2003). They are recognized due to the inhibition of enzymes of arachidonic acid metabolism, including COX and LOX. In the rat paw edema model, rutin, a di-*O*-glycosylated flavonol presents on MAAE, reduced the polymorphonuclear cells chemotaxis, besides it reduced the histamine production, as well as reduced the production and release of cytoprotective prostaglandins (Gautam et al., 2016). Quercetin (aglycone of flavonoid rutin) inhibits the lipopolysaccharide-induced NO production, but its glycoside rutin is inactive in vitro assay, as well as for prostaglandin E2 production by peritoneal macrophages. However, rutin showed the capacity to block lipopolysaccharide-induced NO production *in vivo* assays, since it can be hydrolyzed by glucosidases in the gastrointestinal tract and produce quercetin (Shen et al., 2022; Manach et al., 1997). These hydrolyzation reactions can occur for the other *O*-glycosylated flavonols such as quercetin *O*-hesoxide and quercetin *O*-deoxyhesoxide (Manach et al., 1997).

The antioxidant and anti-inflammatory properties of ellagic acid and its derivatives have also been described in the literature (Mansouri et al., 2020; Murphy et al., 2020) The hydrolysable tannins (e.g. vescalagin and castalagin) are commonly related to anti-inflammatory and analgesic properties (Fidelis et al., 2020; Granica et al., 2015; Piwowarski and Kiss, 2015; Piwowarski et al., 2014). The compound vescalagin reduced TNF-α (66%) and IL-6 (52%), when compared to the control (Chang et al., 2013), that suggests the anti-inflammatory effect of MAAE can also be closely related to vescalagin and others tannins.

MAAE reduced the total leukocyte influx (64%) and both mononuclear cells (monocytes) (46%) and polymorphonuclear cells (77%), similar to the reduction observed for drug indomethacin of 66, 39, and 84%, respectively. After the stimulus, the leukocyte migration occurs in the local carrageenan injection, and initially (up 12 h after stimulus) the polymorphonuclear are predominant and secondarily the mononuclear cells (Cassatella, 1995). Thus, chemotactic mediators are released such as TNF, LTB4, IL-8, and IL-1β, which induced the expression of adhesion compounds by vascular endothelium (PAF, selectins, and others), in this sense, it is possible to suggest that MAAE can inhibit arachidonic acid, COX and LOX followed by reduction and production of prostaglandins (Alcaraz-Quiles et al., 2018; Omoigui et al., 2007).

Flavonoids, compounds presented on MAAE and widely distributed in plants such as *O*-glycosylated quercetin (**13**, **15–17**), are correlated to antioxidant properties and they modulate the inflammatory responses by expression of different factors such as cytokines and chemokines, including TNF-α, IL-1β, IL-6 and IL-8 (Dicarlo et al., 2019; Gandhi et al., 2018; Panche et al., 2016). The hydroethanolic extract from *M. albicans* leaves has been reported to reduce TNF-α and IL-1β levels (Lima et al., 2018). In addition, megastigmanes, here reported for the first time from genus *Miconia,* exhibit important pharmacological properties, including inhibition of LPS-stimulated induction for proinflammatory cytokines and molecules related to leukocyte adhesion (Pan et al., 2019).

A positive correlation is described between the total phenol (TPC) and tannin contents (TTC) with the potential antioxidant and anti-inflammatory. In our study, MAAE revealed TPC and TTC of 327.40 ± 5.56 and 140.29 ± 8.70 mg/g of gallic acid equivalent (GAE), both important compounds for the antioxidant activity. The TPC value from leaf aqueous extract by infusion of *M. albicans* was reported as 318.52 ± 0.002 mg/g of GAE (Alexandre et al., 2021), similar to the results obtained here. While the methanol and hydroethanolic extracts by maceration and turboextraction from leaves of *M. albicans* presented TPC of 70.04 ± 0.12 and 551.3 ± 3.72 mg/g GAE, respectively. The differences in TPC values are relative to changes in the extractor solvent and method, as well as the chemical differences due to seasonality and specimens (harvest site, genetics, and others) as described by Gimenez et al. (2020). The concentration of flavonols (rutin and quercetin), compounds recognized by their antioxidant potential, were higher in the rainy season in the leaves of *M. albicans*, while the triterpenes ursolic and oleanolic acids were higher in the dry season (Gimenez et al., 2020).

Hyperalgesia is a common process in inflammation, where the nociceptors are sensitized in the injured tissue and mediators are produced inducing pain, such as TNF, prostaglandins, IL-1, IL-6, and IL-8 (Huang et al., 2006). MAAE extract decreased the abdominal writhings, demonstrating effect on painful sensitivity, similar to non-steroidal anti-inflammatory drugs. MAAE reduced the abdominal writhings caused by acetic acid and the formalin-induced paw licking time in the second phase, like as indomethacin. The formalin paw licking assay indicated the anti-hyperalgesic activity that suggests a mediation by inhibition of prostaglandin biosynthesis, as well as inhibition of mediator release mechanisms, as suggested by Quintans-Junior and collaborators (2020), which showed a reduction in TNF- and IL-1β levels.

The acute toxicity evaluation of MAAE demonstrated no toxicity. In the repeated-dose oral experiment, the agitation and vocal fremitus were detected after 21 days at a higher dose (1280 mg/kg), but the therapeutic dose (256 mg/kg) showed no toxicity effects. The treatment caused an increment in GLU that could induce an elevation in total CHO, since an increase in blood GLU can accelerate the production of triglycerides. In addition, GLU levels associated with transaminase levels (ALT and AST) can suggest liver damage. ALT and AST are enzymes that indicate hepatic function and their values can be increased in situations of liver damage. Beyond the liver cells, these enzymes are also present in heart, muscle, and lung cells. Thus, the increase in serum ALT and ASP values could indicate damage to these tissues (Song et al., 2014). However, in our study, these enzymes were decreased in treatments at lower doses, suggesting a hepatoprotection, probably due to the action of the polyphenols present in the extract.

5. Conclusion

This work expanded the chemical knowledge from genus *Miconia*, since some tannins (**5–6**, and **8–9**), megastigmanes (**11**–**12**), and trimethylated ellagic acid (**18, 22**) were reported for the first time here for it. The anti-inflammatory and anti-hyperalgesic properties of MAAE were confirmed, as used in folk medicine, corroborating and giving evidence of *M. albicans* to treat inflammatory disorders, and suggesting an alternative potential to assist this treatment. The MAAE showed no acute or repeated-dose toxicities.

Funding

This work was supported by Federal University of Mato Grosso do Sul and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) scholarship 88887.635035/2021–00.

Ethical approval

The experiments with animals were approved by the Ethics Committee on Animal Experimentation of the UFMS (protocol 1.033/2019).

CRediT authorship contribution statement

Djaceli Sampaio de Oliveira Dembogurski: conceived, developed research, analyzed the chemical data, wrote the manuscript, conducted anti-inflammatory, antinociceptive, and toxicological experiments. **Iluska Senna Bonfá:** conducted anti-inflammatory, antinociceptive, and toxicological experiments.. **Luciane Candeloro:** conducted the histological analyzsed. **Eduardo Benedetti Parisotto:** conducted the biochemical analyses, All authors read and approved the manuscript. **Mônica Cristina Toffoli Kadri:** conducted anti-inflammatory, antinociceptive, and toxicological experiments. **Denise Brentan Silva:** conceived, developed research, analyzed the chemical data, wrote the manuscript.

Declaration of competing interest

All authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to Federal University of Mato Grosso do Sul (UFMS), Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do estado de Mato Grosso do Sul (FUNDECT), Instituto Nacional de Ciência e tecnologia em Áreas Úmidas (INAU), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process number 313047/2020-0).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2023.116251.

References

Alcaraz-Quiles, J., Casulleras, M., Oettl, K., Titos, E., Flores-Costa, R., Duran-Guell, M., Claria, J., 2018. Oxidized albumin triggers a cytokine storm in leukocytes through P38 mitogen-activated protein kinase: role in systemic inflammation in decompensated cirrhosis. Hepatology 68 (5), 1937–1952. https://doi.org/10.1002/hep.30135.

Alexandre, A.V., Castro, F.M., Batista, F.L.A., Santos, S.A.A.R., Mendes, F.R.S., Goncalves, N.G.G., Campos, A.R., 2021. Chemical profile and anxiolytic- and anticonvulsant-like effects of *Miconia albicans* (Sw.) triana (Melastomataceae) leaves in adult zebrafish. Phcog. Mag. 17 (73), 146–153.

Almeida, M.Z., Léda, P.H.O., Silva, M.Q.O.R., Pinto, A., Lisboa, M., Guedes, M.L.M.L., Peixoto, A.L., 2014. Species with medicinal and mystical-religious uses in São Francisco do Conde, Bahia, Brazil: a contribution to the selection of species for introduction into the local Unified Health System. Rev. Bras. Farmacogn. 24 (2), 171–184. https://doi.org/10.1016/j.bjp.2014.04.006.

ANVISA, 2010. Agência Nacional de Vigilância Sanitária. Brasília, Brasil. Resolução–RDC N° 10, de 9 de março de 2010.

Beecher, G.R., 2003. Overview of dietary flavonoids: nomenclature, occurrence and intake. J. Nutr. 133 https://doi.org/10.1093/jn/133.10.3248S, 3248S–54S.

Bindu, S., Mazumder, S., Bandyopadhyay, U., 2020. Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: a current perspective. Biochem. Pharmacol. 180, 114147 https://doi.org/10.1016/j.bcp.2020.114147.

Cassatella, M.A., 1995. The production of cytokines by polymorphonuclear neutrophils. Immunol. Today 16, 21–26. https://doi.org/10.1016/0167-5699(95)80066-2.

Castaneda, R., Natarajan, S., Jeong, S.Y., Hong, B.N., Kang, T.H., 2019. Traditional oriental medicine for sensorineural hearing loss: can ethnopharmacology contribute to potential drug discovery? J. Ethnopharmacol. 231, 409–428. https://doi.org/10.1016/j.jep.2018.11.016.

Celotto, A.C., Nazario, D.Z., Spessoto, M.A., Martins, C.H.G., Cunha, W.R., 2003. Evaluation of the *in vivo* antimicrobial activity of crude extracts of three *Miconia* species. Braz. J. Microbiol. 34, 339–340. https://doi.org/10.1590/S1517-83822003000400010.

Cerulli, A., Napolitano, A., Masullo, M., Hosek, J., Pizza, C., Piacente, S., 2020. Chestnut shells (Italian cultivar "Marrone di Roccadaspide" PGI): antioxidant activity and chemical investigation with in depth LC-HRMS/MS^n rationalization of tannins. Food Res. Int. 129, 108787 https://doi.org/10.1016/j.foodres.2019.108787.

Chang, W.C., Shen, S.C., Wu, J.S., 2013. Protective effects of vescalagin from pink wax apple [*Syzygium samarangense* (Blume) Merrill and Perry] fruit against methylglyoxal-induced inflammation and carbohydrate metabolic disorder in rats. J. Agric. Food Chem. 61 (29), 7102–7109. https://doi.org/10.1021/jf4020284.

Chang, S., Wang, M., Tian, Y., Qi, J., Qiu, Z., 2020. Systematic analysis and identifcation of the absorption and metabolic components of Zengye decoction in type 2 diabetic rats by HPLC-ESI-Q-TOF–MS/MS. Chin. Med. 15, 50. https://doi.org/10.1186/s13020-020-00331-z.

Chen, L., Yang, G., Grosser, T., 2013. Prostanoids and inflammatory pain. Prostag. Other Lipid Mediat. 104–105, 58–66. https://doi.org/10.1016/j.prostaglandins.2012.

Crevelin, E.J., Turatti, I.C.C., Crotti, A.E.M., Veneziani, R.C.S., Lopes, J.L.C., Lopes, N.P., Cunha, W.R., 2006. Identification of biologically active triterpenes and sterols present in hexane extracts from *Miconia* species using high-resolution gas chromatography. Biomed. Chromatogr. 20 (8), 827–830. https://doi.org/10.1002/bmc.609.

Dicarlo, M., Teti, G., Verna, G., Liso, M., Cavalcanti, E., Sila, A., Chieppa, M., 2019. Quercetin exposure suppresses the inflammatory pathway in intestinal organoids from winnie mice. Int. J. Mol. Sci. 20 (22), 5771. https://doi.org/10.3390/ijms20225771.

El-Gabalawy, H., Guenther, L.C., Bernstein, C.N., 2010. Epidemiology of immune-mediated inflammatory diseases: incidence, prevalence, natural history, and comorbidities. J. Rheumatol. Suppl. 85, 2–10. https://10.3899/jrheum.091461.

Ferreira, D.S., Esperandim, V.R., Marcal, M.G., Neres, N.B.R., Cunha, N.L., Andrade e Silva, M.L., Cunha, W.R., 2013. Natural products and Chagas' disease: the action of triterpenes acids isolated from *Miconia* species. Univ. Sci. 18 (3), 243–256. https://doi.org/10.11144/Javeriana.SC18-2.npcd.

Fidelis, M., Carmo, M.A.V., Cruz, T.M., Azevedo, L., Myoda, T., Miranda Furtado, M., Granato, D., 2020. Camu-camu seed (*Myrciaria dubia*) - from side stream to an antioxidant, antihyperglycemic, antiproliferative, antimicrobial, antihemolytic, anti-inflammatory, and antihypertensive ingredient. Food Chem. 310, 125909 https://doi.org/10.1016/j.foodchem.2019.125909.

Gandhi, G.R., Neta, M., Sathiyabama, R.G., Quintans, J.S.S., Oliveira, E.S.A.M., Araujo, A.A.S., Gurgel, R.Q., 2018. Flavonoids as Th1/Th2 cytokines immunomodulators: a systematic review of studies on animal models. Phytomedicine 44, 74–84. https://doi.org/10.1016/j.phymed.2018.03.057.

Gao, J., Shi, J.-M., Lu, X.-M., Sun, C.-R., Pan, Y.-J., 2011. Differentiation of common diastereoisomeric ursane-type triterpenoids by high-performance liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 25 (10), 1349–1355. https://doi.org/10.1002/rcm.4978.

Gautam, R., Singh, M., Gautam, S., Rawat, J.K., Saraf, S.A., Kaithwas, G., 2016. Rutin attenuates intestinal toxicity induced by Methotrexate linked with anti-oxidative and anti-inflammatory effects. BMC Compl. Alternative Med. 16, 99. https://doi.org/10.1186/s12906-016-1069-1.

Gimenez, V.M.M., Silva, M.L.A., Cunha, W.R., Januario, A.H., Costa, E.J.X., Pauletti, P. M., 2020. Influence of environmental, geographic, and seasonal variations in the chemical composition of *Miconia* species from Cerrado. Biochem. Systemat. Ecol. 91, 104049 https://doi.org/10.1016/j.bse.2020.104049.

Granica, S., Piwowarski, J.P., Kiss, A.K., 2015. Ellagitannins modulate the inflammatory response of human neutrophils ex vivo. Phytomedicine 22 (14), 1215–1222. https://doi.org/10.1016/j.phymed.2015.10.004.

Greaves, P., 2012. In: Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Studies, fourth ed. Elsevier, Amsterdam, The Netherlands.

Herald, T.J., Gadgil, P., Tilley, M., 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. J. Sci. Food Agric. 92 (11), 2326–2331. https://doi.org/10.1002/jsfa.5633.

Hotamisligil, G.S., 2006. Inflammation and metabolic disorders. Nature 444 (7121), 860–867. https://doi.org/10.1038/nature05485.

Huang, J., Zhang, X., McNaughton, P.A., 2006. Inflammatory pain: the cellular basis of heat hyperalgesia. Curr. Neuropharmacol. 4, 197–206. https://doi.org/10.2174/157015906778019554.

Hunskaar, S., Hole, K., 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103–114. https://doi.org/10.1016/0304-3959(87)90088-1.

Jo, M.S., Lee, S., Yu, J.S., Baek, S.C., Cho, Y.-C., Kim, K.H., 2020. Megastigmane derivatives from the cladodes of *Opuntia humifusa* and their nitric oxide inhibitory activities in macrophages. J. Nat. Prod. 83 (3), 684–692. https://doi.org/10.1021/acs.jnatprod.9b01120.

Koster, R., 1959. Acetic acid for analgesic screening. Fed. SAVE Proc. 18, 412–416.

Lima, R.C.L., Kenneth, T.K., Kato, L., Silva, M.J., Franzyk, H., Staerk, D., 2018. High-resolution PTP1B inhibition profiling combined with HPLC-HRMS-SPE-NMR for identification of PTP1B inhibitors from Miconia albicans. Molecules 23 (7), 1755. https://doi.org/10.3390/molecules23071755.

Manach, C., Morand, C., Memigne, C., Texier, O., Regerat, F., Remesy, C., 1997. Bioavailability of rutin and quercetin in rats. FEBS Lett. 409, 12–16. https://doi.org/10.1016/s0014-5793(97)00467-5.

Mansouri, Z., Dianat, M., Radan, M., Badavi, M., 2020. Ellagic acid ameliorates lung inflammation and heart oxidative stress in elastase-induced emphysema model in rat. Inflammation 43 (3), 1143–1156. https://doi.org/10.1007/s10753-020-01201-4.

Moilanen, J., Sinkkonen, J., Salminen, J.P., 2013. Characterization of bioactive plant ellagitannins by chromatographic, spectroscopic and mass spectrometric methods. Chemoecology 23 (3), 165–179.

Murphy, M.T., Qin, X., Kaul, S., Barrientos, G., Zou, Z., Mathias, C.B., Bose, D.D., 2020. The polyphenol ellagic acid exerts anti-inflammatory actions via disruption of store-operated calcium entry (SOCE) pathway activators and coupling mediators. Eur. J. Pharmacol. 875, 173036 https://doi.org/10.1016/j.ejphar.2020.173036.

Nunes, C.D.R., Arantes, M.B., Pereira, S.M.F., Cruz, L.L., Passos, M.S., Moraes, L.P., Vieira, I.J.C., Oliveira, D.B., 2020. Plants as sources of anti-inflammatory agents. Molecules 25 (16), 3726. https://doi.org/10.3390/molecules25163726.

OECD, 2002. Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264071001-en.

OECD, 2008. Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris. https://doi.org/10.1787/9789264264649-en.

Omoigui, S., 2007. The biochemical origin of pain: the origin of all pain is inflammation and the inflammatory response. Part 2 of 3 - inflammatory profile of pain syndromes. Med. Hypotheses 69 (6), 1169–1178. https://doi.org/10.1016/j.mehy.2007.06.033.

Pan, S.-P., Pirker, T., Kunert, O., Kretschmer, N., Hummelbrunner, S., Latkolik, S.L., Rappai, J., Dirsch, V.M., Bochkov, V., Bauer, R., 2019. C13 megastigmane derivatives from *Epipremnum pinnatum*: β-damascenone inhibits the expression of pro-inflammatory cytokines and leukocyte adhesion molecules as well as NF-κB signaling. Front. Pharmacol. 10, 1351. https://doi.org/10.3389/fphar.2019.01351.

Panche, A.N., Diwan, A.D., Chandra, S.R., 2016. Flavonoids: an overview. J. Nutr. Sci. 5 https://doi.org/10.1017/jns.2016.41, 47/41-47/15.

Pinho-Ribeiro, F.A., Verri Jr., W.A., Chiu, I.M., 2016. Nociceptor sensory neuron-immune interactions in pain and inflammation. Trends Imunnol 38 (1), 5–19. https://doi.org/10.1016/j.it.2016.10.001.

Piwowarski, J.P., Kiss, A.K., 2015. Contribution of C-glucosidic ellagitannins to *Lythrum salicaria* L. influence on pro-inflammatory functions of human neutrophils. J. Nat. Med. 69 (1), 100–110. https://doi.org/10.1007/s11418-014-0873-5.

Piwowarski, J.P., Granica, S., Zwierzynska, M., Stefanska, J., Schopohl, P., Melzig, M.F., Kiss, A.K., 2014. Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich plant materials. J. Ethnopharmacol. 155 (1), 801–809. https://doi.org/10.1016/j.jep.2014.06.032.

Quintans-Junior, L.J., Quintans, J.S.S., Gandhi, S.R., Passos, F.R.S., Heimfarth, L., Pereira, E.W.M., Zengin, G., 2020. Dereplication and quantification of the ethanol extract of *Miconia albicans* (Melastomaceae) by HPLC-DAD-ESI-/MS/MS, and assessment of its anti-hyperalgesic and anti-inflammatory profiles in a mice arthritis-like model: evidence for involvement of TNF-α, IL-1β and IL-6. J. Ethnopharmacol. 258, 112938 https://doi.org/10.1016/j.jep.2020.112938.

Rasines-Perea, Z., Jacquet, R., Jourdes, M., Quideau, S., Teissedre, P.-L., 2019. Ellagitannins and flavano-ellagitannins: red wines tendency in different areas, barrel origin and ageing time in barrel and bottle. Biomolecules 9, 316. https://doi.org/10.3390/biom9080316.

Reichert, C.L., Silva, Carollo, C.A., Weffort-Santos, A.M., Santos, C.A.M., 2018. Metabolic profiling and correlation analysis for the determination of killer compounds of proliferating and clonogenic HRT-18 colon cancer cells from *Lafoensia pacari*. J. Ethnopharmacol. 224, 541–552. https://doi.org/10.1016/j.jep.2018.06.021.

Ribeiro, V.P., Arruda, C., El-Salam, M.A., Bastos, J.K., 2018. Brazilian medicinal plants with corroborated anti- inflammatory activities: a review. Pharm. Biol. 56 (1), 253–268. https://doi.org/10.1080/13880209.2018.1454480.

Rodrigues, J., Rinaldo, D., Silva, M.A., Santos, L.C., Vilegas, W., 2011. Secondary metabolites of *Miconia rubiginosa*. J. Med. Food 14 (7/8), 834–839. https://doi.org/10.1089/jmf.2010.0157.

Santos, V.S., Nascimento, T.V., Felipe, J.L., Boaretto, A.G., Damasceno-Junior, G.A., Silva, D.B., Toffoli-Kadri, M.C., Carollo, C.A., 2017. Nutraceutical potential of *Byrsonima cydoniifolia* fruits based on chemical composition, anti-inflammatory, and antihyperalgesic activities. Food Chem. 237, 240–246. https://doi.org/10.1016/j.foodchem.2017.05.082.

