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SATHIYABAMA RAJIV GANDHI

PHYTOCHEMICAL, ANTI-HYPERALGESIC AND ANTI-INFLAMMATORY POTENTIAL OF *MICONIA ALBICANS* Sw. TRIANA (MELASTOMATACEAE) AND HEALTH BENEFITS OF *MICONIA* GENUS

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The thesis was presented to the Graduate Program in Health Sciences at the Federal University of Sergipe as a partial requirement for obtaining the Doctor of Health Sciences degree.

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Approved on: **29/09/2023**

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Supervisor: Prof. Dr. Lucindo José Quintans Júnior (UFS)

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DEDICATED TO MY

BELOVED FAMILY MEMBERS

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SATHIYABAMA RAJIV GANDHI

ABSTRACT

This study reviews existing research on the therapeutic potential and molecular mechanisms of the *Miconia* genus, a group of medicinal plants with 282 species in Brazil and other tropical American nations. The research focuses on the pharmacological properties of phytochemicals in the *Miconia* genus and their impact on well-being, while addressing safety considerations. The study conducted literature searches across electronic databases and identified 14 species from the genus *Miconia*, with the most frequently used species being *Miconia albicans* Sw. Triana and *Miconia rubiginosa* (Bonpl.) DC. *M. albicans* showed significant efficacy and potential for developing safe drugs to treat pain and inflammation. Another study aimed to identify the anti-arthritic and anti-inflammatory profile of *M. albicans* ethanolic leaf extract (EEMA), which was found to have 23 compounds. The EEMA significantly decreased tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) levels in pleural lavage, lowered mechanical hyperalgesia, nociceptive and hyperalgesic behaviors, and enhanced mobility. In the open-field test, EEMA significantly improved grip strength and decreased the dimension of complete Freund's adjuvant (CFA)-induced ipsilateral knee edema. In conclusion, the *Miconia* genus's rich history in folk medicine, diverse therapeutic potential, and associated molecular mechanisms highlight its promise as a source for safe and effective treatments for pain and inflammation.

Keywords: *Miconia albicans*, therapeutic mechanism, cytokines, antioxidant, arthritic, anti-inflammatory

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LIST OF ABBREVIATIONS

AAPH- 2,2'-azobis (2-amidinopropane) dihydrochloride;

CFA- Complete Freund's Adjuvant;

CID- collision-induced dissociation;

DMARDs- disease-modifying anti-rheumatic drugs;

DEMA- a dried extract of *Miconia albicans*;

DPPH- 1,1-diphenyl-2-picrylhydrazyl;

EEMA- ethanolic leaf extract of *M. albicans*;

GM-CSF- granulocyte macrophage-colony stimulating factor;

IL- interleukins;

MAEE- *Miconia albicans* ethanolic leaf extract;

MAFRE- *Miconia albicans* fruits extract;

MeSH- Medical Subjects Headings;

NSAIDs- Non-steroidal anti-inflammatory drugs;

NFκB- nuclear factor-κB;

Nrf2- nuclear factor erythroid-2-related factor 2;

OA- oleanolic acid;

PRISMA- Preferred Reporting Items for Systematic Reviews and Meta-Analyses;

RA- rheumatoid arthritis;

RNS- reactive nitrogen species;

ROS- reactive oxygen species;

sTNFR- soluble tumour necrosis factor receptor;

TNF-α- tumour necrosis factor-α;

UA- ursolic acid;

VASP- Visual Analogue Scale of Pain;

VEGFR-2- vascular endothelial growth factor receptor-2;

WOMAC- Western Ontario and McMaster Universities Osteoarthritis Index

SUMMARY

1 INTRODUCTION

The Melastomataceae botanical family includes the genus *Miconia*, which includes a variety of blooming perennial arboreal medicinal shrubs that are commonly cultivated in tropical American nations (LEITÃO et al., 2014). With about 276 species represented, 121 of which are endemic, the genus is primarily found in the Cerrado biome, a Brazilian savannah ecosystem in the Atlantic forest of North-Eastern Brazil (REIS et al., 2005; PESSOA et al., 2012). According to VIEIRA et al. (2009), certain *Miconia* fruits are edible and a great source of phenolic compounds. According to RODRIGUES et al. (2011), the Brazilian people frequently employ some species of *Miconia* as medicines to treat various ailments.

Species of the *Miconia* genus, including *Miconia rubiginosa* (Bonpl.) DC. and *Miconia cinnamomifolia* (DC.) Naudin, are used in traditional medicine to alleviate pain, throat infections, colds, and fever (RODRIGUES; CARVALHO, 2007). The leaves of *Miconia albicans* Sw. Triana are used by traditional healers to treat rheumatoid arthritis (RA) and back discomfort, and the stem possesses effective antipyretic properties (DE ALBUQUERQUE et al., 2007; RIBEIRO et al., 2017). Due to its alleged ability to lessen joint pain in elderly people and the burning sensation of joint pain, *M. albicans* has been given the popular names “canela-de-velho” and "branda-fogo" in Brazilian folk medicine. The actual efficacy of many of these treatments, however, has received little research and/or reporting, mainly about its phytochemical profile, anti-inflammatory and analgesic properties.

CUNHA et al. (2019) reviewed previous phytochemical studies and reported 21 investigated species from the genus *Miconia*, such as *Miconia stenostachya* DC., *M. albicans*, *Miconia pepericarpa* Mart. ex DC., *Miconia sellowiana* Naudin, *Miconia fallax* DC., *M. rubiginosa*, *Miconia ligustroides* (DC.) Naudin, *Miconia ferruginata* DC., *Miconia langsdorffii* Cogn.,

Miconia macrothyrsa Benth., *Miconia affinis* DC., *Miconia lepidota* DC., *Miconia pilgeriana* Ule., *Miconia myriantha* Benth., *Miconia alypifolia* Naudin., *Miconia cannabina* Markgr., *Miconia cabucu* Hoehne., *Miconia willdenowii* Klotzsch ex Naudin., *Miconia prasina* (Sw.) DC., *Miconia ioneura* Griseb., *and Miconia trailii* Cogn., containing no less than 79 phytochemicals including flavonoids, triterpenes, steroids, phenolic acids, quinones, tannins, and lignans.