Sengar, N., Joshi, A., Prasad, S.K., Hemalatha, S., 2015. Anti-inflammatory, analgesic and anti-pyretic activities of standardized root extract of *Jasminum sambac*. J. Ethnopharmacol. 160, 140–148. https://doi.org/10.1016/j.jep.2014.11.039.

Serpeloni, J.M., Reis, M.B., Rodrigues, J., Santos, L.C., Vilegas, W., Varanda, E.A., Colus, I.M.S., 2008. In vivo assessment of DNA damage and protective effects of extracts from *Miconia* species using the comet assay and micronucleus test. Mutagenesis 23 (6), 501–507. https://doi.org/10.1093/mutage/gen043.

Serpeloni, J.M., Barcelos, G.R.M., Mori, M.P., Yanagui, K., Vilegas, W., Varanda, E.A., Colus, I.M.S., 2011. Cytotoxic and mutagenic evaluation of extracts from plant species of the *Miconia* genus and their influence on doxorubicin-induced mutagenicity: an in vitro analysis. Exp. Toxicol. Pathol. 63 (5), 499–504. https://doi.org/10.1016/j.etp.2010.03.011.

Shen, S.-C., Lee, W.-R., Lind, H.-Y., Huang, H.-C., Kod, C.-H., Yang, L.-L., Chen, Y.-C., 2022. In vitro and in vivo inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E2 production. European. J. Pharmacol. 446, 187–194. https://doi.org/10.1016/s0014-2999(02)01792-2.

Silva, D.B., Okano, L.T., Lopes, N.P., Oliveira, D.C.R., 2013. Flavanone glycosides from *Bidens gardneri* bak. (Asteraceae). Phytochemistry 96, 418–422. https://doi.org/10.1016/j.phytochem.2013.09.024.

Silva-Correa, C.R., Campos-Reyna, J.L., Villarreal-La Torre, V.E., Calderón-Peña, A.A., González Blas, M.V., Aspajo-Villalaz, C.L., Cruzado-Razco, J.L., Sagástegui-Guarniz, W.A., Guerrero-Espino, L.M., Hilario-Vargas, J., 2021. Potential activity of medicinal plants as pain modulators: a review. Pharmacog. J. 13 (1), 248–263. https://doi.org/10.5530/pj.2021.13.35.

Singh, A.K., Kumar, S., Vinayak, M., 2018. Recent development in antihyperalgesic effect of phytochemicals: anti-inflammatory and neuro-modulatory actions. Inflamm. Res. 67 (8), 633–654. https://doi.org/10.1007/s00011-018-1156-5.

Song, E., Fu, J., Xia, X., Su, C., Song, Y., 2014. Bazhen decoction protects against acetaminophen induced acute liver injury by inhibiting oxidative stress, inflammation and apoptosis in mice. PLoS One 9, e107405. https://doi.org/10.1371/journal.pone.0107405.

Souza, G.E.P., Ferreira, S.H., 1985. Blockade by anti-macrophage sérum of the migration of PMN neutrophils into the inflamed peritoneal cavity. Agents Actions 17, 97–103. https://doi.org/10.1007/BF01966691.

Spínola, V., Pinto, J., Castilho, P.C., 2015. Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLC-DAD–ESI-MS^n and screening for their antioxidant activity. Food Chem. 173, 14–30. https://doi.org/10.1016/j.foodchem.2014.09.163.

Strand, V., 2007. Are COX-2 inhibitors preferable to non-selective non-steroidal anti-inflammatory drugs in patients with risk of cardiovascular events taking low dose aspirin? Lancet 370 (9605), 2138–2151. https://doi.org/10.1016/S0140-6736(07)61909-6.

Tarawneh, A.H., Leon, F., Ibrahim, M.A., Pettaway, S., McCurdy, C.R., Cutler, S.J., 2014. Flavanones from *Miconia prasina*. Phytochem. Lett. 7, 130–132. https://doi.org/10.1016/j.phytol.2013.11.001.

Teixeira, M.A., Chaguri, L.C.A.G., Carissimi, A.S., Souza, N.L., Mori, C.M.C., Gomes, V.M.W., Poli Neto, A., Nonoyama, K., Merusse, J.L.B., 2000. Hematological and biochemical profiles of rats (*Rattus norvegicus*) kept under microenvironmental ventilation system. Braz. J. Vet. Res. Anim. Sci. 37, 341–347. https://doi.org/10.1590/S1413-95962000000500001.

Vasconcelos, M.A.L., Royo, V.A., Ferreira, D.S., Crotti, A.E.M., Andrade e Silva, M.L., Carvalho, J.C.T., Cunha, W.R., 2006. *In vivo* analgesic and anti-inflammatory activities of ursolic acid and oleanoic acid from *Miconia albicans* (Melastomataceae). Z. Naturforsch., C: J. Biosci. 61 (7/8), 477–482. https://doi.org/10.1515/znc-2006-7-803.

Willard, M.D., Tvedten, H., 2012. Small Animal Clinical Diagnosis by Laboratory Methods, fifth ed. Saunders Elsevier, Company, Missouri, USA.

Winter, C.A., Risley, E.A., Nus, G.W., 1962. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med. 111, 544–547. https://doi.org/10.3181/00379727-111-27849.

Wu, X., Xie, J., Qiu, L., Zou, L., Huang, Y., Xie, Y., Zhang, Q., 2021. The anti-inflammatory and analgesic activities of the ethyl acetate extract of *Viburnum taitoense* Hayata. J. Ethnopharmacol. 269, 113742 https://doi.org/10.1016/j.jep.2020.113742.

Younis, W., Alamgeer, Schini-Kerth, V.B., Silva, D.B., Gasparotto Junior, A., Bukhari, I.A., Assiri, A.M., 2020. Role of the NO/cGMP pathway and renin-angiotensin system in the hypotensive and diuretic effects of aqueous soluble fraction from *Crataegus songarica* K. Koch, 249. J. Ethnopharmacol., 112400 https://doi.org/10.1016/j.jep.2019.112400

Supplementary Data

Infusion from *Miconia albicans* (Melastomataceae) leaves exhibits anti-inflammatory and anti-hyperalgesic activities without toxicity

Djaceli Sampaio de Oliveira Dembogurski,[a] Iluska Senna Bonfá,[b] Luciane Candeloro,[c] Eduardo Benedetti Parisotto,[d] Mônica Cristina Toffoli Kadri,[b], Denise Brentan Silva[a,*]

[a]Laboratory of Natural Products and Mass Spectrometry (LaPNEM), Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.
[b]Laboratory of Pharmacology and Inflammation, FACFAN/ Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.
[c]Laboratory of Histology, Biosciences Institute (INBIO), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.
[d]Laboratory of Hematology, Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.

* Corresponding author: Laboratory of Natural Products and Mass Spectrometry, Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Av. Costa e Silva, s/nº, 79070-900, Campo Grande, Mato Grosso do Sul, Brazil. Tel.: +55 (67) 3345-7366.

E-mail address: denise.brentan@ufms.br (D.B. Silva).

Organ weights and histopathology analyses

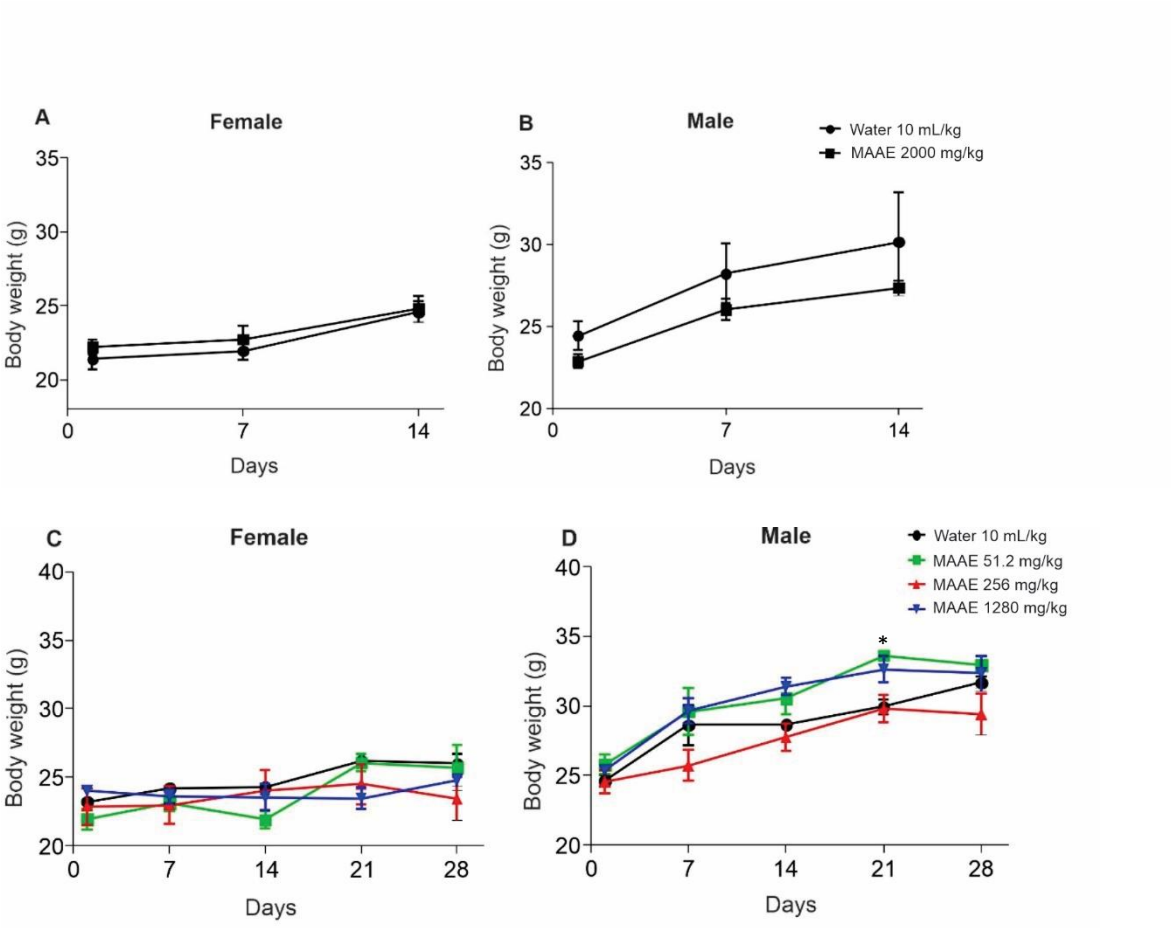
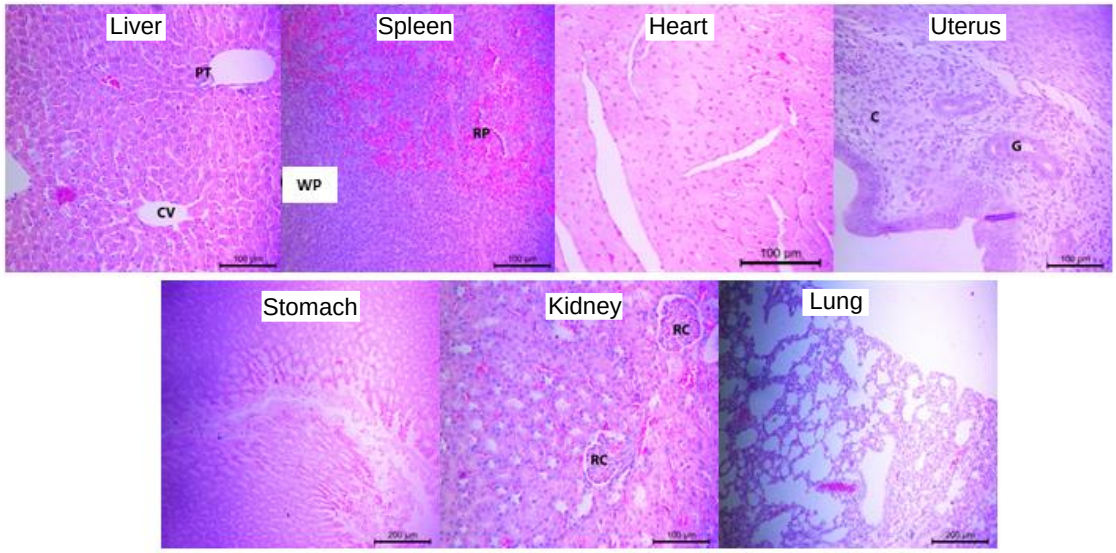


Fig. S1. Body weight of animals after the treatment with aqueous extract of *Miconia albicans* (MAAE). All the animals received water (vehicle) or MAAE by single dose oral for 14 days (**A**, **B**) and repeated dose for 28 days (**C**, **D**). The body weight of female (**A, C**) and male (**B, D**) were evaluated weekly over the fourteen and twenty-eight days. Results were expressed with mean ± EPM. Statistical analyses of two-way variance ANOVA and Bonferroni test (n= 4/group), *p<0.05, when compared to Water group (vehicle).

Acute toxicity (female)



Acute toxicity (male)

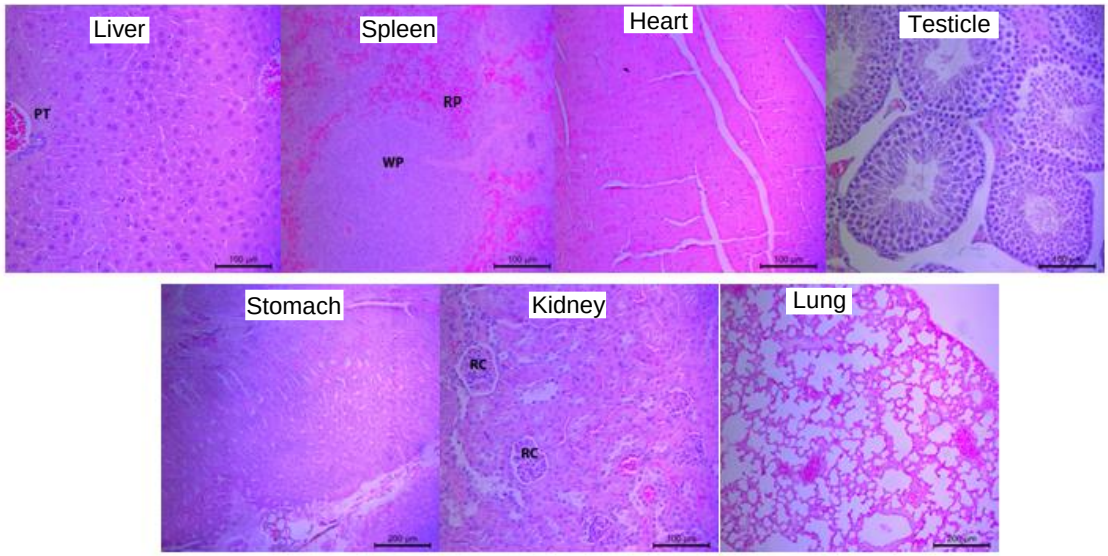
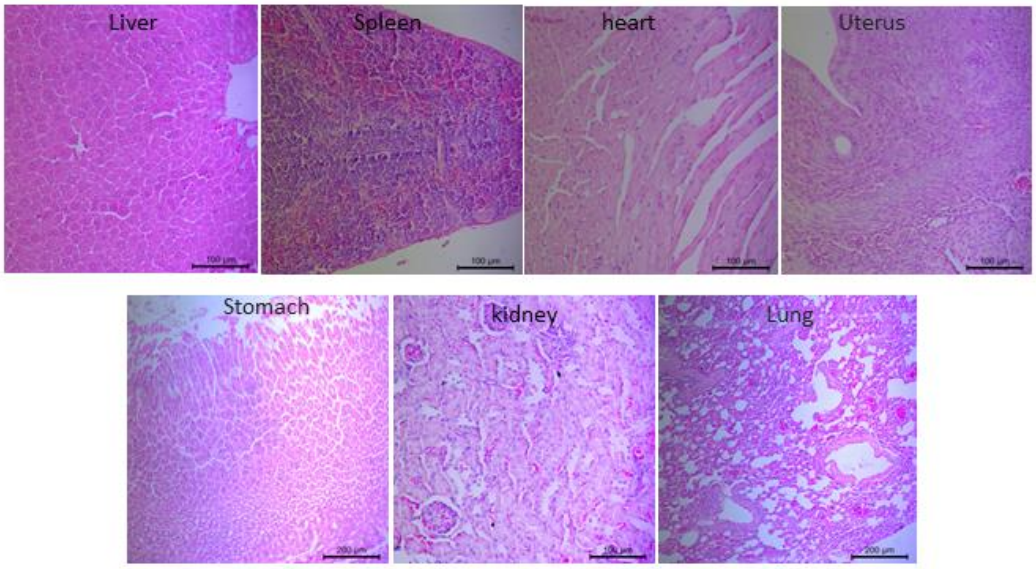


Fig. S2. Cross-sections from liver, spleen, heart, uterus, testicle, stomach, kidney, and lung in MAAE-treated animals (females and males) from the acute toxicity assay. H&E stain.

Vehicle (water) - female



Vehicle (water) - male

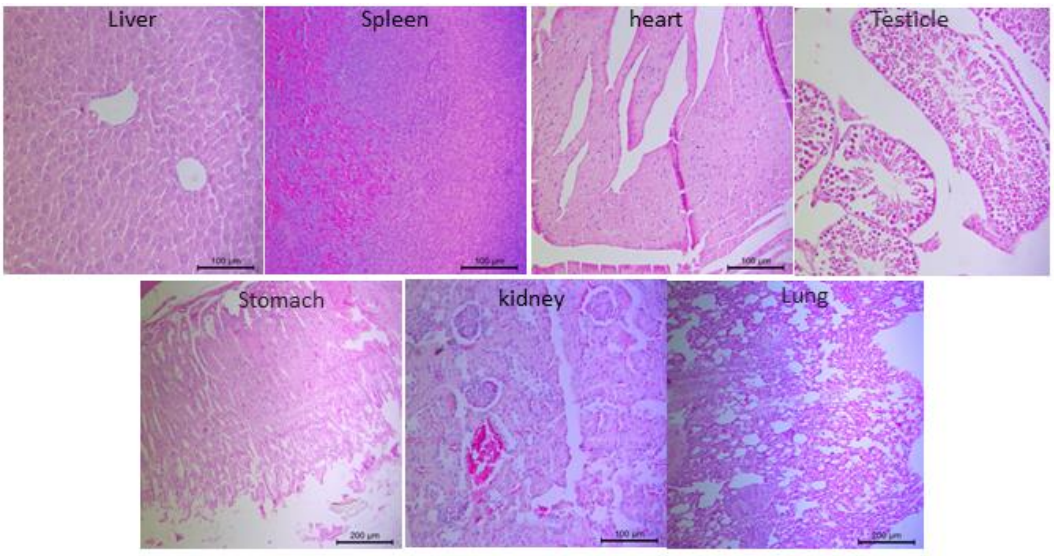
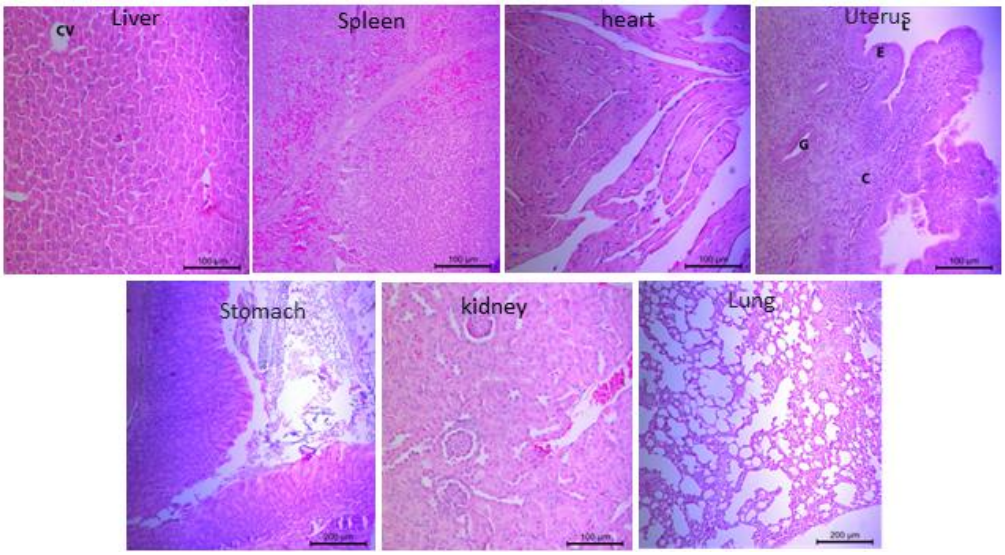


Fig. S3. Cross-sections from liver, spleen, heart, uterus, stomach, kidney, and lung in water-treated animals - control (females and males). H&E stain.

MAAE (dose 1280 mg/kg) – female



MAAE (dose 1280 mg/kg) – male

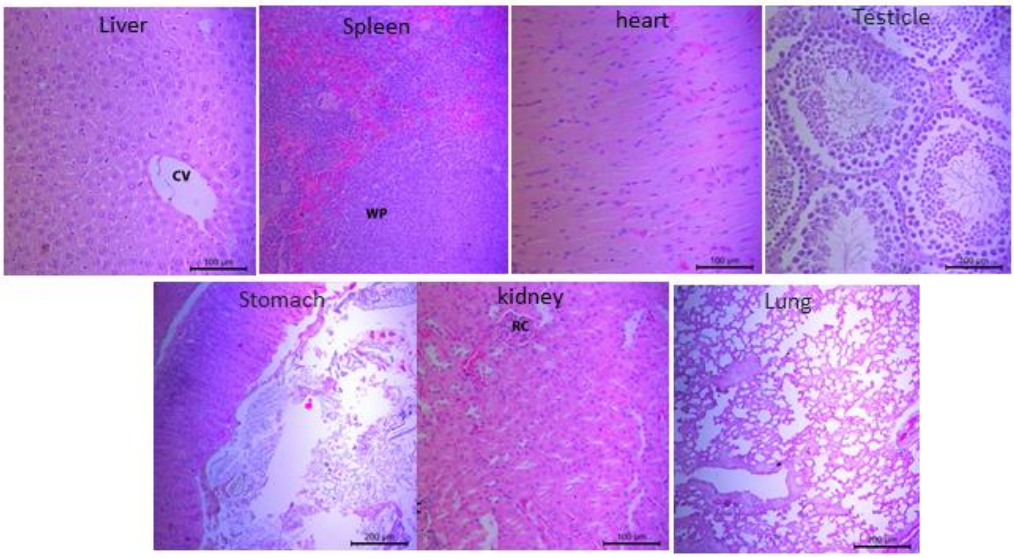
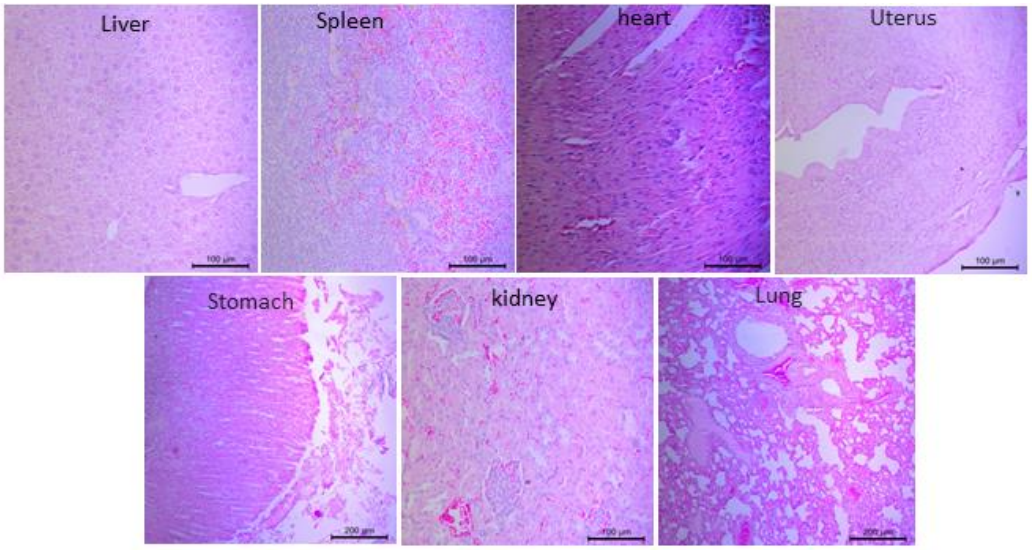


Fig. S4. Cross-sections from liver, spleen, heart, uterus, stomach, kidney, and lung in MAAE-treated animals (females and males) –dose 1280 mg/kg from repeated-dose toxicity assay. H&E stain.

MAAE (dose 256 mg/kg) – female



MAAE (dose 256 mg/kg) – male

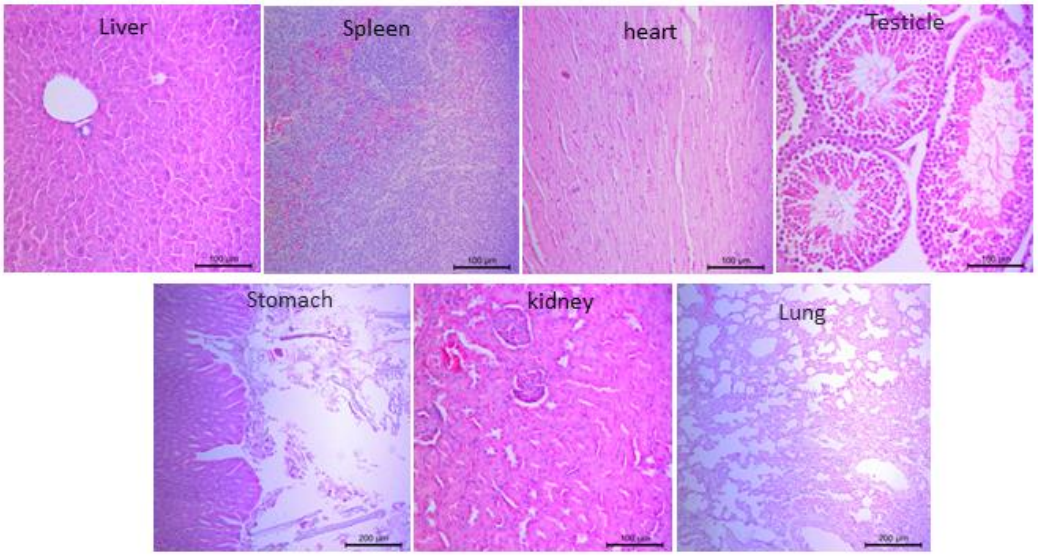
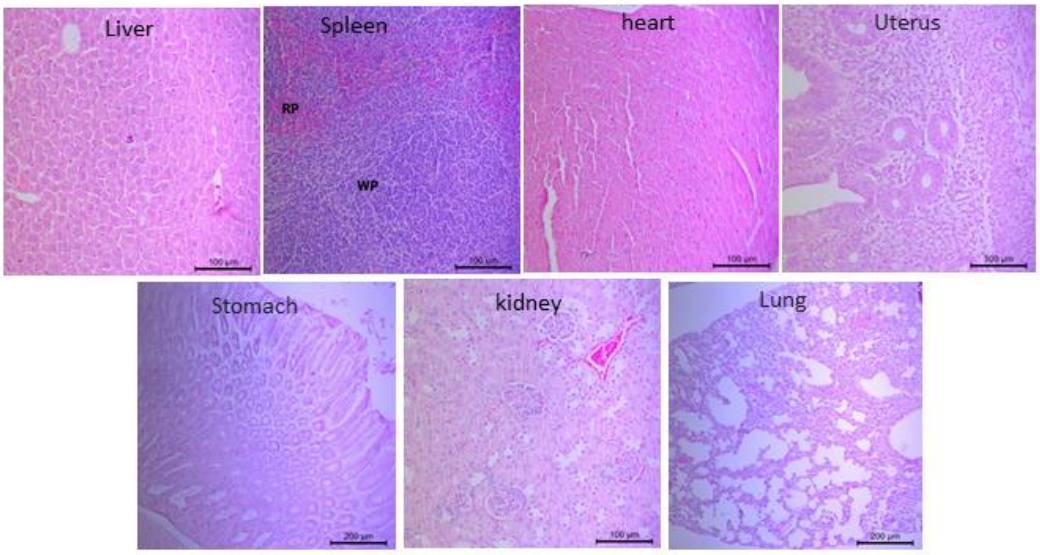


Fig. S5. Cross-sections from liver, spleen, heart, uterus, stomach, kidney, and lung in MAAE-treated animals (females and males) –dose 256 mg/kg from repeated-dose toxicity assay. H&E stain.

MAAE (dose 51.2 mg/kg) – female



MAAE (dose 51.2 mg/kg) – male

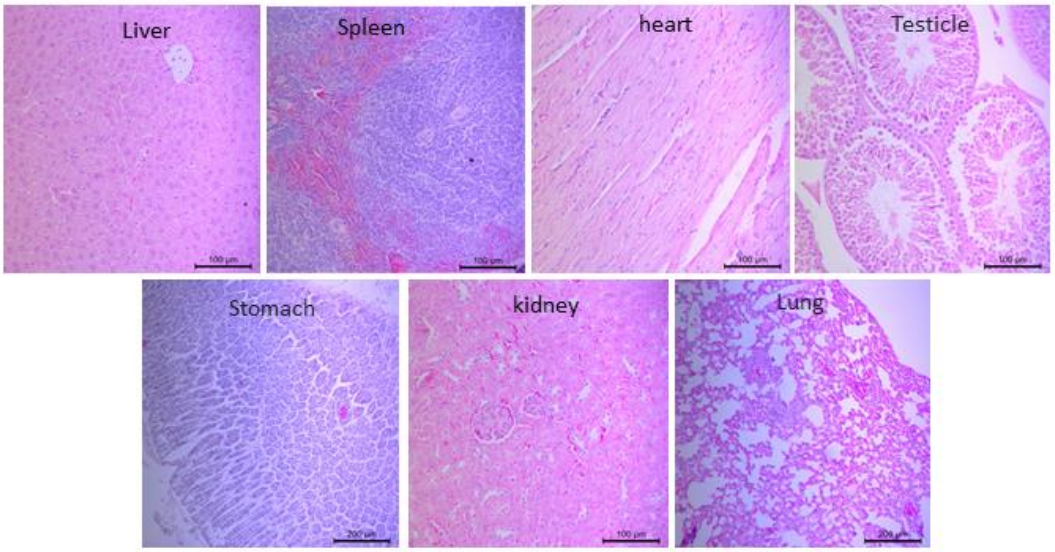


Fig. S6. Cross-sections from liver, spleen, heart, uterus, stomach, kidney, and lung in MAAE-treated animals (females and males) –dose 51.2 mg/kg from repeated-dose toxicity assay. H&E stain.idem

Hematological and biochemical analyses

Table S1: Hematological parameters measured after the treatment with MAAE in acute toxicity study

Parameters	Female		Male	
	Water 10 mL/Kg	MAAE 2000 mg/Kg	Water 10 mL/Kg	MAAE 2000 mg/Kg
WBC (x10^3/µL)	9.53±0.97	9.98±1.96	3.60±0.08	7.73±3.17
RBC (x10^6/µL)	10.20±0.34	10.98±1.41	10.03±0.43	9.53±0.23
HGB (g/dL)	15.40±0.41	14.83±0.35	15.53±0.57	14.68±0.38
HCT (%)	53.20±1.56	50.65±0.96	54.17±2.60	50.70±1.30
MCV (fL)	52.23±0.43	53.26±0.68	54.03±0.58	53.24±0.38
MCH (pg)	15.10±0.14	15.58±0.33	15.53±0.13	15.40±0.13
MCHC (g/dL)	28.97±0.18	29.25±0.15	28.67±0.08	28.98±0.33
PLT (x10^3/µL)	963.30±114.7	1340±143.6	1180±27.55	1104±108.6

MAAE = *Miconia albicans* aqueous extract. WBC= leukocytes; RBC= erythrocyte; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; HGB = hemoglobin concentration; PLT= platelet. The animals received water (control group) or MAAE by single-dose oral. The hematological parameters of female and male were registered in the end of experiment, on the fourteenth day. The results were expressed with mean ± EPM. ANOVA and Bonferroni test (n = 4/group).

Table S2. Hematological parameters effect of MAAE in different repeated-dose oral study

Parameters	Female			
	Water 10mL/Kg	MAAE 51.2 mg/kg	MAAE 256 mg/kg	MAAE 1280 mg/kg
WBC (x10^3/µL)	9.53±0.96	6.90±1.02	6.67±1.27	11.00±1.61
RBC (x10^6/µL)	10.20±0.35	10.50±0.22	9.47±0.12	10.40±0.07
HGB (g/dL)	15.40±0.58	15.70±0.15	14.67±0.17	15.33±0.21
HCT (%)	53.20±1.56	54.13±1.03	50.47±0.86	53.63±0.78
MCV (fL)	52.23±0.30	51.57±0.48	53.47±0.19	51.53±0.47
MCH (pg)	15.10±0.14	14.97±0.17	15.57±0.08	14.77±0.13
MCHC (g/dL)	28.97±0.18	25.52±3.54	25.72±3.39	25.14±3.46
PLT (x10^3/µL)	963.30±114.70	1068±59.40	1065±74	1569.00±97.62***
	Male			
	Water 10mL/Kg	MAAE 51.2 mg/kg	MAAE 256 mg/kg	MAAE 1280 mg/kg
WBC (x10^3/µL)	3.60±0.08	7.75±1.52	7.475±1.28	4.67±0.80$^{\alpha}$
RBC (x10^6/µL)	10.03±0.43	9.98±0.63	9.65±0.24	9.72±0.34
HGB (g/dL)	15.53±0.57	15.15±0.54	14.93±0.41	14.58±0.40
HCT (%)	54.17±1.84	52.48±2.02	52.15±1.20	51.20±1.42
MCV (fL)	54.03±0.58	52.68±0.76	54.13±0.36	52.18±0.87
MCH (pg)	15.53±0.13	15.20±0.24	15.50±0.20	14.83±0.19
MCHC (g/dL)	28.67±0.08	28.86±0.19	28.60±0.50	28.64±0.16
PLT (x10^3/µL)	1180±27.55	1218±68.97	1439±58.80$^{\&}$	1628±45.73**

MAAE = *Miconia albicans* aqueous extract. WBC= leukocytes; RBC= erythrocyte; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; HGB = hemoglobin concentration; PLT= platelet. The animals received water (control group) or MAAE in different repeated-dose oral, daily for twenty-eight days. The hematological parameters of female and male were registered in the end of experiment, on the twenty-eighth day. The results were expressed with mean ± EPM. ANOVA, and then Bonferroni test (n = 4/group). ** p<0.01; *** p<0.001, when compared with control. $^{\alpha}$ p<0.05, when compared with MAAE 1280 mg/kg female. $^{\&}$ p<0.05, when compared with MAAE 256 mg/kg female.

Table S3. Biochemical parameters effect of MAAE in acute toxicity study

Parameters	Female		Male	
	Water 10mL/Kg	MAAE 2000 mg/Kg	Water 10mL/Kg	MAAE 2000 mg/Kg
GLU (g/dL)	82.65±2.27	70.80±0.82	48.05±9.88	43.05±3.20
ALB (g/dL)	2.09±0.30	1.47±1.08	1.71±0.15	1.51±0.03
CHOL (mg/dL)	67.48±0.65	75.32±3.91	66.19±0.56	70.76±3.33
ALT (U/L)	6.06±2.205	4.98±1.33	9.23±2	21.49±2.10**
AST (U/L)	49.08±3.36	43.99±1.20	42.15±2.04	41.73±0.51

MAAE= *Miconia albicans* aqueous extract. GLU= glucose; ALB= albumin; CHOL= total cholesterol; ALT= alanine aminotransferase; AST= aspartate aminotransferase. The animals received water (control group) or MAAE by single-dose oral. The biochemical parameters of female and male were registered in the end of experiment, on the fourteenth day. The results were expressed with mean ± EPM. ANOVA, and then Bonferroni test (n = 4/group). **p<0.01, when compared with control group.

Table S4. Biochemical parameters effect of MAAE in different repeated-dose oral study

Parameter	**Female**			
	Water	MAAE	MAAE	MAAE
	10mL/Kg	51.2 mg/kg	256 mg/kg	1280 mg/kg
GLU (g/dL)	82.65±2.27	94.25±5.90	104.9 ±6.88	81.41±3.37
ALB (g/dL)	2.09±0.30	1.56±0.03	1.50±0.051	1.59±0.08
CHOL (mg/dL)	67.48±0.64	63.76±0.78	64.45±1.09	67.74±4.74
ALT (U/L)	6.06±2.05	2.12±0.67	3.17±0.31	4.81±0.65
AST (U/L)	49.08±3.36	49.72±0.25	49.47±2.90	42.66±2.28
	Male			
	Water	MAAE	MAAE	MAAE
	10mL/Kg	51.2 mg/kg	256 mg/kg	1280 mg/kg
GLU (g/dL)	48.05±9.88	85.96±6.60*	106.4 ±5.43***	82.65±8.57*
ALB (g/dL)	1.71±0.14	1.44±0.03	1.62±0.08	1.50±0.01
CHOL (mg/dL)	66.19±0.56	67.48±3.20	76.47±6.70	90.36±3.79**$^{\alpha}$
ALT (U/L)	14.84±5.96	6.71±0.65	6.42±1.48	16.44±1.18$^{\alpha}$
AST (U/L)	42.15±2.04	42.21±2.00	41.78±0.82	46.86±2.87

MAAE= *Miconia albicans* aqueous extract. GLU= glucose. ALB= albumin. CHOL= total cholesterol. ALT= alanine aminotransferase. AST= aspartate aminotransferase. The animals received water (control group) or MAAE by single-dose oral. The biochemical parameters of female and male were registered in the end of experiment, on the fourteenth day. The results were expressed with mean ± EPM. ANOVA, and then Bonferroni test (n = 4/group). * $p<0.05$; ** $p<0.01$; *** $p<0.001$, when compared with control. $^{\alpha}p<0.05$, when compared with MAAE 1280 mg/kg female.

5. CAPÍTULO 2 – Avaliações das atividades antibacterianas e antibiofilmes de extratos de *Miconia albicans.*

Apresentação de resultados.

5.1. INTRODUÇÃO

Os biofilmes são caracterizados como um sistema microbiano que se adere a uma superfície biótica ou abiótica e produz uma matriz com a capacidade de proteger a colônia de fatores ambientais. Geralmente, as infecções bacterianas agudas estão relacionadas com bactérias planctônicas, que são tratadas com antibióticos. Contudo, se as bactérias atingirem a forma de biofilme, a infecção torna-se crônica, ou piorando uma infecção pré-estabelecida, tornando o quadro mais complexo e de delicada terapia (BOROWSKI *et al*., 2018).

A bactéria gram-negativa *Pseudomonas aeruginosa* é oportunista e provoca infecções nosocomiais nas formas aguda e crônica em pacientes hospitalizados e imunocomprometidos. Apresenta-se como uma das principais bactérias com resistência a diversos antibióticos, dificuldade na erradicação dos biofilmes formados e reinfecção frequente, o que tornou a *P. aeruginosa* um alvo de pesquisa para novos fármacos (ALJALAMDEH *et al*., 2021).

Staphylococcus aureus é outro patógeno muito presente em ambiente hospitalar, conhecido como oportunista, envolvido em diversos tipos de infecções, desde a pele a endocardites. As infecções por *S. aureus* causam internações, além de maior tempo de permanência hospitalar, altos custos com medicamentos e incidência de mortes. Por ser um patógeno frequente e com resistência a diversos antimicrobianos, torna-se um alvo no rastreio de substâncias eficazes de erradicação (PINTO *et al*., 2020; REIS *et al*., 2020).

Considerando os obstáculos emergenciais de resistência bacteriana e ineficácia de classes de antibacterianos no tratamento dessas cepas, encara-se tentativas de buscar novas estratégias de combate as bactérias patogênicas. Desse modo, avaliando estudos antibacterianos envolvendo espécies do gênero *Miconia*, pode-se observar a investigação de *M. salicifolia* (BUSSMANN, *et al*., 2010), *M. ligustroides* (CUNHA *et al*., 2010) e *M. latecrenata* (RODRIGUES *et al*., 2020) frente a cepas patogênicas. No entanto, quando se busca pela espécie *Miconia albicans* não são encontradas pesquisas que analisem o potencial antimicrobiano ou antibiofilme dessa planta.

Miconia albicans é nativa de regiões tropicais, porém abundante no cerrado brasileiro. É conhecida tradicionalmente pelo nome canela de velho. Suas folhas são consumidas em forma de chá na medicina popular, indicadas principalmente para o tratamento de inflamações, artrite e

artrose. As propriedades terapêuticas dessa planta podem variar de acordo com a composição química extraída (CORREA *et al*., 2021). Em estudo anterior desse grupo de pesquisa, pode-se reconhecer a atividade anti-inflamatória e nociceptiva das folhas de *M. albicans* advindas de um extrato aquoso, verificando-se também todo perfil químico da mesma. Nesse estudo, visamos avaliar a atividade antimicrobiana e antibiofilme do extrato aquoso de *M. albicans*, como também de frações obtidas a partir do extrato, frente a cepas patogênicas de *Staphylococcus aureus* e *Pseudomonas aeruginosa*.

5.2. MATERIAL E MÉTODOS

5.2.1. Amostra vegetal e extração

As folhas de *M. albicans*, depois de secas e estabilizadas em estufa de ar circulante por 48 h em 50 °C, foram trituradas em moinho de facas, tamizadas (mesh 32; 0,50 mm) e homogeneizadas. O extrato aquoso das folhas de *M. albicans* foi produzido a partir de alíquotas de 18 g de material vegetal e adição de 500 mL de água ultrapura (MilliQ, Millipore) a 100° C por 15 min e agitando esse material a cada 5 minutos. Posteriormente, o extrato foi filtrado com papel de filtro e submetido a liofilização. Obtendo-se rendimento de 18% de extrato aquoso das folhas de *M. albicans* (EAMA).

Para a obtenção de frações, o extrato EAMA foi submetido a partição líquido-líquido. Assim, 2 g de EAMA foi solubilizado em metanol:água (6:4, v/v) e particionado com os solventes hexano, diclorometano, acetato de etila e butanol. As amostras obtidas foram concentradas em evaporador rotativo e secas por liofilização, resultando nas frações hexano (EAMA-Hx, 19,56 mg), diclorometano (EAMA-DCM, 453,78 mg), acetato de etila (EAMA-Ac, 164,86 mg), butanol

(EAMA-Bu, 140 mg) e a hidrometanólica (EAMA-Me, 161,3 mg), conforme ilustrado na **Figura1.**

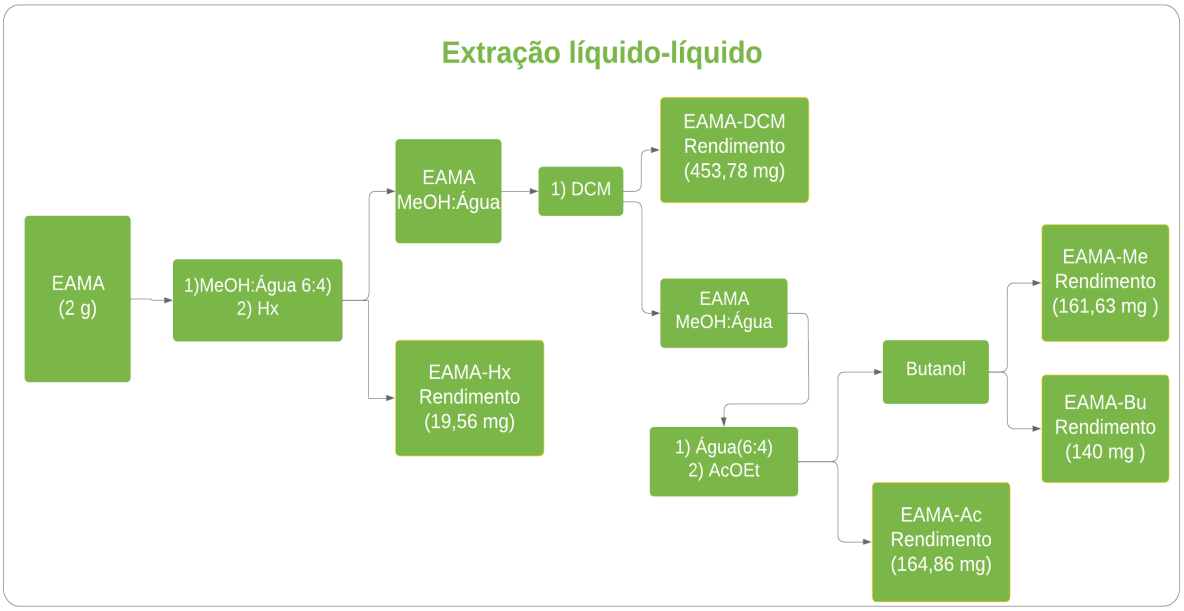


Figura 1: Obtenção de frações com solventes de polaridade crescente (hexano (EAMA-Hx), diclorometano (EAMA-DCM), acetato de etila (EAMA-Ac), butanol (EAMA-Bu) e hidrometanólica (EAMA-Me) a partir do extrato aquoso das folhas de *Miconia albicans* (EAMA).