According to the review, flavonoids from the *Miconia* species are primarily aglycones like quercetin, matteucinol, and kaempferol, while others are glycosylated with sugar units in the carbons 3 or 7 (CUNHA et al., 2019). Ursolic acid (UA), oleanolic acid (OA), α-amyrin, β-amyrin, α-amyrin acetate, β-amyrin acetate, arjunolic acid, sumaresinolic acid, 2-α-hydroxyursolic acid, and maslinic acid are the principal pentacyclic triterpenes and derivatives found from the *Miconia* genus. Additionally, it has been claimed that this genus contains the components gallic acid, ellagic acid, primin, casuarictin, schizandriside, and a number of its derivatives.

The biological and therapeutic potentials of extracts, chemicals, and their derivatives from *Miconia* species have been examined *in vivo* and *in vitro* research. The *Miconia* species showed various biochemical activities such as anti-inflammatory (VASCONCELOS, 2006), anti-diabetic (LIMA et al., 2018); anti-rheumatic (RODRIGUES et al., 2011), anti-mutagenic and anti-tumor (GUNATILAKA et al., 2001; CUNHA et al., 2008; SERPELONI et al., 2011), anti-microbial (CELOTTO et al., 2003; CUNHA et al., 2007; RODRIGUES et al., 2008; QUEIROZ et al., 2011; TRACANNA et al., 2010), schistosomicidal (VIEGAS et al., 2017), antioxidant (SPESSOTO et al., 2003; MANCINI et al., 2008; MOSQUERA et al., 2009; PIERONI et al., 2011), analgesic (VASCONCELOS et al., 2003; VASCONCELOS et al., 2006), anti-malarial (CUNHA et al., 2003; LIMA et al., 2015), anti-nociceptive (SPESSOTO et al., 2003); leishmanicidal (PEIXOTO et al., 2011), trypanocidal (CUNHA et al., 2003; PIERONI et al.,

2011), insecticidal and fungicidal (GULDBRANDSEN et al., 2015; CUNHA et al., 2017) activities. However, little is known about their safety, mechanism of action, and systemic toxicity in prolonged use, limiting their clinical use.

DA SILVA et al. also reported the pharmacological features of the genus *Miconia* in a recent study (2022). Additionally, the authors compiled phytochemical studies pertaining to *Miconia* species. They found 148 chemical substances, including flavonoids and phenolic acids, which are the main components of the species. Based on the identified *in vivo* and *in vitro* pharmacological research, we investigate the phytochemical composition of the members of the *Miconia* genus, their safety, and possible health applications in this systematic review. We place particular emphasis on their associated molecular mechanisms.

On the other hand, the most cited and one of the most important species in ethnopharmacology, *M. albicans*, lacks comprehensive phytochemical and pharmacological data. Despite preliminary indications of its potential for arthritis, our study is the first to conduct a thorough phytochemical analysis using dereplication technique, while also examining its effects on experimental inflammation models, particularly with a focus on alleviating arthritis symptoms, utilizing preclinical models.

2 REVIEW OF LITERATURE

2. 1 Ethnomedicinal uses of *Miconia* genus

Studies on ethnomedicine have documented a variety of uses for *Miconia* species in traditional medicine, including *M. albicans*, one of the species that has been the subject of the most research (ALMEIDA et al., 2014). The species, also known as "canela-de-velho," is traditionally utilized by the Brazilian people to treat many ailments. *M. albicans* leaves are used to make teas, which are mostly drank to relieve arthritis and back discomfort (RIBEIRO et al., 2017; LIMA et al., 2020). Its stem has also been claimed to be used to treat vitiligo and alleviate feverish symptoms (DE ALBUQUERQUE et al., 2007; GOLDENBERG et al., 2008).

M. rubiginosa, also known as "capiroroquinha" locally, was found to be employed in the treatment of throat ailments according to a survey conducted in rural Minas Gerais (Brazil) communities (RODRIGUES et al., 2007). According to BOSCOLO; VALLE (2008), locals of the state of Rio de Janeiro used the leaves of *Miconia cinnamomifolia* (DC.) Naudin to treat their fevers and colds. Herbalists in the state of Rio de Janeiro have reported *Miconia chartacea* Triana for its ethnomedicinal uses (LEITÃO et al., 2009).

2.2 Phytochemical studies of *Miconia* genus

A prior study found that plant species belonging to the *Miconia* genus had yielded 28 glycosylated flavonoids and 10 aglycones, the majority of which were quercetin, matteucinol, and kaempferol. The *Miconia* genus contains numerous bioactive derivatives of the main pentacyclic triterpenes UA, OA, α-amyrin and, β-amyrin. Steroids and their derivatives, such as β-sitosterol, stigmasterol, stigmast-4-ene-3,6-dione, campesterol, gallic acid, ellagic acid, are further identified phytochemicals (CUNHA et al., 2019). High-speed counter-current chromatography analysis of the leaves from *M. rubiginosa* revealed the presence of phytochemicals including flavonoids, gallic acid, casuarictin, and schizandriside

(RODRIGUES et al., 2011). Using UPLC-DAD-QTOF-MS/MS, a study found ellagic acid, gallotannin, and terpenes in the methanol extract of *Miconia minutiflora* (Bonpl.) DC. (GATIS-CARRAZZONI et al., 2018).

The secondary metabolites of triterpenes obtained from plant species in *Miconia* are UA and OA, which are typically found in an isomeric mixture with a pentacyclic structure (CUNHA et al., 2006). Other triterpenes reported are α-amyrin, β-amyrin, lupeol, maslinic acid, epibetulinic acid and arjunolic acid (LIMA et al., 2018). *Miconia* plant species demonstrated the presence of certain phytoconstituents, including a phenolic acid (gallic acid), bioflavonoids, and flavonoid glycosides (quercetin, myricetin, catechin, and kaempferol) (RODRIGUES et al., 2007). In addition, an examination of an ethanol extract of *M. albicans* leaves using the methods HPLC-DAD-ESI-MS/MS revealed 23 natural compounds, the majority of which were rutin and glycoside flavonoids generated from quercetin (QUINTANS-JÚNIOR et al., 2020). Phytochemical studies on this genus reported the presence of triterpenes (TARAWNEH et al., 2014), flavones (ZHANG et al., 2003), coumarins, and benzoquinones (GUNATILAKA et al., 2001). The possible phytoconstituents that have been discovered or extracted from different *Miconia* plant species and are linked to a variety of biological functions are shown in Figure 1A-C. Figure 2 shows the number of publications with their citations and the year they were reported for each species of *Miconia*.

Figure 1A, B & C. Promising phytochemical structures identified/isolated from different *Miconia* plant species that are responsible for a variety of biological functions from the reviewed studies

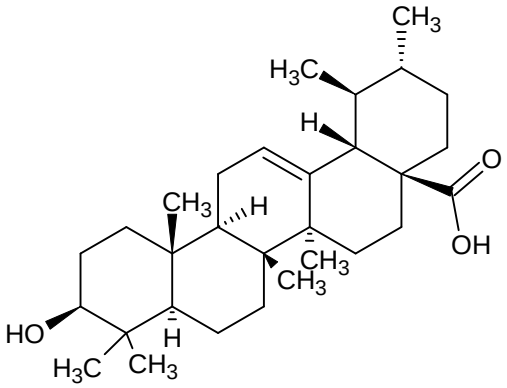
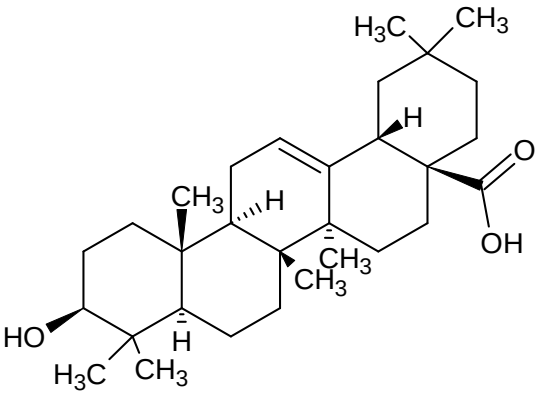
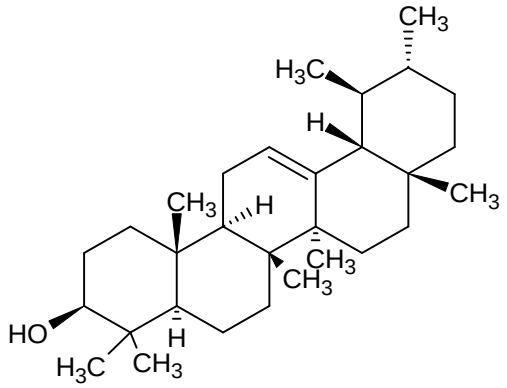
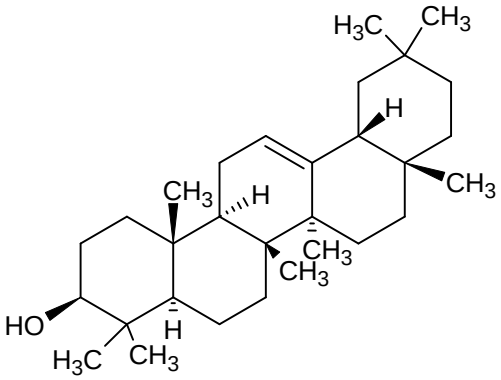
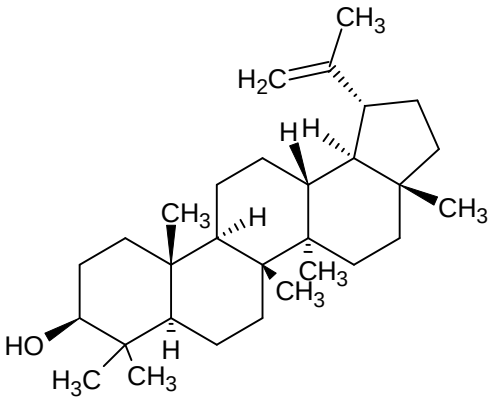
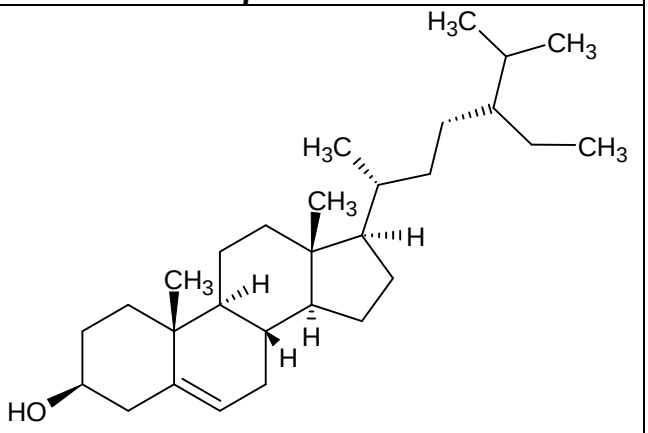
Ursolic acid	Oleanoic acid
	