5.2.2. Cultura de bactérias e condições

Foram utilizadas cepas de *Staphylococcus aureus* ATCC 25904 e *Pseudomonas aeruginosa* ATCC 27853 cultivadas em ágar Mueller Hinton (Oxoid Ltda., Inglaterra) a 37°C por 24h. As bactérias foram diluídas em solução estéril de NaCl 0,9% e preparadas com ajuste de densidade ótica (DO) a 600 nm de 0,150, que corresponde a 3.10^8 UFC/mL.

5.2.3. Formação de biofilme

Para a biomassa total e a viabilidade celular do biofilme foi utilizado o ensaio colorimétrico cristal violeta de acordo com a metodologia de Trentin e colaboradores (2011) com adaptações. Em uma placa de 96 poços (Costar 3599, Corning, Inc., Corning, NYC), 4µL de extrato ou frações (EAMA, EAMA-DCM, EAMA-Ac, EAMA-Bu e EAMA-Me, nas concentrações: 5, 50, 100 e 500 µg/mL) foram solubilizados em dimetilsulfóxido (DMSO) e adicionados a 76 µL de água ultrapura

(MilliQ, Millipore), 40 µL de meio TSB (*Trypcase Soy Broth*) para *Pseudomonas aeruginosa* ou BHI (*Brain Hearth Infusion medium*) para *Staphylococcus aureus* e 80 µL da suspenção bacteriana com DO a 600 nm. Como controle foram utilizados 4 µL de DMSO (veículo), 8 µL/mL de gentamicina para *P. aeruginosa* ou 8 µL/mL de vancomicina para *S. aureus* e 200 µL de meio de cultura como controle de esterilidade. Para o screening inicial foram utilizados todos os extratos e frações em todas as concentrações. A placa preparada foi incubada por 24 h em 37 °C. Simultaneamente, o crescimento bacteriano foi avaliado de acordo com a leitura de absorbância em 600 nm no tempo inicial (0 h) e no tempo final (24 h). Após a incubação e leitura da placa, o conteúdo de células planctônicas foi removido da placa, lavando-se com 200 µL de solução salina estéril (0,9 % NaCl) 3 vezes afim de remover as células não aderentes. As células aderentes foram fixadas por cristal violeta (0,4% por 15 min em temperatura ambiente), enquanto a viabilidade das células aderentes foi avaliada pelo método de MTT (0,3 mg/mL, incubada por 1 h em 37 °C), após o corante ser removido por lavagem com água, é usado 200 µL de etanol (99,5%) por 30 min para ressuspender o corante aderido. A inibição da formação de biofilme foi determinada pela leitura de absorbância em DO 600 nm. Os valores acima de 100% representam a indução do crescimento bacteriano ou da formação de biofilme comparado ao controle negativo (2% DMSO, veículo).

5.3. Resultados

Na avaliação de crescimento microbiano as amostras testadas e submetidas a leitura DO 600 nm mostraram que não houve redução no crescimento bacteriano tanto de *Pseudomonas aeruginosa* quanto de *Staphylococccus aureus.* Em relação a inibição da formação de biofilme para ambas as cepas, igualmente não foram observadas atividades. Os resultados encontram-se ilustrados nas **Figuras 2** e **3**.

Na **Figura 2,** observa-se o efeito do extrato aquoso das folhas de *M. albicans* (EAMA) e das frações EAMA-DCM, EAMA-Ac, EAMA-Me e EAMA-Bu, sobre o crescimento bacteriano e a formação de biofilme da cepa *P. aeruginosa.* O EAMA mostrou elevado crescimento bacteriano nas concentrações de 5, 50, 100 e 500 µL/mL. Quanto a formação de biofilme, EAMA em 100 µL/mL foi a amostra que apresentou maior formação de biofilme testada.

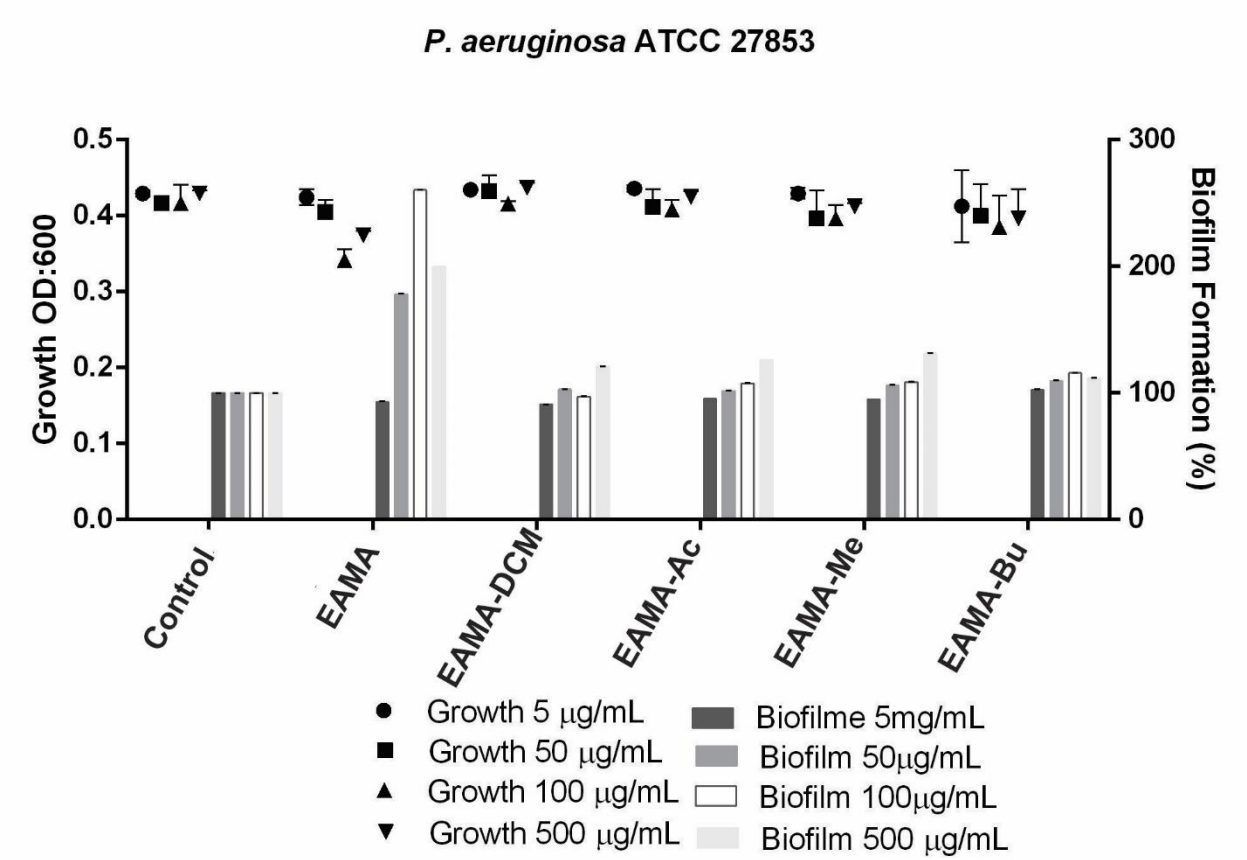


Figura 2: Efeito do extrato aquoso das folhas de *Miconia albicans* (EAMA) e frações diclorometano (EAMA-DCM), acetato de etila (EAMA-Ac), hidrometanólica (EAMA-Me) e butanol (EAMA-Bu) sobre a formação de biofilme e crescimento bacteriano da cepa *P. aeruginosa* ATCC 27853 nasas concentrações de 5, 50, 100 e 500 µg/mL.. Teste t-Student, *p < 0,05.

Na **Figura 3** verifica-se o efeito de todas as amostras testadas sobre a formação de biofilme e crescimento bacteriano de *S. aureus*. Em que, o maior crescimento bacteriano foi observado para o EAMA na concentração de 100 µL/mL. Para biofilme, o EAMA em 100 e 500 µL/mL e EAMA-Bu em 100 µL/mL foram as amostras que apresentaram maior formação de biofilme da cepa *S. aureus.*

As amostras testadas frente ambas as cepas, não podem ser consideradas ativas quanto as atividades antimicrobiana e antibiofilme, uma vez que não houve inibição de crescimento bacteriano tão quanto de biofilme. Pelo contrário ao esperado, houve crescimento bacteriano e formação de biofilme.

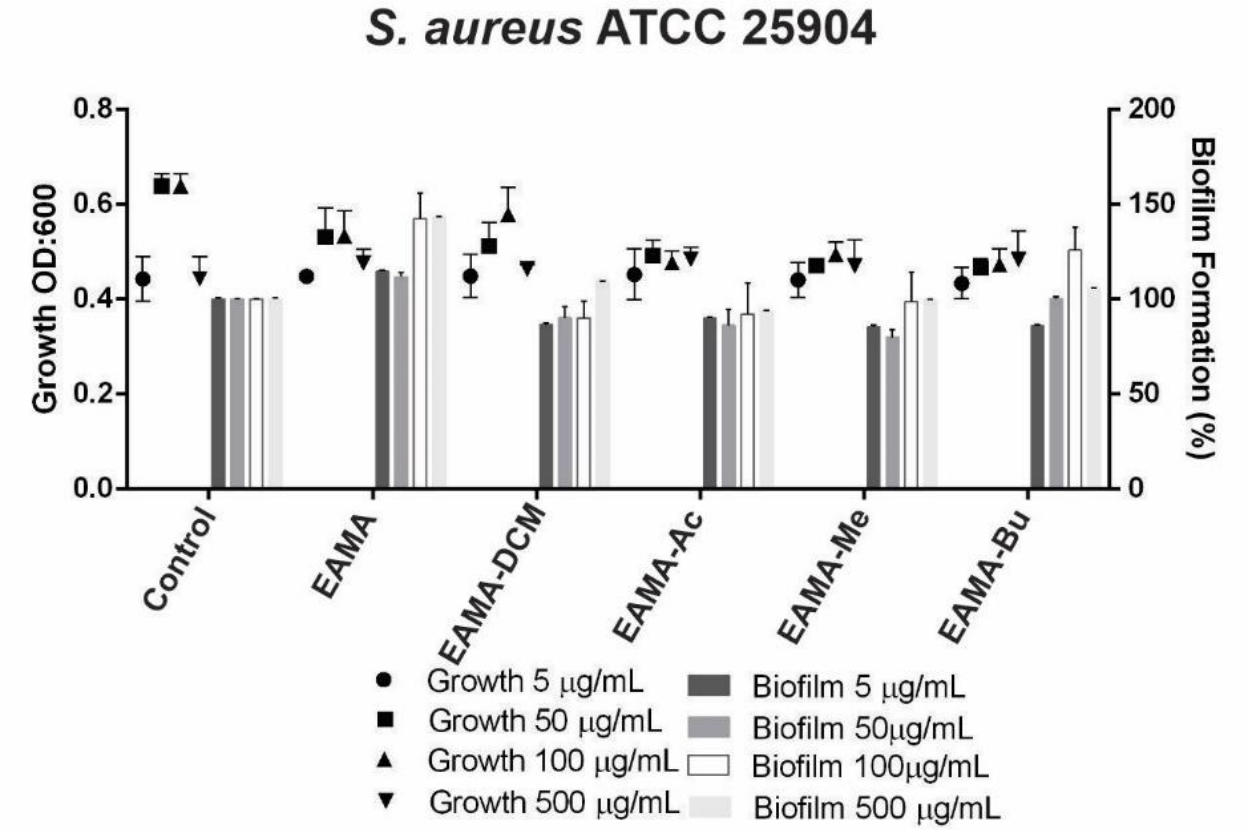


Figura 3: Efeito do extrato aquoso das folhas de *Miconia albicans* (EAMA) e frações diclorometano (EAMA-DCM), acetato de etila (EAMA-Ac), hidrometanólica (EAMA-Me) e butanol (EAMA-Bu) sobre a formação de biofilme e crescimento bacteriano de *S. aureus* ATCC 25904 nas concentrações de 5, 50, 100 e 500 µg/mL. Teste t-Student, *p < 0,05.

5.4. Discussão e conclusão

A investigação de novas estratégias para a prevenção e tratamento de infecções causadas por bactérias formadoras de biofilme é imprescindível nos dias de hoje. Essa busca é frequentemente realizada tanto a partir de produtos naturais quanto compostos sintéticos. Os produtos naturais se apresentam como um amplo leque de opções a serem testadas com grande diversidade e complexidade estrutural frente a essas bactérias especializadas, o que vem sendo comprovado pelos inúmeros trabalhos publicados na literatura e novos candidatos a fármacos desenvolvidos com base em produtos naturais (NEWMAN, CRAIGG, 2020). Esses avanços em termos de números de compostos detectados e identificados em amostras de produtos naturais e avaliados biologicamente tem relação direta com os avanços analíticos (ATANASOV *et al*., 2021).

Na literatura podemos encontrar diversos trabalhos que investigam a atividade antibacteriana com diferentes cepas patogênicas relacionadas a espécies do gênero *Miconia*, no entanto não são contemplados trabalhos que envolvem bactérias formadoras de biofilmes. O que dá abertura para investigação de novas substâncias do gênero vegetal sobre essa linha de pesquisa.

Os estudos demonstram como diferentes produtos naturais dispõe de capacidades antibiótica e antibiofilme, que tais extratos podem ser alvo de novas pesquisa de desenvolvimento farmacêutico e biotecnológico, assim como podem ser utilizados em estratégias de associação com os medicamentos já empregados na terapêutica para maior sucesso no tratamento, tentando minimizar casos de reinfecção e resistência terapêutica.

5.5. REFERÊNCIAS BIBLIOGRÁFICAS

Aljalamdeh, R., Price, R., Jones, M. D., and Bolhuis, A. (2021). The effect of particle size of inhaled tobramycin dry powder on the eradication of *Pseudomonas aeruginosa* biofilms. **European Journal of Pharmaceutical Sciences**, 158, 105680.

Atanasov, G.A., Zotchev, S.B., Dirsch, V.M. (2021). Natural products in drug Discovery: advances and opportunities. **Nature Reviews**, 20, 200-216.

Bussmann, R.W., Malca-Garcia, G., Glenn, A., Sharon, D., Chait, G., Diaz, D., Pourmand, K., Jonat, B., Somogy, S., Guardado, G., Aguirre, C., Chan, R., Meyer, K., Kuhlman, A., Townesmith, A., Effio-Carbajal, J., Frias-Fernandez, F., Benito, M. (2010). Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. **Journal of Ethnopharmacology** 132, 101-108.

Correa, J.G.S., Bianchin, M., Lopes, A.P., Silva, E., Ames, F.Q., Pomini, A.M., Carpes, S.T., Rinaldi, J.C., Melo, R.C., Kioshima, E.S., Bersani-Amado, C.A., Pilau, E.J., Carvalho, J.E., Ruiz, A.L.T.G., Visentainer, J.V., Santin, S.M.O. (2021). Chemical profile, antioxidante and anti-inflammatory properties of *Miconia albicans* (Sw.) Triana (Melastomataceae) fruits extract. **Journal of Ethnopharmacology** 273, 113979.

Cunha, W. R., G. X. de Matos, M. G. Souza, M. G. Tozatti, M. L. Andrade e Silva, C. H. Martins, R. da Silva and A. A. Da Silva Filho (2010). "Evaluation of the antibacterial activity of the methylene chloride extract of *Miconia ligustroides*, isolated triterpene acids, and ursolic acid derivatives." **Pharmaceutical Biology** 48(2): 166-169.

Dembogurski, D. S.O., Silva Trentin, D., Boaretto, A. G., Rigo, G. V., da Silva, R. C., Tasca, T., Silva, D. B. (2018). Brown propolis-metabolomic innovative approach to determine compounds capable of killing Staphylococcus aureus biofilm and Trichomonas vaginalis. **Food Research International**, 111, 661-673.

Farias, K. S., Kato, N. N., Boaretto, A. G., Weber, J. I., Brust, F. R., Alves, F. M., Carollo, C. A. (2019). Nectandra as a renewable source for (+)-alpha-bisabolol, an antibiofilm and anti-Trichomonas vaginalis compound. **Fitoterapia**, 136, 104179.

Newman, D.J. & Craigg, G.M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. **Journal of natural Products**, 83, 770-803.

Pinto, H. B., Brust, F. R., Macedo, A. J., and Trentin, D. S. (2020). The antivirulence compound myricetin possesses remarkable synergistic effect with antibacterials upon multidrug resistant Staphylococcus aureus. **Microbial Pathogenesis**, 149, 104571.

Reis, S. V. D., Couto, N. M. G., Brust, F. R., Trentin, D. S., Silva, J., Arruda, M. S. P., Macedo, A. J. (2020). Remarkable capacity of brosimine b to disrupt methicillin-resistant Staphylococcus aureus (MRSA) preformed biofilms. **Microbial Pathogenesis**, 140, 103967.

Rodrigues, L.A., Almeida, A.C., Gontijo, D.C., Salustiano, I.V., Almeida, A.A., Brandão, G.C., Ribon, A.O.B., Leite, J.P.V. (2020). Antibacterial screening of plants from the Brazilian Atlantic Forest led to the identification of active compounds in *Miconia latecrenata* (DC.) Naudin. **Natural Product Research**, 35, 24.

Trentin, D.S., Giordani, R. B., Zimmer, K. R., da Silva, A. G., da Silva, M. V., Correia, M. T., Macedo, A. J. (2011). Potential of medicinal plants from the Brazilian semi-arid region (Caatinga) against Staphylococcus epidermidis planktonic and biofilm lifestyles. **Journal of Ethnophamacology**, *137*(1), 327-335.

Von Borowski, R. G., Macedo, A. J., and Gnoatto, S. C. B. (2018). Peptides as a strategy against biofilm-forming microorganisms: Structure-activity relationship perspectives. **European Journal of Pharmaceutical Sciences**, 114, 114-137.

6. CAPÍTULO 3 – LC-MS-based untargeted metabolomics of the medicinal plant *Miconia albicans* (Swartz) Triana shows seasonal differences

Manuscrito a ser submetido para publicação.

LC-MS-based untargeted metabolomics of the medicinal plant *Miconia albicans* (Swartz) Triana (Melastomataceae) shows seasonal differences

Djaceli Sampaio de Oliveira Dembogurski[1§], André da Silva Nunes[1§], Amanda Galdi Boaretto[1], Flavio Macedo Alves[2], Denise Brentan da Silva[1*]

[1]Laboratory of Natural Products and Mass Spectrometry (LaPNEM), Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.

[2]Laboratory of Botany, Biosciences Institute (INBIO), Federal University of Mato Grosso do Sul, Mato Grosso do Sul, Brazil.

[§]Author with same contribution

* Corresponding author: Laboratory of Natural Products and Mass Spectrometry, Faculty of Pharmaceutical Sciences, Food and Nutrition (FACFAN), Federal University of Mato Grosso do Sul, Av. Costa e Silva, s/nº, 79070-900, Campo Grande, Mato Grosso do Sul, Brazil. Tel.: +55 (67) 3345-7366.

E-mail address: denise.brentan@ufms.br (D.B. Silva).

Abstract

Miconia albicans (Swartz) Triana (Melastomataceae) is an ethnopharmacological species relevant in popular medicine of Brazil, which is used to treat inflammation and pain. It can be found in Biome Cerrado. In this context, it was here explored the influence of dry and rainy season in the variation of secondary metabolites from *M. albicans* by metabolomics and chemometric tools. The leaves of *M. albicans* were collected during two years and submitted to analyses by HPLC-DAD-MS techniques and application of statistical analysis. Twenty-five compounds were annotated, including flavonoids, megastigmanes, tannins, and triterpenes. The higher ion intensities of flavonoids and megastigmanes were observed in dry period, while tannins were higher in the rainy season. The variations detected emphasize the interference of temporal variables in the chemical profile of plants.

Keywords: megastigmane, flavonoid, hydrolysable tannin, dry season, rainy season.

6.1. Introduction

Miconia albicans (Swartz) Triana, a medicinal species of Melastomataceae family, has a cosmopolitan distribution. It is native to the Brazilian Cerrado that is a biome with two well-characterized seasons, a dry and rainy season (LIMA *et al*., 2020). This plant is popularly known as “canela de velho" and used to treat inflammation, joint pain, infections, and arthritis in the traditional medicine, representing an important vegetal drug widely used by population that can be easily found in the brazilian markets (SERPELONI *et al*., 2011). Besides, this plant is widely investigated for several biological activities (CORREA *et al*., 2021), but few studies have attempted to understand the influence of the seasonal variation on the secondary metabolism of it.

The chemical composition of medicinal plants can be affected by biotic and abiotic factors. These factors include seasonality, altitude, temperature, rainfall, age, UV radiation, atmosphere composition, herbivory, and pathogen attack, which can impact the qualitative and quantitative chemical composition of a plant (GOBBO-NETO, LOPES, 2007). Thus, the period/time of harvest can significantly change the chemical composition and impact the therapeutic value of a medicinal plant-extract.

The effects of seasonal, environmental and geographic intervention of *M. albicans* and *M. chamissois*, were evaluated by identification and quantification of flavonoids (rutin, quercetin, matteucinol, miconioside) and triterpenes (ursolic acid, oleanolic acid). Samples of leaves were collected in three different environments (dry and humid), twice a year in São Paulo State, Brazil. The highest levels of flavonoid rutin occured in *M. albicans* when collected during the rainy season. The flavanone matteucinol were detected in *M. chamissois*, mainly for plants collected in the dry season. In addition, the triterpene ursolic acid was detected when collected during the dry season in dry and humid environments, on *M. albicans* and *M. chamissois*. These findings show the influence of spatial and temporal variations in the chemical composition, emphasizing the importance of applying different metabolomics techniques to evaluate a better plant potential (GIMENEZ *et al*., 2020).