α-amyrin	**β-amyrin**
	
Lupeol	**β-sitosterol**
	

Figure. 1A

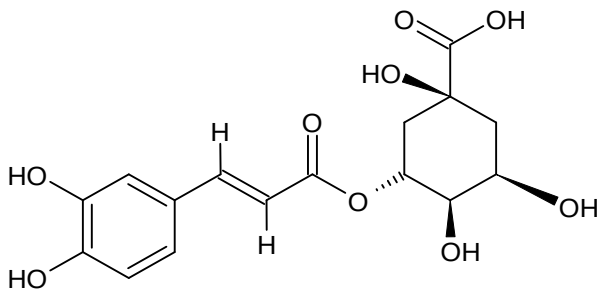
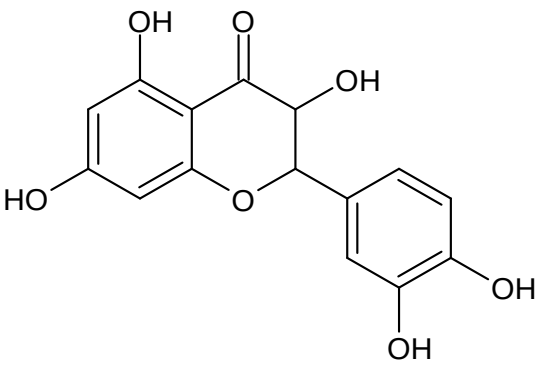
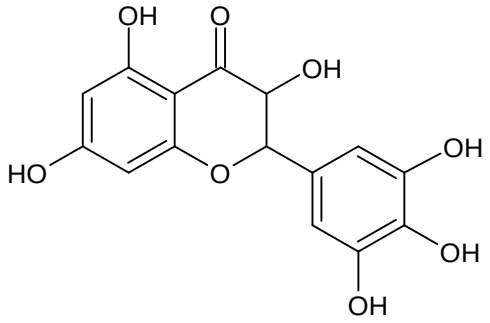
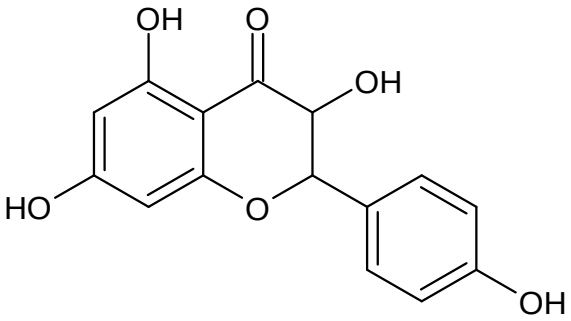
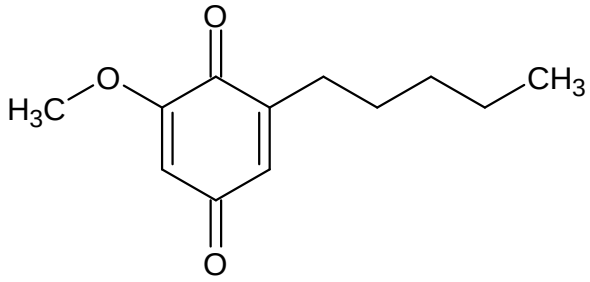
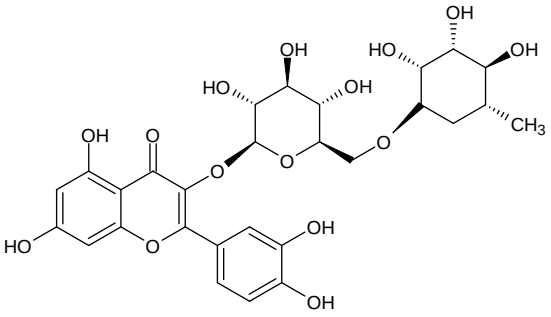
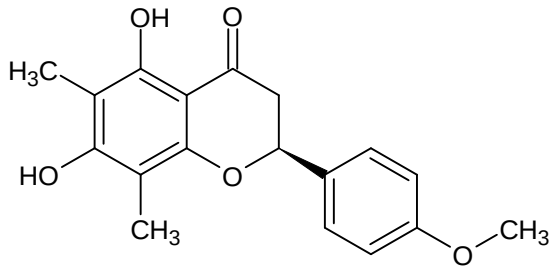
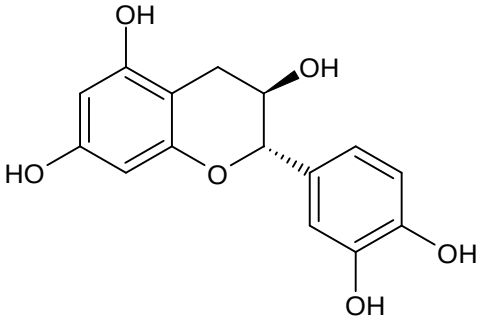
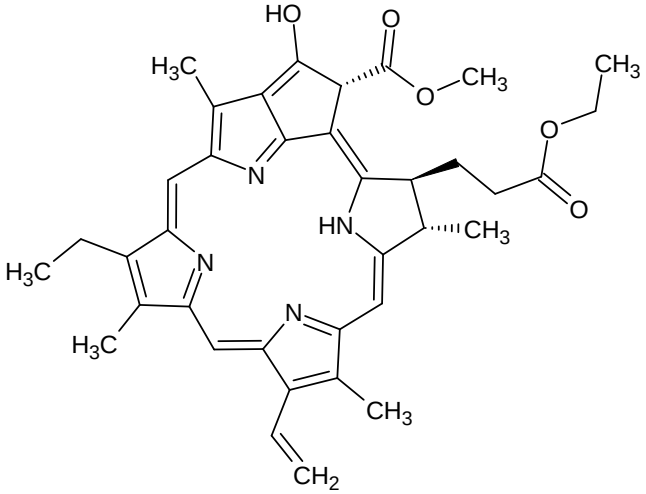
Chlorogenic acid	Quercetin
	
Myricetin	**Kaempferol**
	
Primin	**Rutin**
	

Figure. 1B

Matteucinol	Catechin
	
Pheophorbide A ethyl ester	
	
Ellagitannin	

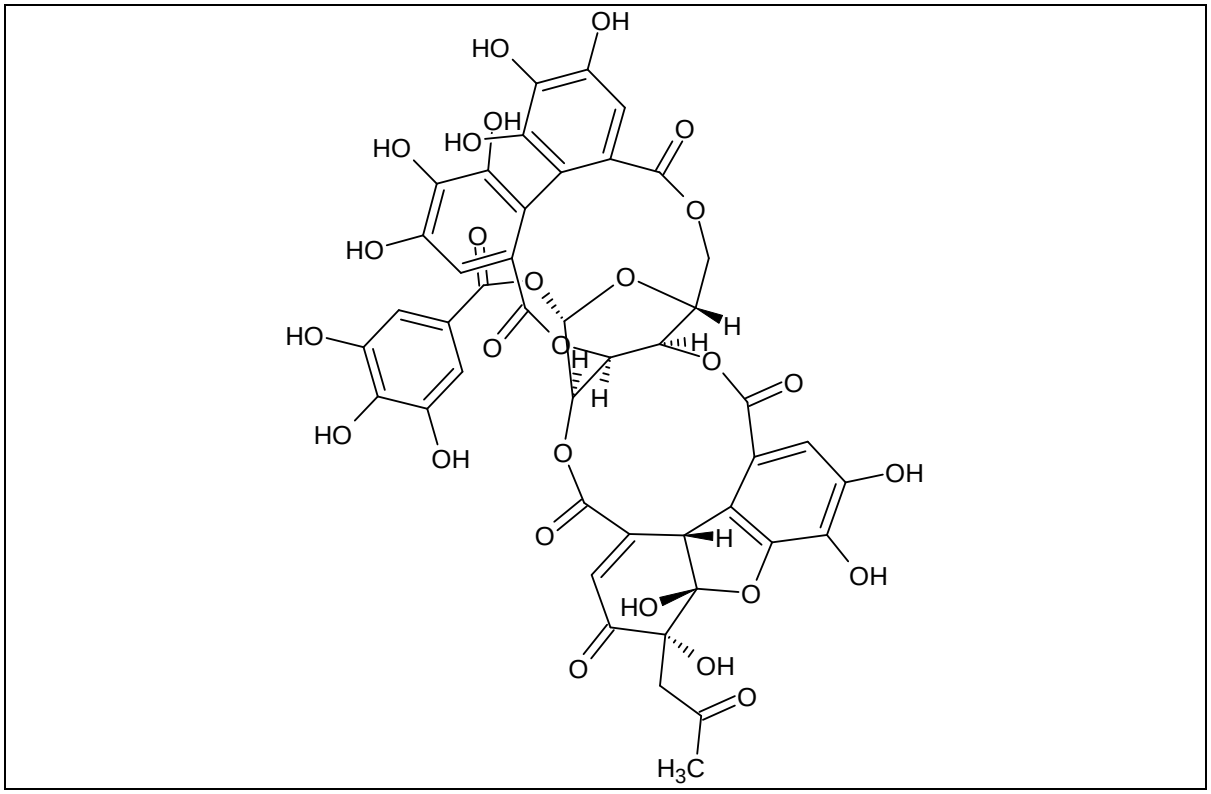


Figure. 1C

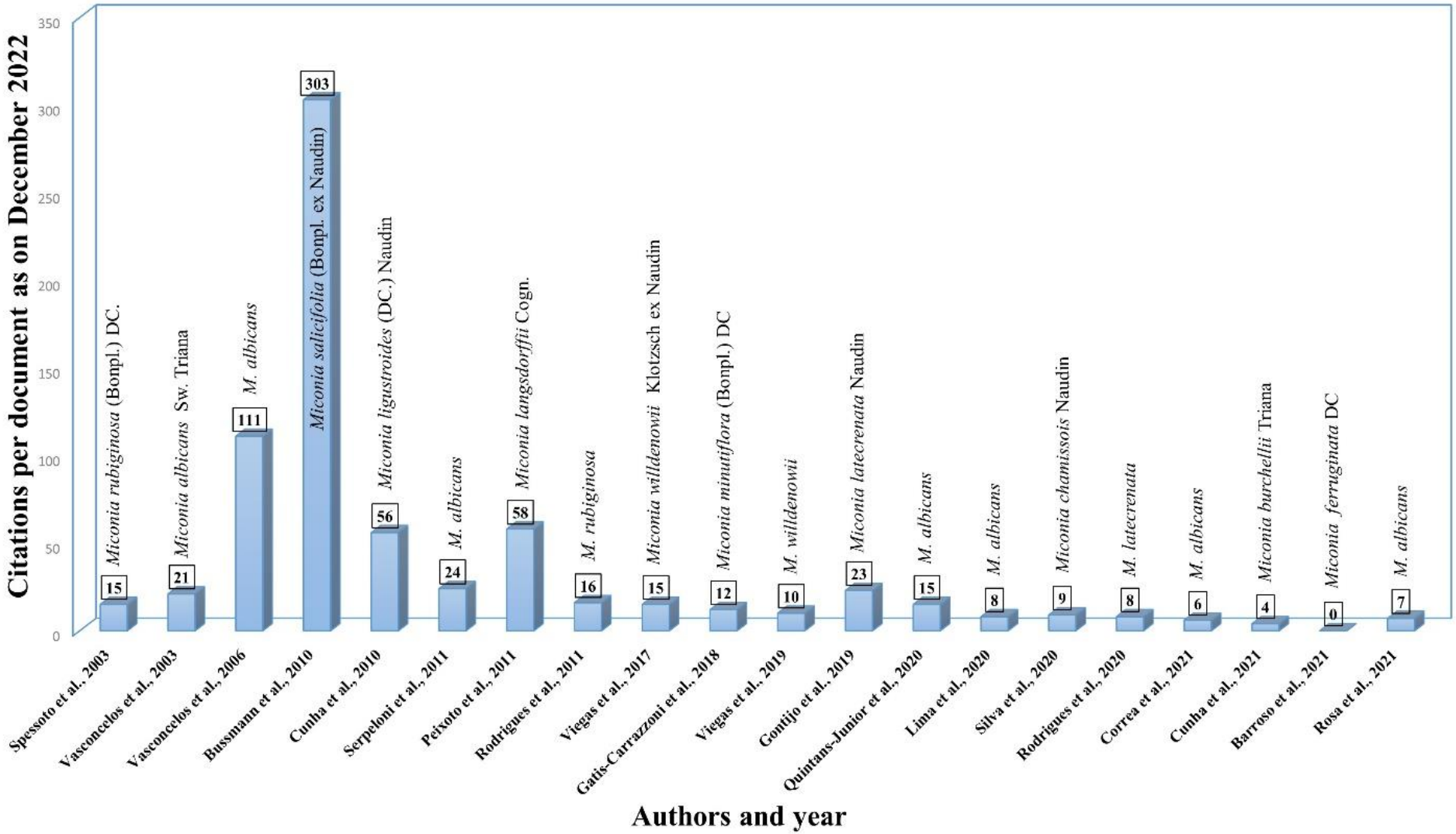


Figure 2. A graph depicting the volume of content referencing the genus *Miconia*, the number of citations it received, and the year it was published

3 OBJECTIVES

3.1 General Objectives

- To study the medicinal uses and related molecular mechanism of *Miconia* genus and perform phytochemical and pharmacological study with *Miconia albicans* Sw. Triana (Melastomataceae)

3.2 Specific Objectives

- To discuss literature on *in vivo* and *in vitro* studies on *Miconia's* therapeutic potential
- To analyze phytochemical data, compile safety concerns, and analyze *Miconia* genus biological activities
- To study the chemo profiles of *M. albicans* using HPLC-DAD-ESI-MS/MS
- To evaluate the anti-arthritic and anti-inflammatory profile of the ethanolic leaf extract of *M. albicans*
- To investigate the effect of *M. albicans* on TNF-α and IL-6 cytokines

4 STUDY 1: Health functions and related molecular mechanisms of *Miconia* genus: a systematic review

The study has been published in the journal *Heliyon* with an impact factor of 4 (Appendix A). https://doi.org/10.1016/j.heliyon.2023.e14609

4.1 Methodology

4.1.1 Search strategy

Literature searches were conducted using the four main electronic databases, namely PubMed, Embase, Scopus, and Web of Science and limited to Medical Subjects Headings (MeSH) and Descriptores en Ciencias de la Salud (DCS) (Health Sciences Descriptors) to identify studies published to December 2022. Specific keywords such as '*Miconia'*, 'biological activities', 'therapeutic mechanisms', 'animal model', 'cell-line model', 'antinociceptive', 'hyperalgesia', 'anti-inflammatory', and 'inflammation' were used. Additionally, a manual search was done online and in Google Scholar searching both academic and preprint literature to detect any articles not found in the databases. The data were collected from online journals published in English, irrespective of the region and publication type.