As demonstrated by Quintans-Junior and collaborators (2020), *M. albicans* leaves of Sergipe (Brazil), have a relation about chemical profile and pharmacological activities. By HPLC-DAD-ESI-MS/MS were identified twenty-three compounds between flavonoids and tannins. In relation to the biological properties, anti-arthritic, anti-inflammatory and anti-hyperalgesic assays. The tests

evaluated on carrageenan induced pleurisy in mice, showed decreased levels of TNF and IL-6, reduced knee edema induced by intra-articular injection of CFA, and reduced the hyperalgesic symptoms in mice, in 50 and 100 mg/kg of ethanolic extract. The findings were correlated with the presence of flavonols, which corroborates data from the literature.

In this sense, this work explores a set of data collected, analyzing the influence of rainfall, temperature, air humidity, and wind on the presence of secondary metabolites, temperature and light of *Miconia albicans* individuals.

6.2. Materials and methods

6.2.1. Plant material

Leaves of *Miconia albicans* were collected in the Private Natural Heritage Reserve (latitude 20°50'75.25", longitude 54°61'85.30") localized in Federal University of Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brazil. This reserve is an urban fragment of Brazilian Savanna. The collections started in July 2018 until February 2020, four individuals were collected once per month, in the afternoon, during a period of ten months. Leaves were collected, at the same stage of development, from different points of each individual. After collection, plant material was stabilized by drying in a circulating air oven (50 ºC), for 48 hours, powered by a knife mill and stored at room temperature for later extraction and chemical analysis.

The species was identified by Dr. Flavio Macedo Alves and a voucher was deposited at CGMS herbarium of Federal University of Mato Grosso do Sul at number CGMS 79407, under authorization by SISGEN (National Management System Genetic Heritage and Associated Traditional Knowledge) with protocol number of registration ACD1712.

6.2.2. Climate information

Climatic data the months of collection, to July 2018 from February 2020, was acquired at the available online database Mato Grosso do Sul Weather and Climate Monitoring Center (Centro de

Monitoramento do Tempo e do Clima de Mato Grosso do Sul, CEMTEC-MS). Were acquired the data of instantaneous temperature (IT), gust of wind (GW), minimum temperature (MAINT), rainfall (RA), days without rain (DWR), maximum temperature (MAXT), maximum humid (MAXH), minimum humid (MINH), instantaneous humid (IH), for each month. Environmental data were used to assess how the months are grouped within Cerrado seasons.

6.2.3. Sample preparation

The extracts from leaves were obtained with 10 mg of powdered plant and extracted using 1 mL of methanol and ultrapure water 7:3 (v/v) in an ultrasonic bath for 10 min (De Vos *et. al*., 2007). Subsequently, the extracts were centrifuged at 10.000 rpm for 3 min, the supernatant was removed and filtered with PTFE filter (Millex 0.22 µm, Millipore). A pool (quality control) was prepared from the mixture of 25 µL of each sample, homogenized, and injected at each five samples. For the injections, the samples were randomized.

6.2.4. Analyses of extracts by liquid chromatography coupled to diode array detector and mass spectrometry (LC-DAD-MS):

Chromatographic analyses were performed on an UFLC LC-20AD Shimadzu Prominence coupled to a diode array detector and a high-resolution mass spectrometer with electrospray ionization source (MicrOTOF-Q III – Bruker Daltonics, Billerica, MA, USA). The analytical column used was a Kinetex C18 (2.6 µm, 100A, 150 x 2.1 mm, Phenomenex), which was maintained at 50 °C and a flow rate of 0.3 mL/min was applied. Acetonitrile (B) and as solvent B and ultrapure water (B), both added formic acid 0.1% (v/v), were used as mobile phase and the following elution gradient program was used: 0-2 min – 3% of B, 2-25 min – 3 to 25% of B, 25-40 min – 25 to 80% of B, and 40-43 min 80% of B. The MS analyses were performed in negative ion mode for the data processing and this method was selected from the initial analysis of pool in positive and negative ion mode, since the negative ion mode revealed a higher number of peaks. For these analyses, N_2 was applied as nebulizer gas (4 Bar), collision gas, and dry gas (9 L/min). The capillary voltage was 2.500 kV.

6.2.5. Data proceeding

Firstly, the data were analyzed in the software “DataAnalysis 4.2” (Bruker Daltonics). The raw data were aligned by “Metalign 3.0” (LOMMEN, KOOLS, 2012), resulting in 1003 entrances

and, subsequently, the data was reduced by "MSClust" (TIKUNOV *et al*., 2012). After that, duplicate peaks and with intensity less than 750 were removed, gerating 22 compounds. The resulting data matrix was used in the statistical analysis in the "software R V.4.1.3" (R CORE TEAM, 2022).

To assess the interrelationship between the samples, we performed a Principal Components Analysis (PCA). The PCA was performed using the "stats" package and the graphs plotted using the "factoextra" package in the "software R". Peak data were transformed by logarithm (log_{10}) for further calculation of components. Analyzes of the effects of seasonality on the intensity of metabolites were performed using the permutational analysis of variance (PERMANOVA) based on Bray Curtis dissimilarities.

To evaluate the influence of seasonality on classes or metabolites, we used the Wilcoxon signed-rank test from the "rstatix" package. The p threshold considered was 0.05. Box-plots were plotted to evaluate the dispersion of peaks or classes.

6.2.6. Annotation of the compounds

The annotation of the compounds were performed by spectral data (UV, MS, and MS/MS) compared to data published (DEMBOGURSKI *et al*., 2023) and deposited in GNPS. The accurate MS data were applied to determine the molecular formula, considering errors and mSigma up to 8 ppm and 30, respectively. The injection of some authentic standards were also performed to confirm the identity of them.

6.3. Results and discussion

6.3.1. Annotation of the constituents from *Miconia albicans*

The annotation of the compounds applied in the statistical analyses were described in the **Table 1**, and their spectral data were also summarized. The annotated compounds and monitored were twenty-five, which included O-glycosylated flavonols, triterpenes, hydrolysable tannins, megastigmanes, ellagic acid and its derivatives.

The compounds **1** and **2** were putatively annotated as di-*O*-hexoside and citric acid, which presented intense deprotonated ions at *m/z* 341.1061 ($C_{12}H_{22}O_{11}$) and 191.0229 ($C_6H_8O_7$), in addition peak **2** was confirmed by the authentic standard. The peak **8** revealed an absorption band at 280 nm in the UV spectrum, compatible with chromophore flavan-3-ol, and ion at *m/z* 289.0709 ($C_{15}H_{14}O_6$), which was confirmed by the authentic standard as epicatechin.

The peaks **3** and **7** revealed absorption bands at λ_{max} 280 nm and the deprotonated ions at *m/z* 933.00627, 947.0811, 915.0478, and the double charged ion *m/z* 602.0252. These compounds revealed fragmentation patterns of hydrolysable tannins typically of elagitannins, which was confirmed by the product ion at *m/z* 301 that are basically composed of ellagic acid yielded from losses of hexahydroxydiphenyl units (HHDP) with subsequently cyclization reactions on the mass spectrometer (Cerulli *et al.,* 2020). A The compounds **3, 4**, **5,** and **7** were annotated as nonahydroxytriphenoy (NHTP)-hexahydroxydiphenoyl (HHDP)-hexoside (vescalagina or castalagin isomer), *O*-methyl NHTP-HHDP hexoside, and C-procyanidin NHTP-HHDP-hexoside, which revealed spectral data compatible with the data described in the literature (CERULLI *et al.,* 2020, RASINES-PEREA *et al*., 2019).

The peaks **9** and **10** reported no UV absorption, and their protonated ions at *m/z* 387.1992 and 391.2295 exhibited the molecular formula $C_{20}H_{32}O_{10}$ and $C_{20}H_{36}O_{10}$. They showed losses of an *O*-hexosyl group yielding the fragment ions *m/z* 207 $[M+H-162-H_2O]^+$ and 229 $[M+H-162]^+$. These fragments are relative to aglycones with thirteen carbons and compatible to megastigmanes (JO *et al*., 2020), which were annotated as dihydroxy-megastigmadienone *O*-hexoside (roseoside) and trihydroxymegastigmanene *O*-hexoside (CHANG *et al*., 2020; SPÍNOLA *et al*., 2015). The peaks **14** and **16** showed UV spectrum at $\lambda_{max} \approx 280$ nm, deprotonated ions at *m/z* 537.1966 and 541.2277 that were compatible to the molecular formulas $C_{26}H_{34}O_{12}$ and $C_{26}H_{38}O_{12}$. The fragment ions at *m/z* 385 $[M-H-galloyl]^-$ and 371 $[M-H-galloyl-H_2O]^-$ suggested the presence of galloyl substituent for **14** and **16**, respectively (WEI *et al*., LI *et al*., 2015).

The chromatographic peaks **12**, **13**, **15**, and **17** showed two absorption bands in the UV spectrum at $\lambda_{max} \approx 270$ and 350 nm that are aligned to flavonols. The losses of 162 and 146 *u* indicated the *O*-hexosyl and *O*-deoxyhexosyl substituents, respectively (Silva *et al*., 2013). According to

Younis *et al*., (2020), the fragment ion at *m/z* 303 was attributed to quercetin. Thus, they were annotated as *O*-hexosyl quercetin (**12** and **15**), rutin (**13**), and *O*-deoxyhexosyl quercetin (**17**).

The UV spectra of **18** and **19** revealed two absorption bands similar to ellagic acid chromophore (with $\lambda_{max} \approx$ 250 nm and 370 nm) (REICHERT *et al*., 2018). From the protonated ions *m/z* 345 of **19**, the fragment ions at *m/z* 330, 315 and 300 were observed, which are yielded from subsequent losses of radical methyl (15 *u*) (REICHERT *et al*., 2018; MOILANEN *et al*., 2013). From the deprotonated ion *m/z* 461 (**18**) was observed a loss of deoxyhexosyl (146 *u*) and subsequent loss of a radical methyl (15 *u*) to yield the fragment ion at *m/z* 300. Thus, the compounds **18** and **19** were annotated as *O*-deoxyhexosyl *O*-methyl ellagic acid and tri-*O*-methyl ellagic acid.

The compounds **20** and **21** revealed the deprotonated ions at *m/z* 711.3980 $[M-H+HCOOH]^-$, which were compatible to the molecular formula $C_{36}H_{58}O_{11.}$ The fragment ions at *m/z* 503 are yielded from losses of 208 *u*, associated to hexosyl and formic acid (HCOOH), indicating the aglycone triterpenic. Therefore, these compounds were annotated as *O*-glycosylated triterpenes (Gao *et al*., 2011). The peak **23** showed no UV absorption, the deprotonated ion at *m/z* 487.3431 $[M+H-]^-$ and molecular formula $C_{30}H_{48}O_5$ from the of 409 *u*, was assigned arjunolic acid (CORREA *et al*., 2021).

Table 1. Compounds annotated from *Miconia albicans* leaves by LC-DAD-MS.

Peak	RT (min)	MF	Compound	Class	UV (max)	Negative (*m/z*)		Positive (*m/z*)	
						MS [M-H]-	MS/MS	MS [M+H]+	MS/MS
1	1.2	$C_{12}H_{22}O_{11}$	di-*O*-hexoside		-	341.1061	-	-	-
2	1.5	$C_6H_8O_7$	Citric acid*		-	191.0229	-	-	-
3	3.6	$C_{41}H_{26}O_{26}$	NHTP-HHDP-hexoside (vescalagin or castalagin isomer)	Tannin	282	933.0615	467, 421, 300, 275, 257, 231	935.072	469, 439, 307, 277
4	5.2	$C_{41}H_{26}O_{26}$	NHTP-HHDP-hexoside (vescalagin or castalagin isomer)	Tannin	280	933.0615	467, 421, 300, 275, 257, 231	935.0731	615, 495, 469, 439, 307, 277

Peak	RT (min)	MF	Compound	Class	UV (max)	Negative (*m/z*)		Positive (*m/z*)	
						MS [M-H]-	MS/MS	MS [M+H]+	MS/MS
5	7.1	$C_{42}H_{28}O_{26}$	*O*-methyl NHTP-HHDP-hexoside (*O*-methyl vescalagin or castalagin isomer)	Tannin	280	947.0811	493, 467, 421, 301, 275, 203	949.0886	-
6	7.6	$C_{41}H_{24}O_{25}$	Hydrolyzable tannin	Tannin	280	915.0478	457, 169	-	-
7	10.3	$C_{56}H_{38}O_{31}$	C-procyanidin NHTP-HHDP-hexoside	Tannin	280	602.0252	493, 467, 301, 289, 275, 249	-	-
8	13.2	$C_{15}H_{14}O_6$	Epicatechin*	Flavan-3-ol	280	289.0709	163	291.0861	-
9	14.6	$C_{20}H_{32}O_{10}$	Dihydroxy-megastigmadienon	Megastigmane	-	385.1894	205, 153	387.2031	207, 189, 161

Peak	RT (min)	MF	Compound	Class	UV (max)	Negative (*m/z*)		Positive (*m/z*)	
						MS [M-H]-	**MS/MS**	**MS [M+H]+**	**MS/MS**
			e *O*-hexoside (roseoside)						
10	18.2	$C_{20}H_{36}O_{10}$	Trihydroxymegastigmanene *O*-hexoside	Megastigmane	-	389.2173	189	391.234	229, 211, 193, 175
11	18.5	$C_{14}H_6O_8$	Ellagic acid*	Ellagic acid derivative	250, 365	300.999	283, 245, 229, 217, 201, 185, 173, 161	303.0172	
12	19.3	$C_{21}H_{20}O_{12}$	Quercetin *O*-hexoside	Flavonoid	270, 356	463.086	300, 271, 255, 243, 179, 151	465.1003	303
13	19.4	$C_{27}H_{30}O_{16}$	Rutin*	Flavonoid	270, 352	609.1455	463, 301, 151	677.1591	465, 303

Peak	RT (min)	MF	Compound	Class	UV (max)	Negative (*m/z*)		Positive (*m/z*)	
						MS [M-H]-	MS/MS	MS [M+H]+	MS/MS
14	19.5	$C_{26}H_{34}O_{12}$	Mallophenol B	Megastigmane	280	537.1966	385, 313, 271, 211, 169	539.2107	-
15	19.8	$C_{21}H_{20}O_{12}$	Quercetin *O*-hexoside	Flavonoid	270, 356	463.086	300, 271, 255, 151	465.1003	303
16	21.2	$C_{26}H_{38}O_{12}$	Clypearoside A	Megastigmane	280	541.2277	371, 363, 331, 313, 271, 253, 227, 169	543.2459	
17	22.1	$C_{21}H_{20}O_{11}$	Quercetin *O*-deoxyhexoside	Flavonoid	262, 350	447.091	300, 271, 255, 163	449.1057	303, 257, 229
18		$C_{21}H_{18}O_{12}$	O-deoxyhexosyl *O*-methyl ellagic acid	Ellagic acid derivative	265, 360	461.0727	315, 300	463.091	-
19	25.4	$C_{17}H_{12}O_8$	Tri-*O*-methyl ellagic acid	Ellagic acid derivative	219, 365	343.0442	311, 298	345.0585	330, 315, 300, 285

Peak	RT (min)	MF	Compound	Class	UV (max)	Negative (*m/z*)		Positive (*m/z*)	
						MS [M-H]-	MS/MS	MS [M+H]+	MS/MS
20	30.7	$C_{36}H_{58}O_{11}$	*O*-hexosyl triterpene	Triterpene	-	711.3984	503	689.3878	505, 487, 469, 451, 439, 405, 261, 215, 187
21	30.8	$C_{36}H_{58}O_{11}$	*O*-hexosyl triterpene	Triterpene	-	711.398	503	689.3878Na	505, 487, 469, 451, 439, 405, 261, 215, 187
22	31.7	$C_{36}H_{58}O_{10}$	*O*-hexosyl triterpene	Triterpene	-	695.4032	487	-	-
23	35.5	$C_{30}H_{48}O_5$	Triterpene (arjunolic acid)	Triterpene	-	487.3431	409	-	-
24	39.6	$C_{28}H_{44}O_{11}$	Unknown	-	-	555.2847	299, 255, 225, 206, 164	-	-

Peak	RT (min)	MF	Compound	Class	UV (max)	Negative (*m/z*)		Positive (*m/z*)	
						MS [M-H]-	MS/MS	MS [M+H]+	MS/MS
25	39.6	$C_{25}H_{46}O_{10}$	Fatty acid derivative	Fatty acid	-	505.3024	255, 249	-	-

RT: retention time; MF: molecular formula; *confirmed by authentic standard. HHDP: hexahydroxydiphenoyl; NHTP, nonahydroxytriphenoyl

6.3.2. Metabolomics analyses from *Miconia albicans* correlated to climatic factors

First, we analyze the climatic factors to verify how the dry and rainy periods are distributed according to the harvests of plant materials used in our study. The climatic data were obtained from the registers of the climatic monitoring center (CEMTEC-MS) and these data were ordinated by Non-Metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarities. As a result, we observed that December 2018 (dec-18) presented low rainfall (RA) and many days without rain (DWR), consequently this month was grouped with the dry season (**Figure 1**), although December is classified into the rainy season (RATTER *et al*., 1997) in the literature. These observations demonstrated the importance to collect real data to classify the climatic factors and to apply them in the study. Additionally, October 2018 (oct-18) grouped with the rainy months due to a high rainfall registered, while October 2019 (oct-19) was a dry period due to many days without rain were registered and low rainfall. This is a transitional month between the dry and rainy season, so a variation of climatic conditions in this month is normal. Therefore, from the multivariate analysis of climatic data we classified August 2018 (aug-18), December 2018 (dec-18), June 2019 (jun-19), August 2019 (aug-19), and October 2019 (oct-19) as dry season, and the rainy season was constituted by the months October 2018 (oct-18), February 2019 (feb-19), April (apr-19), November 2019 (nov-19), and February 2020 (feb-20).

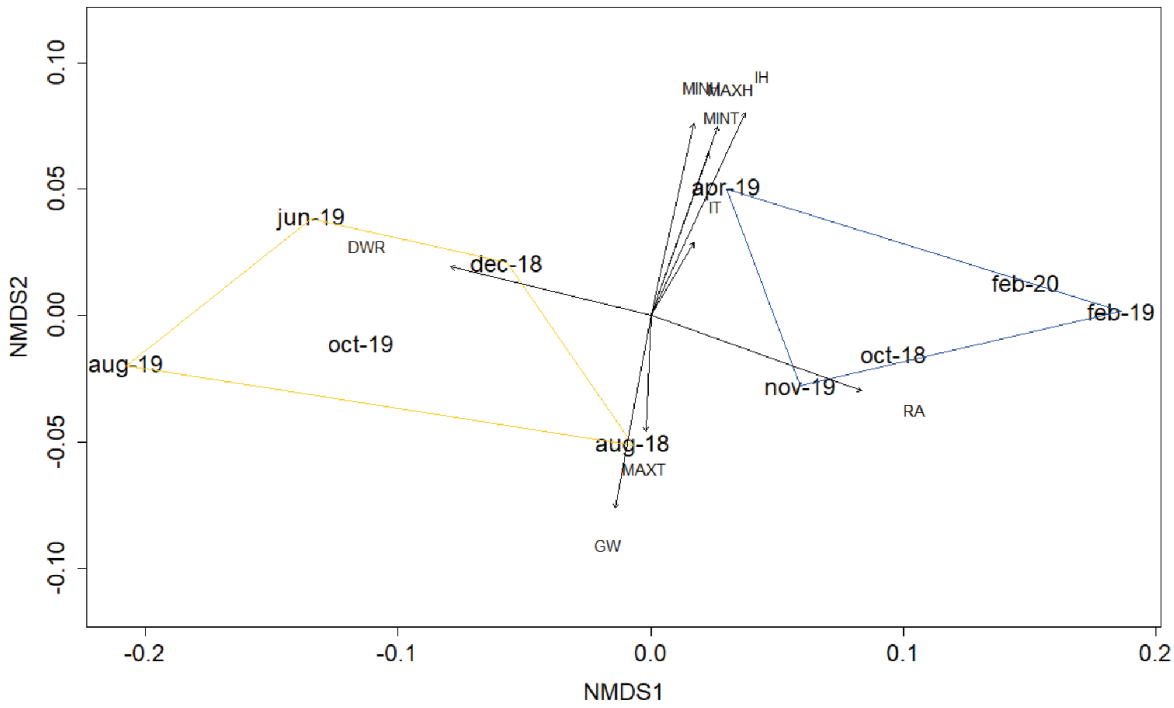
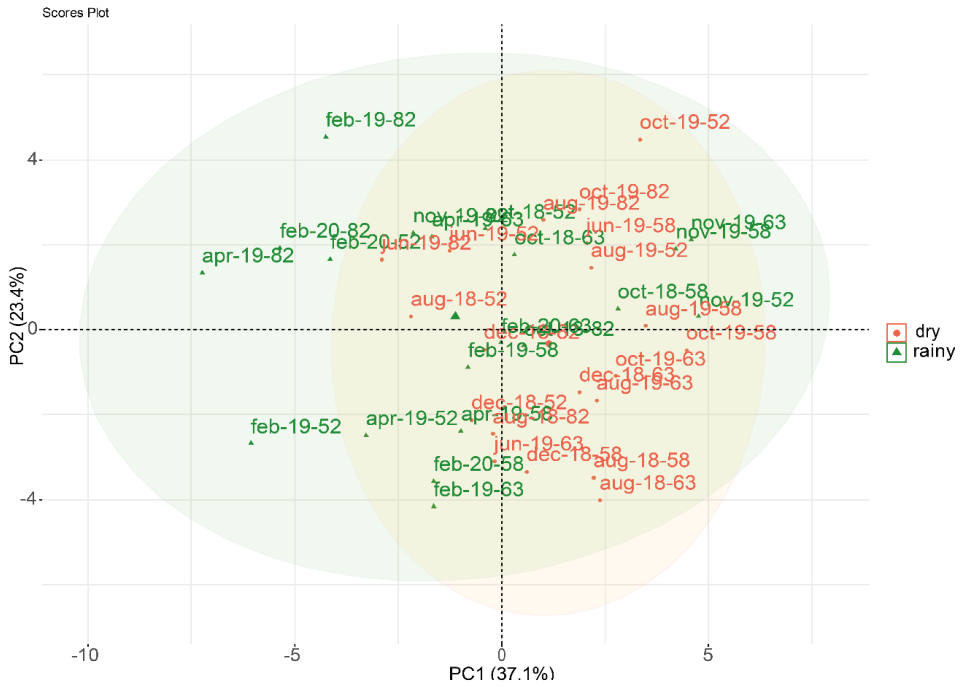


Figure 1 - NMD (stress = 0.02337093) of the environmental data of the collection period. The ordering suggests seasonal variation. Inserted variables: instantaneous temperature (IT), gust of wind (GW), minimum temperature (MAINT), rainfall (RA), days without rain (DWR), maximum temperature (MAXT), maximum humid (MAXH), minimum humid (MINH), instantaneous humid (IH).