4.1.2 Study selection

Initially, two authors (JSSQ and RQG) performed the literature search in the databases. Then the extracted titles, abstracts, and relevant full-text published articles were reviewed independently by three investigators (SRG, JSSQ, and GRG), with any disagreement being settled by consensus or, failing this, by a fifth author (LJQJ); the final selection of articles for systematic review was made in consultation with all co-authors. Only original research papers investigating the *Miconia* genus, its well-defined chemical composition, and its potential therapeutic mechanisms using *in vivo* and *in vitro* experimental models were included in this

review. Review articles, meta-analyses, book chapters, conference proceedings, editorials/letters, patents, and case reports were excluded.

4.1.3 Data extraction

One of the authors (SRG) summarized data extraction from shortlisted studies individually. Table 1 and 2 summarizes the critical information from the selected studies: (a) plant extracts/natural molecules isolated from the plants, (b) the animals/strains or cell lines used, (c) the doses/routes of administration, (d) the proposed mechanisms of action, (e) the first author's name and year of publication.

4.2 Results

4.2.1 Search results

Figure 3 shows a PRISMA flow chart showing the process used in this systematic review's database search and articles assessment (MOHER et al., 2009). According to the PRISMA statement, the systematic investigation included *in vivo* and *in vitro* studies relating to the mechanism of action of plant species belonging to the *Miconia* genus. Among the 88 potentially relevant articles from four databases (PubMed: 52, Embase: 13, Scopus: 13, Web of Science: 10), 12 were discarded for being duplicates and 40 were excluded after comprehensive screening for the following reasons: (a) studies unrelated to the objectives and aim of this systematic review; (b) studies established to be reviews, editorials, conference proceedings, and meta-analyses. 36 full-text articles were then evaluated independently, with 21 studies being identified that met the eligibility criteria of this review.