The chemical data were analyzed by multivariate statistics such as by the unsupervised Principal Component Analysis (PCA) to observe interrelationships between the sample group regarding the secondary metabolites present in *M. albicans* individuals collected in dry and rainy seasons. However, the PCA showed no clear separation of groups related to the variables inserted, but we noted a tendency to divide individuals related to the groups of dry and rainy season (**Figure 2A**). The strong variables observed from biplot were, for example, the peaks **7, 8**, **9**, **10**, and **14 (Figure 2B**). It is important to notice that in the Cerrado there are two distinct seasons, but the group separation based on the secondary metabolites from the plants collected in all the months was not clear, which was verified as a seasonal graduation. Seasonality, as an independent factor when considered all collection months, was significant (PERMANOVA, F=4.3327, R_2=0.10235, P=0.005). However, this explains a small variation in the intensity of the metabolites (R_2=0.10235).

A



B

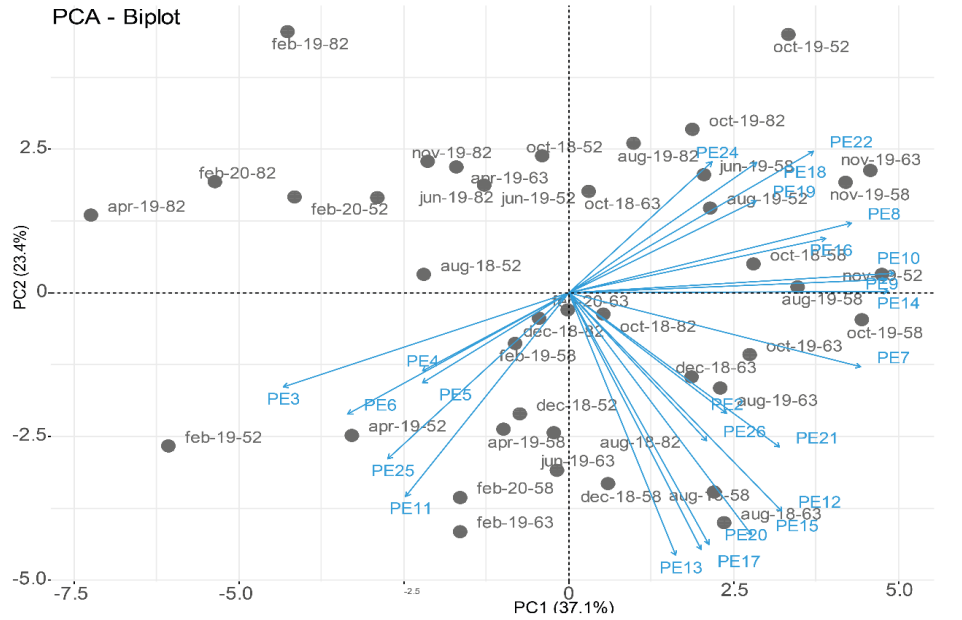


Figure 2 - (A) Principal component analysis (PCA) from the samples of *Miconia albicans* analyzed by LC-MS/MS that were collected in different months of rainy (oct-18, feb-19, feb-20, apr-19, and nov-19) and dry season (aug-18, dec-18, oct-19, jun-19, and aug-19). (B) Biplot of principal component 1 (PC1) and principal component 2 (PC2). Months: february (feb), april (apr), june (jun), october (oct), november (nov), and december (dec). Years: 2018 (18), 2019 (19), and 2020 (20). Individuals: 53, 58, 63, and 82.

Although the interrelationship between the samples does not show a multivariate separation between the samples, we performed the Wilcoxon signed-rank test to verify if the classes of metabolites are distinct between dry and rainy samples, adding the metabolites intensities and comparing this total of each compound class (**Figure 3).** In terms of this, the classes of flavonoids, tannins, and triterpenes showed no statistically significant differences. On the other hand, the megastigmanes were significantly distinct between the seasons ($p<0.05$), presenting higher intensity in the dry season**.**

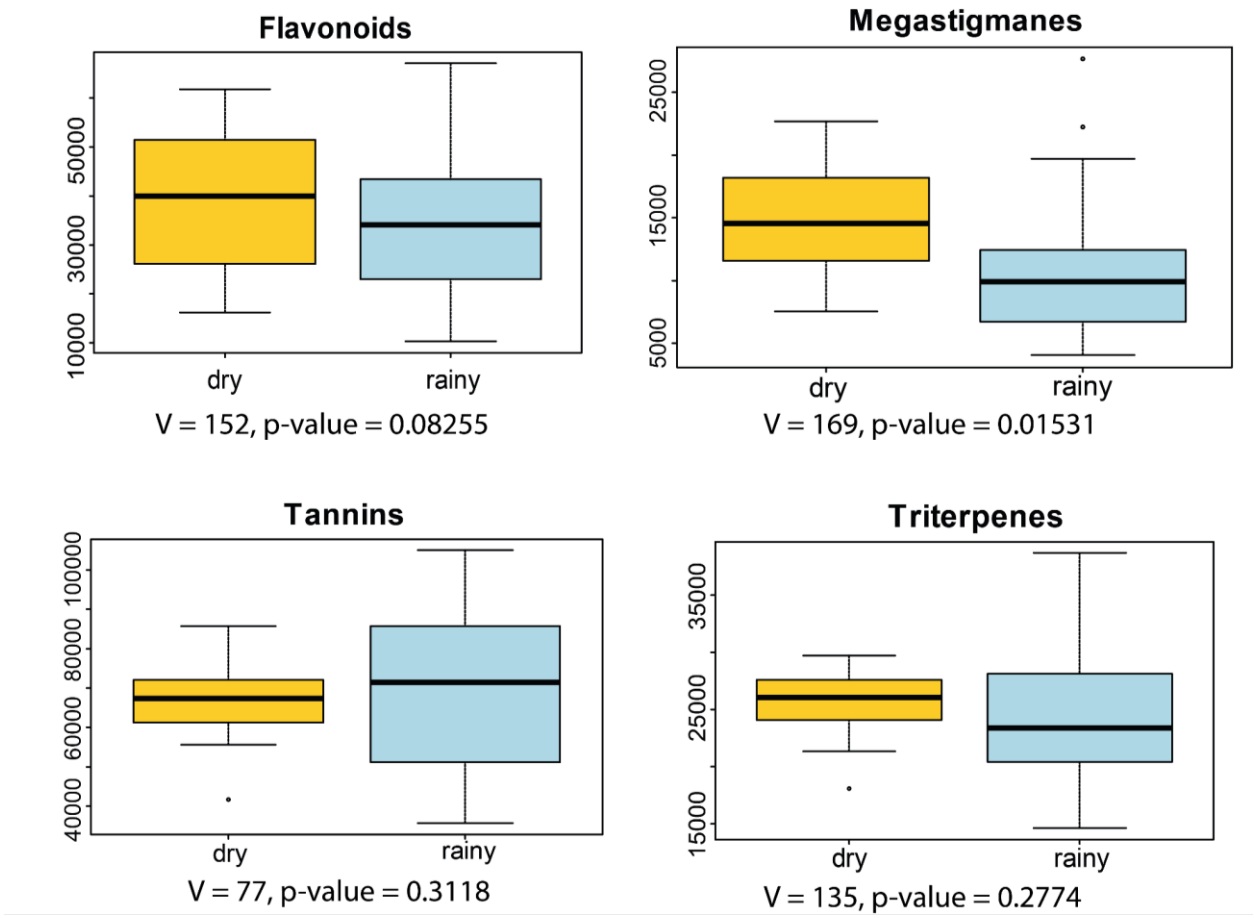


Figure 3 - Boxplot of classes (flavonoids, megastigmanes, tannins, and triterpenes) in relation to the dry and rainy season from the all samples (rainy season: oct-18, feb-19, feb-20, apr-19, and nov-19; dry season: aug-18, dec-18, oct-19, jun-19, and aug-19).

The only samples collected in the driest and rainiest months were analyzed together to determine if the seasonality can be an influence factor on the secondary metabolites of *M. albicans*. The driest season was considered aug-18, aug-19, and oct-19, while the rainiest season was feb-19, apr-19, and feb-20, according to the ordinated data (NMDS). In this way, we obtained a PCA from only these samples, which revealed a distinction between the samples of dry and rainy season. The PC1 explained 46.7% and PC2 26.4% of these differences, and the strong variables observed in biplot for the separation of dried season samples were the peaks **7** (*O*-methyl NHTP-HHDP-hexoside), **8** (epicatechin), **9** (dihydroxy-megastigmadienone *O*-hexoside), **10** (trihydroxymegastigmanene *O*-hexoside), **14** (mallophenol B)**,** and **16** (clypearoside A) (**Figure 4**).

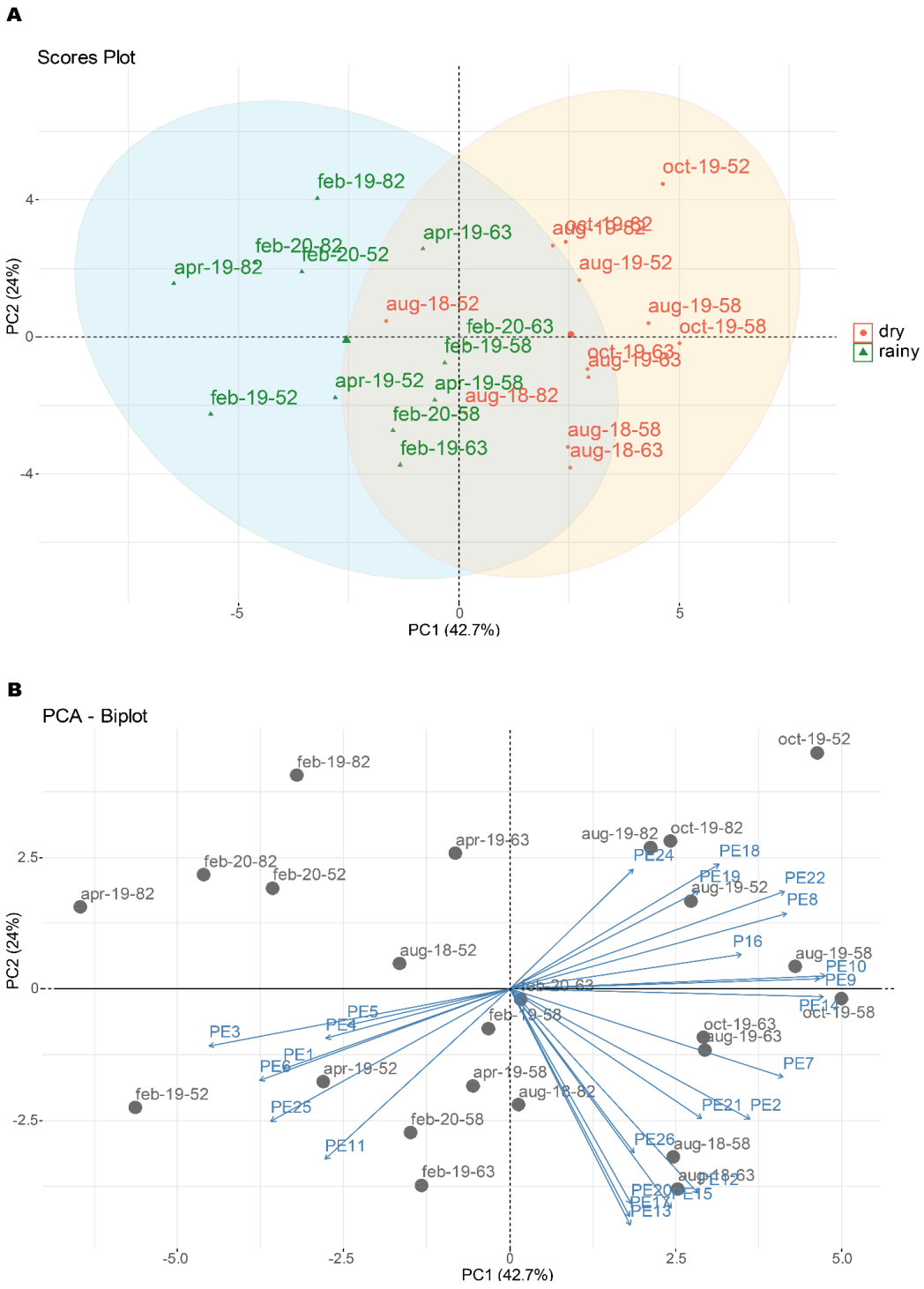


Figure 4. (**A**) Principal component analysis (PCA) from the samples of *Miconia albicans* analyzed by LC-MS/MS that were collected in different months of rainy (feb-19, feb-20, and apr-19) and dry season (aug-18, oct-19, and aug-19). (**B**) Biplot of principal component 1 (**PC1**) and principal component 2 (**PC2**). Seasonality, as an independent factor when considered all collection months, was significant (PERMANOVA, F=10.296, R_2=0.3188, P=9.999e-05), explaining about 31% of the metabolites used in processing.

From the ion intensities of all metabolites added by class and the application of the Wilcoxon test, the groups of *M. albicans* collected in driest and rainiest season presented some statistical significant differences, such as flavonoids and tannins that exhibited statistical relevance **(Figure 5**). The flavonoids revealed higher ion intensity in the dry season (p=0.034) while tannins are more intense in the rainy period (p=0.012). The megastigmanes confirmed their higher increment during the dry season (p=0.0005). However, the triterpenes showed higher ion intensities in the dry season, but they did not show valid statistical significance (p = 0.129) **(Figure 5)**. Some of these patterns can also be observed in the (**Figure 5)** in which the megastigmanes were strongly correlated with the group separation in PC1, such as the glycosylated megastigmanes **9** (dihydroxy-megastigmadienone *O*-hexoside), **10** (trihydroxymegastigmanene *O*-hexoside), and **14** (mallophenol B). The glycosylated megastigmane **14** has an additional galloyl substituent, like as the megastigmane **16**.

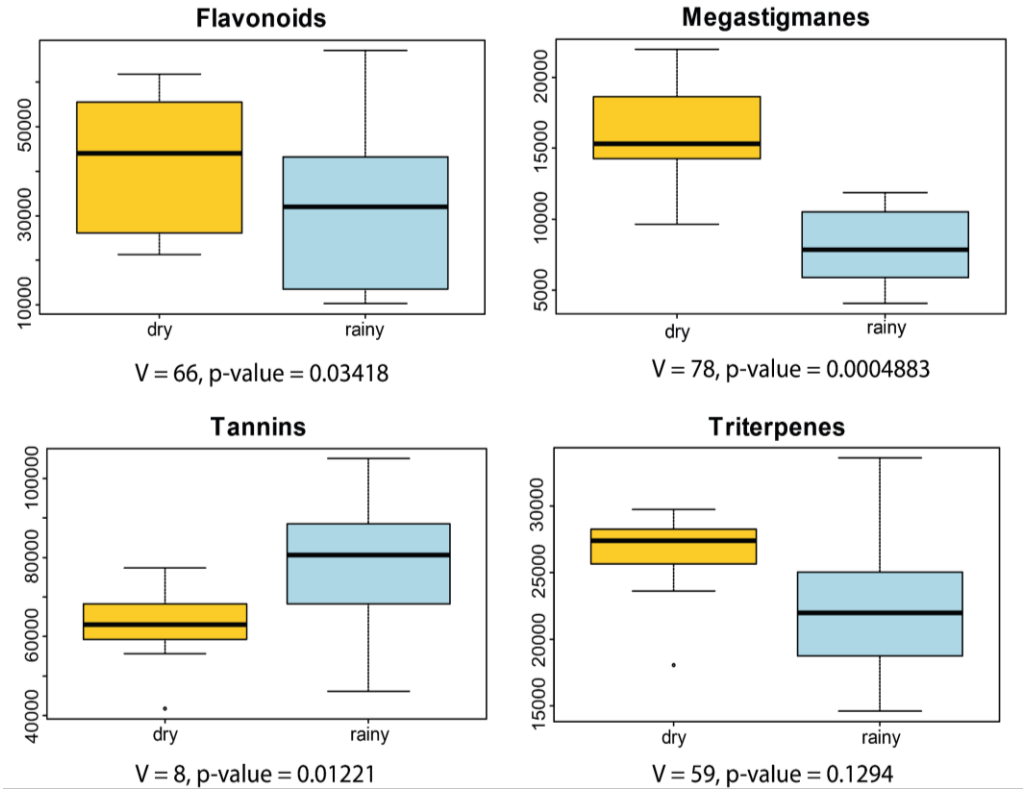


Figure 5 - Boxplot of classes of metabolites (flavonoids, megastigmanes, tannins, and triterpenes) annotated from the *Miconia albicans* samples in relation to dry and rainy season, applying the materials collected in dry (aug-18, aug-19, and oct-19) and rainy periods (feb-19, apr-19, and feb-20).

Flavonoids showed higher intensity in the dry period (p = 0.03418), observed in **Figure 5**. In plants this class of compounds acts as a protector against solar radiation, as well as giving the bitter and astringent taste, that makes the plants unattractive to herbivory, this strategy is an important characteristic of this leaves during the budding and the fruiting stage. Another functions of flavonoids in plants are the antimicrobial and growing regulations, promoting physiological survival (MAHGOUB *et al*., 2023, QIAO *et al*., 2022).

Megastigmanes exhibited high intensity in leaves collected during the dry season. This class of compounds biosynthesized via oxidative cleavage of the carotenoids (ZHAO *et al*., 2022), although this study detected for the first time these compounds in genus *Miconia.* The occurrence of megastigmanes is a chemotaxonomic marker to *Vitis* (Vitaceae) species (CONG *et al*., 2012), were used to a significantly markes to *Aleurites* (Euphorbiace) species (Silva *et al*., 2012) too. There is no mention about the seasonal variation in plants involving this class, highlighting the relation between megastigmanes and dry stress in *Miconia* genus.

Tannins are the compounds revealed largest intensity in *M. albicans* during the rainy season (p = 0.01221), illustrated in **Figure 5**. These compounds annotated from *M. albicans* are hydrolysable tannins of ellagitannin type, which are yielded from the hexoside central with units of hexahydroxydiphenoyl and additional galloyl units can be in the structures. Tannin accumulation is related to several types of stress effects such, nutrient poor soil, high solar exposure and herbivore attack, which is closely related to the cerrado biome (DEHGHANIAN *et al*., 2022). Hydrolysable tannins specifically, have importante function in defense, are more concentrated in young leaves, but low levels are sufficient to defend against herbivory (VISAKORPI *et al*., 2019).

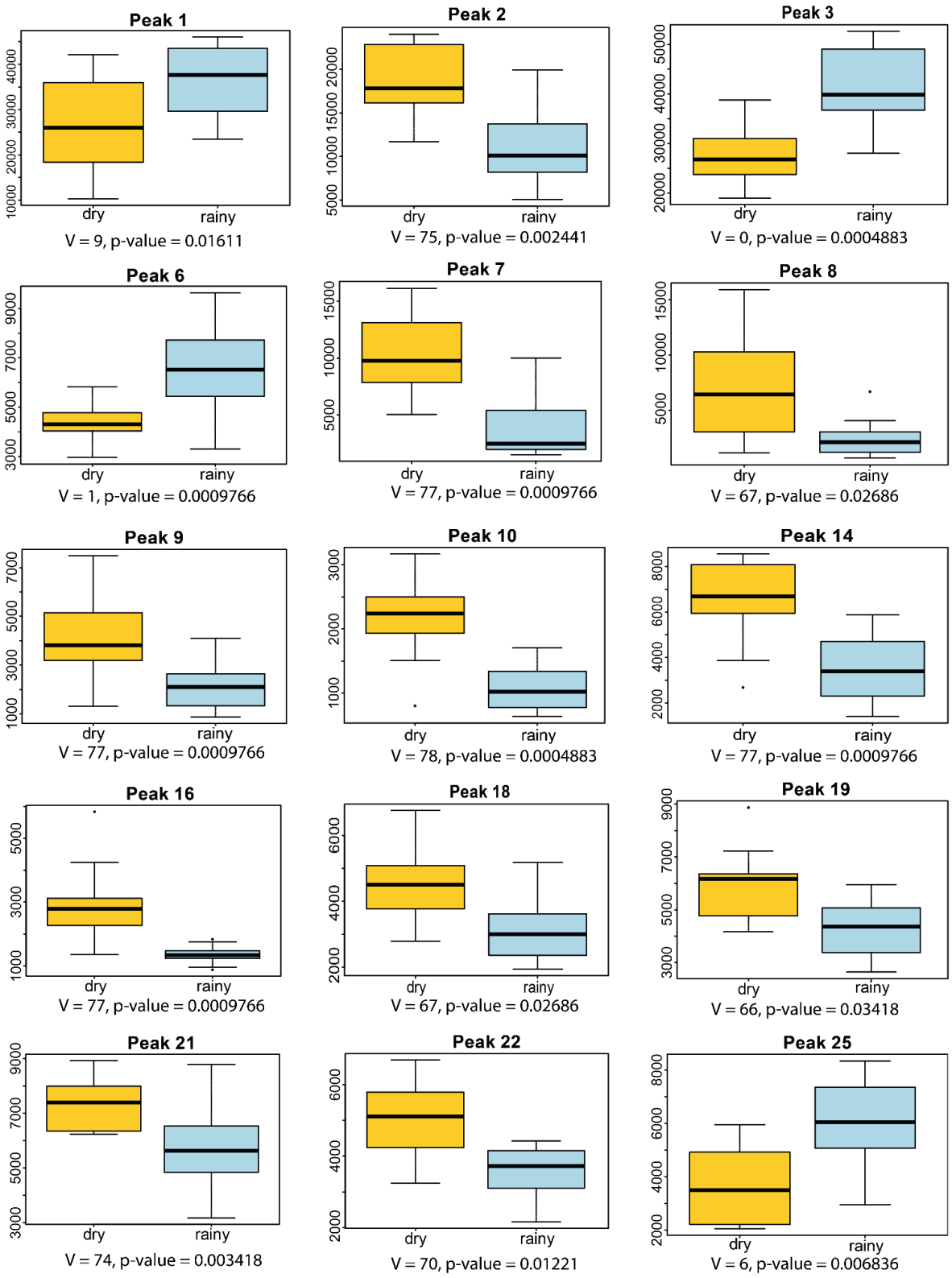


Figure 6 - Boxplots from the annotated peaks of *Miconia albicans* collected in dry and rainy season.

The boxplots from some important compounds are illustrated in **Figure 6**. The highlightly metabolites for dry season include: **2** (citric acid), **7** (C-procyanidin NHTP-HHDP-hexoside), **8** (epicatechin), **9** (dihydroxy-megastigmadienone *O*-hexoside), **10** (trihidroxymegastigmane O-hexoside), **14** (mallophenol B), **16** (clypearoside A), **18** (O-deoxyhexosyl O-methyl ellagic acid), **19** (tri-*O*-methyl ellagic acid), **21** (*O*-hexosyl triterpene) and **22** (*O*-hexosyl triterpene), while for the rainy season include **3** (NHTP-HHDP-hexoside), **6** (hydrolyzable tannin), and **25** (fatty acid derivative).

Differences were observed in leaves of *M. albicans* collected during dry and rainy season, showing that seasonality influences the composition of the plant. This is closely related to the stress caused by water deprivation, high temperatures, high solar radiation, and nutrient poor soil of Cerrado (PINTO, KOLB, 2016).

6.4. References

Cerulli, A., Napolitano, A., Masullo, M., Hosek, J., Pizza, C., & Piacente, S. (2020). Chestnut shells (Italian cultivar "Marrone di Roccadaspide" PGI): Antioxidant activity and chemical investigation with in depth LC-HRMS/MSn rationalization of tannins. **Food Research International**, 129, 108787.

Chang, S., Wang, M., Tian, Y., Qi, J., Qiu, Z., (2020). Systematic analysis and identifcation of the absorption and metabolic components of Zengye decoction in type 2 diabetic rats by HPLC-ESI-Q-TOF–MS/MS. **Chinese Medicine**, 15, 50.

Cong, H., Zhang, S., Wang, Y., Xuan, L. (2012). Megastigmane glycosides and triterpenoids from *Vitis quinguangularis* Reahd. **Biochemical Systematics and Ecology**, 45, 111-114.

Correa, J.G.S., Bianchin, M., Lopes, A.P., Silva, E., Ames, F.Q., Pomini, A.M., Carpes, S.T., Rinaldi, J.C., Melo, R.C., Kioshima, E.S., Bersani-Amado, C.A., Pilau, E.J., Carvalho, J.E., Ruiz, A.L.T.G., Visentainer, J.V., Santin, S.M.O. (2021). Chemical profile, antioxidant and anti-inflammatory properties of *Miconia albicans* (*Sw*.) Triana (Melastomataceae) fruits extracts. **Journal of Ethnopharmacology**, 273, 113979

Dehghanian, Z., Habibi, K., Dehghanian, M., Aliyar, S., Lajayer, B.A., Astatkie, T., Minkina, T., Keswani, C. (2022). Reinforcing the bulwark: unravelling the efficient applications of plant phenolics and tannins against environmental stresses. **Heliyon**, 8, e090994.

Dembogurski, D.S., Bonfa, I.S., Candeloro, L., Parisotto, E.B., Kadri, M.C.T., Silva, D.B. (2023). Infusion from *Miconia albicans* (Melastomataceae) leaves exhibits anti-inflammatory and anti-hyperalgesic activities without toxicity. **Journal of Ethnopharmacology**, 308, 116251.

Gao, J., Shi, J.-M., Lu, X.-M., Sun, C.-R., Pan, Y.-J., (2011). Differentiation of common diastereoisomeric ursane-type triterpenoids by high-performance liquid chromatography/tandem mass spectrometry. **Rapid Communications in Mass Spectrometry**, 25(10), 1349-1355.

Gimenez, V. M. M., M. L. A., Silva, W. R. Cunha, A. H. Januario, E. J. X. Costa and P. M. Pauletti (2020). "Influence of environmental, geographic, and seasonal variations in the chemical composition of *Miconia* species from Cerrado." **Biochemical Systematics and Ecology** 91: 104049.

Gobbo-Neto, L. & Lopes, N.P. (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Química Nova**, 30 (2), 374-381.

Li, L., Ying, P., Chong, H., Chunting, L., Qingbo, L., Linguang, L., Jie, W., Shaojiang, S. (2015). Antioxidant activity of chemical constituents isolated from *Pithecellobium clypearia*. **Records of Natural Products**, 9 (3), 284-291.

Lima, T. C., Matos, S. S., Carvalho, T. F., Silveira-Filho, A. J., Couto, L. P. S. M., Lima, B. S., Silva, A. M. O. (2020). Evidence for the involvement of IL-1β and TNF-α in anti-inflammatory effect and antioxidative stress profile of the standardized dried extract from Miconia albicans Sw. (Triana) Leaves (Melastomataceae). **Journal of Ethnopharmacology**, 259, 112908.

Lommen, A. & Kools, H.J.J.M. (2012). MetAlign 3.0: performance enhancement by efficient use of advances in computer hardware. **Metabolomics**, 8, 719-726.

Mahgoub, Y.A., Shawky, E., Ghareeb, D.A., Darwish, F.A., Sebakhy, N.A., El-Hawiet, A.M. (2023). UPLC-MS/MS multivariate data analysis reveals phenological growth stages affect silymarin bioactive components of the different organs of two *Silybum marianum* genotypes. **Microchemical Journal**, 187, 108436.

Mendez-Lopez, A. Y., Lagunes-Espinoza, L. d. C., González-Esquinca, A. R., Hernández-Nataren, E., & Ortiz-García, C. F. (2023). Phenological characterization of chipilín (Crotalaria longirostrata Hook. & Arn.) and relationship between the phenological stage and chemical composition of leaves. **South African Journal of Botany**, 154, 140-148.

Moilanen, J., Sinkkonen, J., Salminen, J. P., (2013). Characterization of bioactive plant ellagitannins by chromatographic, spectroscopic and mass spectrometric methods. **Chemoecology** 23(3), 165-179.

Pinto, G.F.F. & Kolb, R.M. (2016) Seasonality affects phytotoxic potencial of five native species of Neotropical savanna. **Botany**, 94: 1-9.

Qiao, Q., Si, F., Wu, C., Wang, J., Zhang, A., Tao, J., Zhang, L., Liu, Y., Feng, Z. (2022). Transcriptome sequencing and flavonoid metabolism analysis in the leaves of three different cultivars of *Acer truncatum*. **Plant Physiology and Biochemistry**, 171, 1-13.

Quintans-Junior, L. J., J. S. S. Quintans, S. R. Gandhi, F. R. S. Passos, L. Heimfarth, E. W. M. Pereira, B. S. Monteiro, S. K. S. Dos, M. C. Duarte, L. S. Abreu, Y. M. Nascimento, J. F. Tavares, M. S. Silva, I. R. A. Menezes, H. D. M. Coutinho, A. A. N. Lima and G. Zengin (2020). "Dereplication and quantification of the ethanol extract of *Miconia albicans* (Melastomaceae) by HPLC-DAD-ESI-/MS/MS, and assessment of its anti-hyperalgesic and anti-inflammatory profiles in a mice arthritis-like model: Evidence for involvement of TNF-α, IL-1β and IL-6." **Journal of Ethnopharmacology** 258: 112938.

Tikunov, Y., Laptenok, S., Hall, R., Bovy, A. & De Vos, R.J.M. (2012). MSClust: a tool for unsupervised mass spectra extraction of chromatography-mass spectrometry ion-wise aligned data. **Metabolomics**, 8, 714-718.

R Core Team (2022) R: A language and environment for statistical computing, Version 4.1.3. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Rasines-Perea, Z., Jacquet, R., Jourdes, M., Quideau, S., Teissedre, P.-L., (2019). Ellagitannins and Flavano-Ellagitannins: Red Wines Tendency in Different Areas, Barrel Origin and Ageing Time in Barrel and Bottle. **Biomolecules,** 9, 316.

Ratter, J.A., Ribeiro, J.F., Bridgewater, S. (1997). The Brazilian cerrado vegetation and threats to its biodiversity. **Annals of Botany**, 80, 223-230.

Reichert, C. L., Silva, Carollo, C. A., Weffort-Santos, A. M., Santos, C. A. M., (2018). Metabolic profiling and correlation analysis for the determination of killer compounds of proliferating and clonogenic HRT-18 colon cancer cells from *Lafoensia pacari*. **Journal of Ethnopharmacology**, 224, 541-552.

Serpeloni, J. M., G. R. M. Barcelos, M. P. Mori, K. Yanagui, W. Vilegas, E. A. Varanda and I. M. d. S. Colus (2011). "Cytotoxic and mutagenic evaluation of extracts from plant species of the *Miconia* genus and their influence on doxorubicin-induced mutagenicity: An in vitro analysis." **Experimental and Toxicologic Pathology** 63(5): 499-504.

Silva, D.B., Fernandes, E.F.A., Ferreira, L.S., Callejon, D.R., Guaratini, T., Lopes, J.N.C., Meyre-Silva, C., Filho, V.C., Lopes, N.P. (2012). Megastigmanes from *Aleurites moluccana* (L.) Willd. (Euphorbiaceae). **Biochemical Systematics and Ecology**, 40, 34-37.

Silva, D.B., Okano, L.T., Lopes, N.P., Oliveira, D.C.R., (2013). Flavanone glycosides from *Bidens gardneri* Bak. (Asteraceae). **Phytochemistry** 96, 418-422.

Spínola, V., Pinto, J., Castilho, P. C., (2015). Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLCDAD–ESI-MSn and screening for their antioxidant activity. **Food Chemistry,** 173, 14-30.

Visakorpi, K., Riutta, T., Martinez-Bauer, A.E., Salminen, J., Gripenberg, S. (2019). Insect community structure covaries with host plant chemistry but is not affected by prior herbivory. **Ecology**, 100 (8), e02739.

De Vos, R.C., Moco, S., Lommen, A., Keurentjes, J.J., Bino, R.J. & Hall, R.D.J.N.p. (2007). Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. **Nature protocols,** 2, 778-791.

Zhao, R., Peng, C., Li, L. Li, X., Meng, C., Liu, J., Xiong, L. (2022). A new arborinane-type triterpenoid glycoside and three known megastigmanes from the roots of *Cyathula officinalis* K.C.Kuan. **Biochemical Systematics and Ecology,** 104, 104489.

Younis, W., Alamgeer, Schini-Kerth, V. B, Silva, D. B., Gasparotto Junior, A., Bukhari, I. A., Assiri, A. M., (2020). Role of the NO/cGMP pathway and renin-angiotensin system in the hypotensive and diuretic effects of aqueous soluble fraction from *Crataegus songarica* K. Koch. **Journal of Ethnopharmacology**, 249, 112400.

Wei, K., Li, W., Koike, K., Liu, L., Fu, X., Lin, L., Chen, Y., Nikaido, T. (2004). Two new galloylglucosides from the leaves of *Mallotus furetianus*. **Chemical and Pharmaceutical Bulletin**, 52 (6), 776-779.

6. CONCLUSÃO GERAL

Neste estudo foi abordada a investigação farmacológica de *Miconia albicans*, e seus aspectos químicos relacionados com as interferências ambientais na planta.

Foram conduzidos ensaios *in vivo* avaliando a capacidade anti-inflamatória, analgésica e toxicidade, utilizando como tratamento o extrato aquoso das folhas de *M. albicans* no intuito de mimetizar a forma que a população utiliza.

No ensaio de edema de pata induzido por carragenina, pode-se verificar a redução de 55% (0,016 ± 0.004) do edema nos animais tratados com extrato na dose de 256 mg/kg. Quanto ao teste de influxo leucocitário induzido por carragenina, foi revelado que o pré-tratamento (256 mg/kg) causou 78% (555 ± 211,2 células/mm³) de redução das células polimorfonucleares. Assim então, os resultados confirmam a atividade inflamatória já conhecida da infusão dessa planta.

Verificando o efeito analgésico, conduziu-se o método de contorção abdominal induzida por ácido acético, em que o extrato (1280 mg/kg) mostrou reduzir 42% (57,6 ± 7,6 número de contorções) das contorções. Desse modo, no teste de lambida de pata induzido por formalina, o tratamento com 256 mg/kg de extrato foi capaz de reduzir em 23% (200,9 ± 11,1 s) o tempo de lambida de pata. Com esses resultados, é possível observar o efeito hiperalgésico moderado dessa espécie vegetal em relação aos fármacos de controle.

Na investigação de toxicidade, inicialmente os animais receberam uma dose única de 2000 mg/kg e foram monitorados diariamente por 14 dias. Não apresentaram nenhum sinal clínico ou comportamental que inferisse toxicidade, sem mortes e sem alterações nos exames histológicos, bioquímicos e hematológicos. Na toxicidade de doses repetidas, foram administradas três doses diferentes (51,2; 256 e 1280 mg/kg), que foi analisada ao longo de 28 dias. Também não foram registradas mortes, nem alterações clínicas e comportamentais. Referente as investigações histológicas, bioquímicas e hematológicas, todas mostraram-se sem alterações significativas. O que revelou a ausência de toxicidade no tratamento com *M. albicans* em diferentes doses.

Pesquisou-se a capacidade antimicrobiana e antibiofilme do extrato aquoso das folhas de *M. albicans* e frações advindas do mesmo, frente as bactérias de *Staphylococcus*

aures e *Pseudomonas aeruginosa*. Os resultados demonstraram que não houve efeito frente a essas bactérias, tanto na redução, quanto na erradicação de biofilmes formados.

No estudo químico das folhas de *M. albicans*, pode-se anotar 25 metabólitos por meio da técnica de HPLC-DAD-MS, dentre esses, encontram-se flavonoides, taninos, megastigmanos e triterpenos. Com destaque a classe de megastigmanos, grupo de compostos biossintetizados pela via dos carotenoides, que pela primeira vez foi identificado no gênero *Miconia.* Os principais megastigmanos identificados em *Miconia albicans* do cerrado de Campo Grande-MS, foram cliperosídeo A, malofenol B e roseosídeo, que anteriormente haviam sido mencionados em espécies das famílias Euphorbiaceae e Fagaceae.

E no intuito de verificar as variações químicas que a planta sofre diante as modificações ambientais, foi realizado também um monitoramento mensal com coleta das folhas da espécie, e informações em base de dados oficiais de parâmetros climáticos. A análise metabolômica foi desenvolvida aplicando técnicas de HPLC-DAD-MS e análises multivariadas. Ao final, foi possível verificar tendências de agrupamento, que mostraram classes de metabólitos mais intensas em determinados períodos. Como, os flavonoides e megastigmanos mais intensos em período de seca, e taninos com maior intensidade em período de cheia. Mostrando que o estresse ambiental influencia na composição química das plantas, que reagem fisiologicamente aos fatores externos.

Todos esses dados demonstram a necessidade da pesquisa e investigação de plantas medicinais de conhecimento etnofarmacológico. Evidenciando a necessidade atual de novas moléculas bioativas que atuem frente a diversas patologias, a pesquisa realizada aplicou técnicas que se mostram eficientes para validar e constatar características de *Miconia albicans.*

7. REFERÊNCIAS BIBLIOGRÁFICAS

Alessandri, A. L., L. P. Sousa, C. D. Lucas, A. G. Rossi, V. Pinho and M. M. Teixeira (2013). "Resolution of inflammation: mechanisms and opportunity for drug development." **Pharmacology e Therapeutics** 139(2): 189-212.

Almeda, F. (1993) "Melastomataceae." **Flora del Bajío y de Regiones Adyacentes** 7: 1-1256.

Aparecida Resende, F., C. A. de Andrade Barcala, M. C. da Silva Faria, F. H. Kato, W. R. Cunha and D. C. Tavares (2006). "Antimutagenicity of ursolic acid and oleanolic acid against doxorubicin-induced clastogenesis in Balb/c mice." **Life Sciences** 79(13): 1268-1273.

Barros, T.F., Lin, V., Borges, J.S., Primon-Barros, M., Zanuncio, V.S.S., Silva, D.B., Trentin, D.S. (2023). "*Staphylococcus aureus* antibiofilm agents from *Combretum* species (Combretaceae) by metabolomics profiling. " **Industrial Crops & Products** 193, 116280.

Baruch, Z., R. R. Pattison and G. Goldstein (2000). "Responses to Light and Water Availability of Four Invasive Melastomataceae in the Hawaiian Islands." **International Journa of Pharmaceutics** 161(1): 107-118.

Bomfim-Patricio, M.C., Salatino, A., Martins, A.B., Wurdack, J.J., Salatino, M.L.F. (2000). "Flavonoids of *Lavoisiera*, *Microlicia* and *Trembleya* (Melastomataceae) and their taxonomic meaning." **Biochemical Systematics and Ecology** 29: 711-726.

Borowski, R. G. V., M. P. Barros, D. B. da Silva, N. P. Lopes, K. R. Zimmer, C. C. Staats, C. B. de Oliveira, E. Giudice, R. Gillet, A. J. Macedo, S. C. B. Gnoatto and A. R. Zimmer (2020). "Red pepper peptide coatings control *Staphylococcus epidermidis* adhesion and biofilm formation." **International Journa of Pharmaceutics** 574: 118872.

Boscolo, O.H. & Valle, L.S. (2018) "Plantas de uso medicinal em Quissamã, Rio de Janeiro, Brasil." **Iheringia, Série Botânica** 63(2), 263-277.

Campos-Silva, R., F. R. Brust, D. S. Trentin and A. J. Macedo (2019). "Alternative method in *Galleria mellonella larvae* to study biofilm infection and treatment." **Microbial Pathogenesis** 137: 103756.

Carvalho, A. C. B., T. N. Lana, J. P. S. Perfeito and D. Silveira (2018). "The Brazilian market of herbal medicinal products and the impacts of the new legislation on traditional medicines." **Journal of Ethnopharmacology** 212: 29-35.

Cechinel-Zanchett, C.C., Faloni, S. & Cechinel-Filho, V. (2017). "Ethnopharmacological, phytochemical, pharmacological and toxicological aspects of *Bauhinia forficata*: A mini-review covering the last five years." **Natural Products Communications** 13(7): 911-916.

Coussens, L. M. & Z. Werb (2002). "Inflammation and cancer." **Nature** 420(6917): 860-867.

Crevelin, E. J., I. C. C. Turatti, A. E. M. Crotti, R. C. S. Veneziani, J. L. C. Lopes, N. P. Lopes and W. R. Cunha (2006). "Identification of biologically active triterpenes and sterols present in hexane extracts from *Miconia* species using high-resolution gas chromatography." **Biomedical Chromatography**. 20(8): 827-830.

Cunha, W.R., Martins, C., Ferreira, D.S., Crotti, A.E.M., Lopes, N.P., Albuquerque, S. (2003). "*In vitro* trypanocidal activity of triterpenes from *Miconia* species." **Planta Medica** 2003; 69, 470-472.

Cunha, N. L., C. J. Uchoa, L. S. Cintra, H. C. Souza, J. A. Peixoto, C. P. Silva, L. G. Magalhaes, V. M. Gimenez, M. Groppo, V. Rodrigues, A. A. Silva Filho, E. S. M. L. Andrade, W. R. Cunha, P. M. Pauletti and A. H. Januario (2012). "*In vitro* schistosomicidal activity of some brazilian cerrado species and their isolated compounds." **Evidence-Based Complementary and Alternative Medicine** 2012: 173614.

Cunha, W. R., E. J. Crevelin, G. M. Arantes, A. E. Crotti, M. L. Andrade e Silva, N. A. Furtado, S. Albuquerque and S. Ferreira Dda (2006). "A study of the trypanocidal activity of triterpene acids isolated from *Miconia* species." **Phytotherapy Research** 20(6): 474-478.

Cunha, W. R., G. X. de Matos, M. G. Souza, M. G. Tozatti, M. L. Andrade e Silva, C. H. Martins, R. da Silva and A. A. Da Silva Filho (2010). "Evaluation of the antibacterial activity of the methylene chloride extract of *Miconia ligustroides*, isolated triterpene acids, and ursolic acid derivatives." **Pharmaceutical Biology** 48(2): 166-169.

Cunha, W. R., M. L. A. Silva, F. M. dos Santos, Í. M. Montenegro, A. R. A. Oliveira, H. R. Tavares, H. S. Leme dos Santos and J. C. da Silva Bizário (2008). "In Vitro. Inhibition of Tumor Cell Growth by *Miconia fallax*." **Pharmaceutical Biology** 46(4): 292-294.

Duffin, R., Leitch, A.E., Fox, S., Haslett, C., Rossi, A.G. (2010). "Targeting granulocyte apoptosis: mechanisms, models, and therapies." **Immunological Reviews** 236:28-40.

Erhabor, J. O., R. Komakech, Y. Kang, M. Tang and M. G. Matsabisa (2020). "Ethnopharmacological importance and medical applications of *Myrothamnus flabellifolius* Welw. (Myrothamnaceae)-A review." **Journal of Ethnopharmacology** 252: 112576.

Ezenyi, I. C., V. Verma, S. Singh, S. E. Okhale and B. Adzu (2020). "Ethnopharmacology-aided antiplasmodial evaluation of six selected plants used for malaria treatment in Nigeria." **Journal of Ethnopharmacology** 254: 112694.

Fernandes, J. M., L. M. Cunha, E. P. Azevedo, E. M. G. Lourenço, M. F. Fernandes-Pedrosa and S. M. Zucolotto (2019). "*Kalanchoe laciniata* and *Bryophyllum pinnatum*: an updated review about ethnopharmacology, phytochemistry, pharmacology and toxicology." **Revista Brasileira de Farmacognosia** 29(4): 529-558.

Ferreira, D. d. S., V. R. Esperandim, M. G. Marcal, N. B. d. R. Neres, N. L. Cunha, M. L. Andrade e. Silva and W. R. Cunha (2013). "Natural products and Chagas' disease: the action of triterpenes acids isolated from *Miconia* species." **Universitas Scientiarum** 18(3): 243-256.