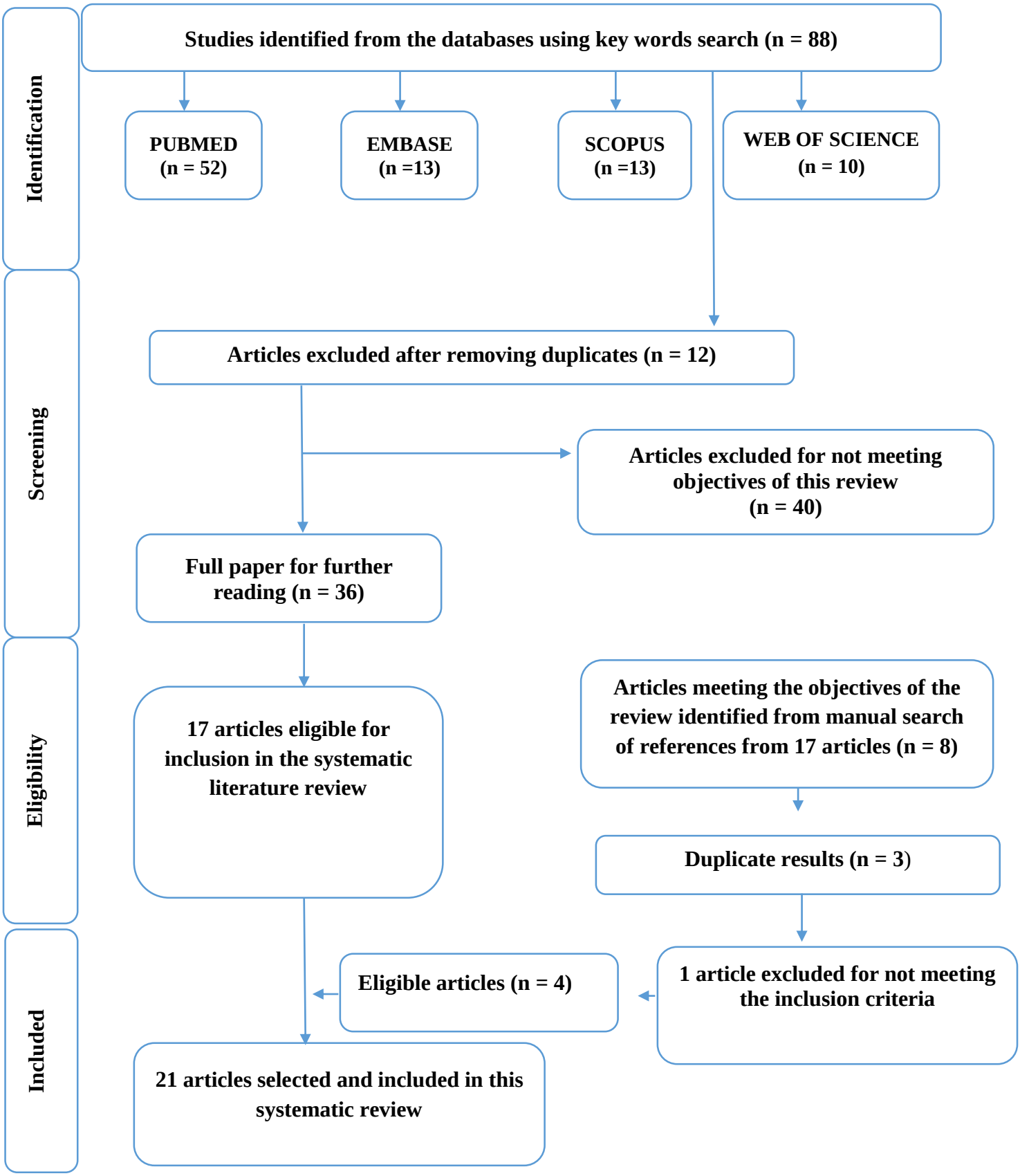


Figure 3. A PRISMA flow chart diagram of the selection process of eligible studies.

4.2.2 Evaluation of selected studies

Our review found that the following 11 plant species from this *Miconia* genus have been reported as containing various promising bioactive phytochemicals in reports conducted in both *in vitro* and *in vivo* experiments: *M. albicans, M. rubiginosa, Miconia salicifolia* (Bonpl. ex Naudin) Naudin., *M. ligustroides, M. stenostachya, M. cabucu, M. langsdorffii, M. willdenowii, M. minutiflora, Miconia latecrenata* Naudin., and *Miconia burchellii* Triana. The following biological activities were evaluated in eight *in vivo* studies, including one human osteoarthritis clinical study by GOMES et al. (2021); anti-inflammatory, anti-arthritic, antioxidant, and antinociceptive peripheral and central analgesic activity; while the *in vitro* studies investigated the extracts of plant species from *Miconia* for their antibacterial, anti-leishmanial, cytotoxic, mutagenic, schistosomicidal, anti-inflammatory, antioxidant, and anti-proliferative activities. The twenty-one articles reviewed here originated from Brazil (n = 20) and the USA (n = 1). Traditional populations and users of medicinal plants in the Northeast region of Brazil depend on folk medicine widely; most of these species are commonly found for sale in public markets (FERREIRA et al., 2021). Our results also show that plant species belonging to the *Miconia* genus that treat joint diseases (those with an inflammatory profile) are more popular with Brazilian folk medicine practitioners than medicinal plants used to treat other illnesses. This is because there is an increase in the elderly population and more people are getting these kinds of diseases (MOURA et al., 2016; ZANK; HANAZAKI, 2017).