Furtado, R. A., E. P. Rodrigues, F. R. Araujo, W. L. Oliveira, M. A. Furtado, M. B. Castro, W. R. Cunha and D. C. Tavares (2008). "Ursolic acid and oleanolic acid suppress preneoplastic lesions induced by 1,2-dimethylhydrazine in rat colon." **Toxicology Pathology** 36(4): 576-580.

Gao, Z., Y. Luan, P. Yang, L. Wang, H. Zhang, S. Jing, L. Wang, T. Wang and D. Wang (2020). "Targeting staphylocoagulase with isoquercitrin protects mice from *Staphylococcus aureus*-induced pneumonia." **Applied Microbiology and Biotechnology** 104(9): 3909-3919.

Garcez, F. R., W. S. Garcez, N. C. Yoshida and P. O. Figueiredo (2016). "The Chemical Diversity from the Vegetation of Mato Grosso do Sul and its Relevance as a Source of Bioactive Molecules." **Revista Virtual de Química** 8(1).

Gasparotto Junior, A., P. de Souza and F. Livero (2019). "*Plinia cauliflora* (Mart.) Kausel: A comprehensive ethnopharmacological review of a genuinely Brazilian species." **Journal of Ethnopharmacology** 245: 112169.

Gatis-Carrazzoni, A., F. V. B. Mota, T. C. C. Leite, T. B. de Oliveira, S. C. da Silva, I. V. A. Bastos, M. B. de Souza Maia, P. S. Pereira, P. P. M. Neto, E. C. de Oliveira Chagas, T. M. S. Silva, M. S. do Nascimento and T. G. da Silva (2019). "Anti-inflammatory and antinociceptive activities of the leaf methanol extract of *Miconia minutiflora* (Bonpl.) DC. and characterization of compounds by UPLC-DAD-QTOF-MS/MS." **Naunyn-Schmiedebergs Archives of Pharmacology** 392(1): 55-68.

Gilbert, A., T. T. Herve, Y. N. William, S. F. Leonard, K. Jules-Roger and K. Albert (2014). "Antidiarrhoeal and antibacterial activity of aqueous and methanolic leaves extracts of Dissotis thollonii Cogn. (Melastomataceae)." **Asian Pacific Journal of Tropical Biomedicine** 4: S672-S678.

Gimenez, V. M. M., M. L. A., Silva, W. R. Cunha, A. H. Januario, E. J. X. Costa and P. M. Pauletti (2020). "Influence of environmental, geographic, and seasonal variations in the chemical composition of *Miconia* species from Cerrado." **Biochemical Systematics and Ecology** 91: 104049.

Goldenberg, R., M. Reginato and F. A. Michelangeli (2020). "*Miconia lucenae* (Melastomataceae), a new species from montane Atlantic Forest in Espirito Santo, Brazil." **PeerJ** 8: e8752.

Goldenberg, R., Baumgratz, J.F.A., Souza, M. L.D.R. (2012). "Taxonomia de Melastomataceae no Brasil: retrospectiva, perspectivas e chave de identificação para gêneros." **Rodriguésia** 63(1): 145-161.

Gontijo, D. C., P. C. Gontijo, G. C. Brandao, M. A. N. Diaz, A. B. de Oliveira, L. G. Fietto and J. P. V. Leite (2019). "Antioxidant study indicative of antibacterial and antimutagenic activities of an ellagitannin-rich aqueous extract from the leaves of *Miconia latecrenata*." **Journal of Ethnopharmacology** 236: 114-123.

Gordon, A., B. Schadow, C. E. Quijano and F. Marx (2011). "Chemical characterizationγ and antioxidant capacity of berries from *Clidemia rubra* (Aubl.) Mart. (Melastomataceae)." **Food Research International** 44(7): 2120-2127.

Guldbrandsen, N., M. De Mieri, M. Gupta, T. Seiser, C. Wiebe, J. Dickhaut, R. Reingruber, O. Sorgenfrei and M. Hamburger (2015). "Screening of Panamanian Plant Extracts for Pesticidal Properties and HPLC-Based Identification of Active Compounds." **Scientia Pharmaceutica** 83(2): 353-367.

Hakala, E., Hanski, L.L., Yrjonen, T., Vuorela, H.J., Vuorela, P.M. (2015). "The lignan-containing extract of *Schisandra chinenses* berries inhibits the growth of *Clamydia pneumonie*." **Natural Product Communications** 10(6): 1001-1004.

He, Y., Q. Wang, Y. Ye, Z. Liu and H. Sun (2020). "The ethnopharmacology, phytochemistry, pharmacology and toxicology of genus Albizia: A review." **Journal of Ethnopharmacology** J 257: 112677.

Ishii, R., Saito, K., Horie, M., Shibano, T., Kitanaka, S., Amano, F. (1999). "Inhibitory effects of hydrolyzable tannins from *Melastoma dodecandrum* Lour, on nitric oxide production by a murine macrophage-like cell line, RAW264.7, actived with lipopolysaccharide and interferon-γ." **Biological and Pharmaceutical Bulletin** 22(6): 647-653.

Li, X., Joshi, A.S., ElSohly, H.N., Khan, S.I., Jacob, M.R., Zhang, Z., Khan, I.A., Ferreira, D., Walker, L.A., Broedel, S.E.J., Raulli, R.E., Cihlar, R.L. (2002). "Fatty acid synthase inhibitors from plants: isolation, structure elucidation and SAR studies." **Journal of Natural Products** 65: 1909-1914.

Lima, C.L.R., T. K. K, L. Kato, M. Jose das Silva, H. Franzyk and D. Staerk (2018). "High-Resolution PTP1B Inhibition Profiling Combined with HPLC-HRMS-SPE-NMR for Identification of PTP1B Inhibitors from *Miconia albicans*." **Molecules** 23(7).

Lima, T. C., S. S. Matos, T. F. Carvalho, A. J. Silveira-Filho, L. P. S. M. Couto, B. S. Lima, F. A. Silva, L. J. Quintans-Junior, J. S. S. Quintans, L. Heimfarth, F. R. S. Passos, S. R. Gandhi and A. M. O. Silva (2020). "Evidence for the involvement of IL-1β and TNF-α in anti-inflammatory effect and antioxidative stress profile of the standardized dried extract from *Miconia albicans* Sw. (Triana) Leaves (Melastomataceae)." **Journal of Ethnopharmacology** 259: 112908.

Luan, H. and T. Horng (2021). "Dynamic changes in macrophage metabolism modulate induction and suppression of Type I inflammatory responses." **Current Opinion in Immunology** 73: 9-15.

Macêdo, M. J. F., D. A. Ribeiro, M. d. O. Santos, D. G. d. Macêdo, J. G. F. Macedo, B. V. d. Almeida, M. E. Saraiva, M. N. S. d. Lacerda and M. M. d. A. Souza (2018). "Fabaceae medicinal flora with therapeutic potential in Savanna areas in the Chapada do Araripe, Northeastern Brazil." **Revista Brasileira de Farmacognosia** 28(6): 738-750.

Queiroz, G.M., M. G. de Souza, L. Casemiro, G. de Queiroz, T. de Carvalho and W. Cunha (2011). "Absence of the antibacterial activity of the crude extracts and compounds isolated from *M. rubiginosa* against extended-spectrum β-lactamase producing enterobacteria." **Journal of Pharmaceutical Negative Results** 2(1).

McEvoy, L., D. F. Carr and M. Pirmohamed (2021). "Pharmacogenomics of NSAID-Induced Upper Gastrointestinal Toxicity." **Frontiers in Pharmacology** 12: 684162.

Milanez, C. R. D., C. R. Marcati and S. R. Machado (2017). "Trabeculae and Al-accumulation in the wood cells of Melastomataceae species from Brazilian savanna." **Botany** 95(5): 521-530.

Mimura, M. R. M., A. Salatino and M. L. F. Salatino (2004). "Distribution of flavonoids and the taxonomy of *Huberia* (Melastomataceae)." **Biochemical Systematics and Ecology** 32(1): 27-34.

Moura, V. M., L. Y. de Souza, N. da Costa Guimaraes, I. G. Dos Santos, P. D. de Almeida, R. B. de Oliveira, R. H. Mourao and M. C. Dos-Santos (2017). "The potential of aqueous extracts of Bellucia dichotoma Cogn. (Melastomataceae) to inhibit the biological activities of Bothrops atrox venom: A comparison of specimens collected in the states of Para and Amazonas, Brazil." **Journal of Ethnopharmacology** 196: 168-177.

Moura, V. M., L. A. Freitas de Sousa, M. Cristina Dos-Santos, J. D. Almeida Raposo, A. Evangelista Lima, R. B. de Oliveira, M. N. da Silva and R. H. Veras Mourao (2015). "Plants used to treat snakebites in Santarem, western Para, Brazil: an assessment of their effectiveness in inhibiting hemorrhagic activity induced by Bothrops jararaca venom." **Journal of Ethnopharmacology** 161: 224-232.

Morais, M. G., A. A. Saldanha, L. S. Azevedo, I. C. Mendes, J. P. C. Rodrigues, P. A. Amado, K. S. Farias, V. S. S. Zanuncio, N. S. Cassemiro, D. B. D. Silva, A. C. Soares and L. Lima (2022). "Antioxidant and anti-inflammatory effects of fractions from ripe fruits of *Solanum lycocarpum* St. Hil. (Solanaceae) and putative identification of bioactive compounds by GC-MS and LC-DAD-MS." **Food Research International** 156: 111-145.

Moura, L., J. X. da Silva Neto, T. D. P. Lopes, S. R. Benjamin, F. C. R. Brito, F. E. A. Magalhaes, E. Florean, D. O. B. de Sousa and M. I. F. Guedes (2019). "Ethnobotanic, phytochemical uses and ethnopharmacological profile of genus *Cnidoscolus* spp. (Euphorbiaceae): A comprehensive overview." **Biomedicine & Pharmacotherapy** 109: 1670-1679.

Moura. V. M., A. F. S. Luciana, B.O. Ricardo, M. M. d. S. Ana, d. M. C. Hipoacute crates, N. S. Milton, P. Sergio and H. V. M. o. Rosa (2013). "Inhibition of the principal enzymatic and biological effects of the crude venom of *Bothrops atrox* by plant extracts." **Journal of Medicinal Plants Research** 7(31): 2330-2337.

Murata, M. (2018). "Inflammation and cancer." **Environmental Health and Preventive Medicine** 23(1): 50.

Murugan, R. and T. Parimelazhagan (2013). "Study of anti-nociceptive, anti-inflammatory properties and phytochemical profiles of *Osbeckia parvifolia* Arn. (Melastomataceae)." **Industrial Crops and Products** 51: 360-369.

Ndjateu, F. S. T., R. B. N. Tsafack, B. K. Nganou, M. D. Awouafack, H. K. Wabo, M. Tene, P. Tane and J. N. Eloff (2014). "Antimicrobial and antioxidant activities of extracts and ten compounds from three Cameroonian medicinal plants: *Dissotis perkinsiae* (Melastomaceae), *Adenocarpus mannii* (Fabaceae) and *Barteria fistulosa* (Passifloraceae)." **South African Journal of Botany** 91: 37-42.

Newman, J. H. and A. Zloza (2017). "Infection: a Cause of and Cure for Cancer." **Current Pharmacology Reports** 3(6): 315-320.

Pieroni, L.G., Rezende, F.M., Ximenes, V.F., Dokkedal, A.L. (2011). "Antioxidant activity and total phenols from the methanolic extract of *Miconia albicans* (Sw.) Triana leaves. " **Molecules** 16, 9439-9450.

Psomas, G. (2020). "Copper(II) and zinc(II) coordination compounds of non-steroidal anti-inflammatory drugs: Structural features and antioxidant activity." **Coordination Chemistry Reviews** 412.

Queiroz, G.M., Souza, M.G.M., Carvalho, T.C., Casemiro, L.A., Cunha, W.R., Martins, C.H.G. (2011). "Absence of the antibacterial activity of the crude extracts and compounds isolated from *M. rubiginosa* against extended-spectrum β-lactamase producting enterobacteria." **Journal of Pharmaceutical Negative Results** 2, 1.

Quintans-Junior, L. J., J. S. S. Quintans, S. R. Gandhi, F. R. S. Passos, L. Heimfarth, E. W. M. Pereira, B. S. Monteiro, S. K. S. Dos, M. C. Duarte, L. S. Abreu, Y. M. Nascimento, J. F. Tavares, M. S. Silva, I. R. A. Menezes, H. D. M. Coutinho, A. A. N. Lima and G. Zengin (2020). "Dereplication and quantification of the ethanol extract of *Miconia albicans* (Melastomaceae) by HPLC-DAD-ESI-/MS/MS, and assessment of its anti-hyperalgesic and anti-inflammatory profiles in a mice arthritis-like model: Evidence for involvement of TNF-α, IL-1β and IL-6." **Journal of Ethnopharmacology** 258: 112938.

Reis, S. V. D., N. M. G. Couto, F. R. Brust, D. S. Trentin, J. Silva, M. S. P. Arruda, S. C. B. Gnoatto and A. J. Macedo (2020). "Remarkable capacity of brosimine b to disrupt methicillin-resistant *Staphylococcus aureus* (MRSA) preformed biofilms." **Microbial Pathogenesis** 140: 103967.

Resende, F.A., Barcala, C.A.M.A., Faria, M.C.S., Kato, F.H., Cunha, W.R., Tavares, D.C. (2006). "Antimutagenicity of ursolic acid and oleanolic acid against doxorubicin-induced clastogenesis in Balb/c mice. " **Life Sciences** 79, 1268-1273.

Rezende, A.R., Romero, R., Goldenberg, R. (2013). "Sinopse de *Miconia* seção *Miconia* DC. (Melastomataceae) no estado de Minas Gerais, Brasil." **Bioscience Journal** 30(1): 273-287.

Rezende, F. M., M. J. P. Ferreira, M. H. Clausen, M. Rossi and C. M. Furlan (2019). "Acylated Flavonoid Glycosides are the Main Pigments that Determine the Flower Colour of the Brazilian Native Tree Tibouchina pulchra (Cham.) Cogn." **Molecules** 24(4).

Ribeiro Neto, J. A., B. R. Pimenta Taroco, H. Batista dos Santos, R. G. Thome, E. Wolfram and R. I. Maciel de A. Ribeiro (2020). "Using the plants of Brazilian Cerrado for wound healing: From traditional use to scientific approach." **Journal of Ethnopharmacology** 260: 112547.

Ribeiro, R. C., M. L. N. Figueiredo, A. Picorelli, D. M. T. Oliveira and F. A. O. Silveira (2016). "Does seed coat structure modulate gut-passage effects on seed germination? Examples from Miconieae DC. (Melastomataceae)." **Seed Science Research** 26(2): 139-147.

Rodrigues, V.E.G. & Carvalho, D.A. (2001). "Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do alto Rio Grande – Minas Gerais." **Ciência e Agrotecnologia** 25(1): 102-123.

Rodrigues, J., D. Rinaldo, M. Aparecido da Silva, L. Campaner dos Santos and W. Vilegas (2011). "Secondary Metabolites of Miconia rubiginosa." **Journal of Medicinal Food** 14(7/8): 834-839.

Rodrigues, J., D. Rinaldo, L. C. dos Santos and W. Vilegas (2007). "An unusual C6-C6" linked flavonoid from *Miconia cabucu* (Melastomataceae)." **Phytochemistry** 68(13): 1781-1784.

Schneider, R., M. Primon-Barros, R. G. Von Borowski, S. Chat, S. Nonin-Lecomte, R. Gillet and A. J. Macedo (2020). "Pseudonajide peptide derived from snake venom alters cell envelope integrity interfering on biofilm formation in *Staphylococcus epidermidis*." **BMC Microbiology** 20(1): 237.

Serna, D. M. and J. H. Martinez (2015). "Phenolics and Polyphenolics from Melastomataceae Species." **Molecules** 20(10): 17818-17847.

Serpeloni, J. M., G. R. M. Barcelos, M. P. Mori, K. Yanagui, W. Vilegas, E. A. Varanda and I. M. d. S. Colus (2011). "Cytotoxic and mutagenic evaluation of extracts from plant species of the *Miconia* genus and their influence on doxorubicin-induced mutagenicity: An in vitro analysis." **Experimental and Toxicologic Pathology** 63(5): 499-504.

Serpeloni, J. M., M. Bisarro dos Reis, J. Rodrigues, L. Campaner dos Santos, W. Vilegas, E. A. Varanda, A. L. Dokkedal and I. M. S. Colus (2008). "In vivo assessment of DNA damage and protective effects of extracts from Miconia species using the comet assay and micronucleus test." **Mutagenesis** 23(6): 501-507.

Silva, A. G., V. A. O. Silva, R. J. S. Oliveira, A. R. de Rezende, R. C. R. Chagas, L. P. S. Pimenta, W. Romao, H. B. Santos, R. G. Thome, R. M. Reis and R. I. M. de Azambuja Ribeiro (2020). "Matteucinol, isolated from *Miconia chamissois*, induces apoptosis in human glioblastoma lines via the intrinsic pathway and inhibits angiogenesis and tumor growth in vivo." **Investigational New Drugs** 38(4): 1044-1055.

Silva, D. B., L. T. Okano, N. P. Lopes and D. C. R. de Oliveira (2013). "Flavanone glycosides from *Bidens gardneri* Bak. (Asteraceae)." **Phytochemistry (Elsevier)** 96: 418-422.

Silva, T. L. d., C. S. D. Costa, M. G. C. d. Silva and M. G. A. Vieira (2022). "Overview of non-steroidal anti-inflammatory drugs degradation by advanced oxidation processes." **Journal of Cleaner Production** 346.

Spessoto, M. A., D. S. Ferreira, A. E. Crotti, M. L. Silva and W. R. Cunha (2003). "Evaluation of the analgesic activity of extracts of *Miconia rubiginosa* (Melastomataceae)." **Phytomedicine** 10(6-7): 606-609.

Tabach, R., J. M. Duarte-Almeida and E. A. Carlini (2017). "Pharmacological and Toxicological Study of Maytenus ilicifolia Leaf Extract. Part I - Preclinical Studies." **Phytotherapy Research** 31(6): 915-920.

Tarawneh, A. H., F. Leon, M. A. Ibrahim, S. Pettaway, C. R. McCurdy and S. J. Cutler (2014). "Flavanones from *Miconia prasina*." **Phytochemistry Letters** 7: 130-132.

Tolouei, S. E. L., R. A. C. Palozi, C. A. S. Tirloni, A. A. M. Marques, M. I. Schaedler, L. P. Guarnier, A. O. Silva, V. P. de Almeida, J. Manfron Budel, R. I. C. Souza, A. C. Dos Santos, D. B. Silva, E. L. B. Lourenco, P. R. Dalsenter and A. Gasparotto Junior (2019). "Ethnopharmacological approaches to *Talinum paniculatum* (Jacq.) Gaertn. - Exploring cardiorenal effects from the Brazilian Cerrado." **Journal of Ethnopharmacology** 238: 111873.

Trentin, D.S., Giordan, R.B., Macedo, A.J. (2013). "Biofilmes bacterianos patogênicos: aspectos gerais, importância clínica e estratégias de combate." **Revista Liberato** 14(22): 113-238.

Trojan-Rodrigues, M., T. L. Alves, G. L. Soares and M. R. Ritter (2012). "Plants used as antidiabetics in popular medicine in Rio Grande do Sul, southern Brazil." **Journal of Ethnopharmacology** 139(1): 155-163.

Viegas, F. P. D., A. T. de Castro, A. P. Castro, Í. Siqueira, W. Rosa, P. F. Espuri, L. F. L. Coelho, M. J. Marques and M. G. Soares (2017). "In vitro schistosomicidal activity of the crude extract, fractions and Primin, the major active benzoquinone constituent from the leaves of Miconia willdenowii (Melastomaceae)." **South African Journal of Botany** 111: 365-370.

Viegas, F. P. D., P. F. Espuri, J. C. Oliver, N. C. Silva, A. L. T. Dias, M. J. Marques and M. G. Soares (2019). "Leishmanicidal and antimicrobial activity of primin and primin-containing extracts from Miconia willdenowii." **Fitoterapia** 138: 104297.

Vieira, W. T., M. V. S. Nicollini, M. G. C. da Silva, L. de Oliveira Nascimento and M. G. A. Vieira (2022). "Natural polysaccharides and proteins applied to the development of gastroresistant multiparticulate systems for anti-inflammatory drug delivery – A systematic review." **European Polymer Journal** 172.

Von Borowski, R. G., A. J. Macedo and S. C. B. Gnoatto (2018). "Peptides as a strategy against biofilm-forming microorganisms: Structure-activity relationship perspectives." **European Journal of Pharmaceuticals Sciences** 114: 114-137.

Wang, X. & Lin, Y. (2008). "Tumor necrosis factor and cancer, buddies or foes?." **National Institutes of Health** 29(11): 1275-1288.

Wang, L., W. Chen, M. Li, F. Zhang, K. Chen and W. Chen (2020). "A review of the ethnopharmacology, phytochemistry, pharmacology, and quality control of *Scutellaria barbata* D. Don." **Journal of Ethnopharmacology** 254: 112260.

Wolfe, M.M., Lichtenstein, D.R. & Singh, G. (1999). "Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs." **The New England Journal of Medicine** 340, 24.

Yeung, A. W. K., M. Heinrich, A. Kijjoa, N. T. Tzvetkov and A. G. Atanasov (2020). "The ethnopharmacological literature: An analysis of the scientific landscape." **Journal of Ethnopharmacology** 250: 112414.

Zhang, Z., H. N. ElSohly, X.-C. Li, S. I. Khan, S. E. Broedel, Jr., R. E. Raulli, R. L. Cihlar and L. A. Walker (2003). "Flavanone Glycosides from *Miconia trailii*." **Journal of Natural Products** 66(1): 39-41.

Zampieri, M. C. T., J. E. S. Sarkis, R. C. B. Pestana, A. R. Tavares and G. F. A. Melo-de-Pinna (2013). "Characterization of *Tibouchina granulosa* (Desr.) Cong. (Melastomataceae) as a biomonitor of air pollution and quantification of particulate matter adsorbed by leaves." **Ecological Engineering** 61: 316-327.

Zappi, D. C., F. L. R. Filardi, P. Leitman, V. C. Souza, B. M. T. Walter, J. R. Pirani, M. P. Morim, L. P. Queiroz, T. B. Cavalcanti, V. F. Mansano, R. C. Forzza, M.C. (2015). "Growing knowledge: an overview of seed plant diversity in Brazil." **Rogriguésia** 66(4):1085-1113.