4.3 DISCUSSION

4.3.1 *In vivo* studies

Our literature search identified 8 *in vivo* studies relating to the biological activities of the extracts and isolated compounds from *Miconia*, such as analgesic and antioxidant activity of *M. rubiginosa* (SPESSOTO et al., 2003), the antinociceptive and peripheral analgesic activity of *M. albicans* (VASCONCELOS et al., 2003), the analgesic and anti-inflammatory activity of *M. albicans* (VASCONCELOS et al., 2006), anti-arthritic and anti-inflammatory activity of *M. albicans* (QUINTANS-JÚNIOR et al., 2020), antioxidant and anti-inflammatory activity of *M. albicans* (CORREA et al., 2021), anti-inflammatory and antinociceptive of *M. minutiflora* (GATIS-CARRAZZONI et al., 2018), anti-inflammatory and antioxidant effect of *M. albicans* (LIMA et al., 2020), and anti-osteoarthritis activity of *M. albicans* (GOMES et al., 2021). Most *in vivo* studies included in our current review mainly focus on the analgesic and anti-inflammatory methodologies, which might be because plants of the *Miconia* genus are most commonly used to treat pain and inflammation in folk medicine, however, many plants with few chemical and pharmacological studies that validate the ethnopharmacological use (CUNHA et al., 2019).

4.3.2 Analgesic and anti-inflammatory activity

The analgesic effect of *M. rubiginosa* extract was studied in rats and mice using the acetic acid-induced writhing and hot plate tests, classical experimental models of analgesic screening were widely used to validate this pharmacological property (BARS et al., 2001; SPESSOTO et al., 2003). The extract (200 mg/kg/body wt.) showed a potent increase in the pain threshold and antinociceptive effect and inhibited acetic acid-induced writhing in mice. The presence of triterpenes (UA and OA, α- amyrin and β-amyrin) and sterols (lupeol and β-sitosterol) seems

responsible for the central and peripheral analgesic and anti-inflammatory profiles. Previous studies have indicated that these triterpenes play a role in modulating pro-inflammatory mediators and mitigating the deleterious effects of inflammation and cell proliferation (KANGSAMAKSIN et al., 2017; SANDEEP, 2020). The results suggest that the possible mechanism of action might be due to the reducing levels of prostaglandin, namely, $PGE_{2\alpha}$ and $PGF_{2\alpha}$ synthesis, the two critical prostanoids in the inflammatory cascade following the acetic acid injection, as well as through central inhibitory mechanisms. The synergistic action of natural molecules in the extract could have been accountable for the observed analgesic effect in biological activity studies (SANDEEP, 2020). Consequently, the analgesic activity study of the crude extracts of aerial parts of *M. rubiginosa* resulted in abdominal writhing inhibition and antinociceptive effects (SPESSOTO et al., 2003). *M. rubiginosa* crude extracts due to the presence of anti-inflammatory phytochemicals might be involved in the peripheral analgesic activity (DIAZ et al., 2000). Furthermore, the extracts of *M. rubiginosa* significantly reversed the acetic acid-induced writhing in mice. *M. rubiginosa* induced a significant increase in pain threshold throughout the experimental period, with a substantial increase in the percentage of protection. The presence of triterpenes and sterols in *M. rubiginosa* is the essential active principle responsible for the witnessed analgesic effect, possibly mediated by the modulation of prostaglandin synthesis, along with effects on central inhibitory mechanisms. Literature studies report that these compounds have analgesic and anti-inflammatory properties (GEETHA; VARALAKSHMI, 2001). There is consistent evidence that lupeol and β-sitosterol regulate tumor necrosis factor-α (TNF-α), vascular endothelial growth factor receptor-2 (VEGFR-2), and pro-inflammatory cytokines activity in cell proliferation, and the production of factors that drive the inflammatory process and, consequently, the pain process (KANGSAMAKSIN et al., 2017).

A similar study performed by VASCONCELOS et al. (2003), using *M. albicans* crude extracts (200 mg/kg/body wt.) in Swiss albino mice and Wistar rats, did not exhibit any analgesic effect on the central nervous system (CNS); however, there were clear peripheral analgesic effects, and a reduction in pain behaviour, and anti-inflammatory activities. Crude extract of the aerial parts of *M. albicans* has been shown to have analgesic effects due to the presence of triterpene acids and β-sitosterol (VASCONCELOS et al., 2003). β-sitosterol has been shown to have outstanding anti-inflammatory properties while reducing critical inflammatory mediators of the pain-related process (LIU et al., 2019).

The potential analgesic and anti-inflammatory activities of UA and OA, isolated from the aerial parts of *M. albicans* crude methylene chloride extract, were evaluated in a carrageenan-induced paw edema animal model. The compounds produced a significant anti-inflammatory effect and a prostaglandin synthesis inhibition. Thus, they showed a significant reduction in inflammatory pain, which seems to be confirmed by the amelioration of the increase in inflammatory mediators, such as the prostaglandins, induced by carrageenan and the subsequent inhibition of the inflammatory signaling pathways which is one of the main triggers for the pain profile of these animal models. The analgesic effect of the extract was mostly based on its peripheral mediated mechanism, which is compatible with the presence of the UA and OA compounds found (VASCONCELOS et al., 2006). UA and OA are inhibitors of key pro-inflammatory pathways in joint pain, such as the nuclear factor erythroid-2-related factor 2 (Nrf2) and nuclear factor-κB (NFκB) pathways (KIM et al., 2010; LI et al., 2016).

Similarly, LIMA et al. (2020) demonstrated that *M. albicans* extract exhibits a potential therapeutic activity against RA in carrageenan-induced paw edema. The study revealed that the extract reduced TNF-α, IL-1β, and consequently, the inflammatory nociception and edema caused by carrageenan injection. These results corroborated, at least in part, those reported by VASCONCELOS et al. (2006), suggesting that *M. albicans* has potential therapeutic uses in

chronic, difficult-to-treat conditions that are very disabling for patients, such as arthritis or other joint pain. The chemical study of the extract produced a vast number of flavonoids with polyphenol structures, and the authors suggest that its bioactivity may be explicitly credited to the presence of the composition of flavonoids containing polyphenols structure, such as rutin and quercetin. Flavonoids are anti-inflammatory and are used to mitigate chronic inflammatory diseases (GANDHI et al., 2018; CARVALHO et al., 2021). Thus, these findings reinforce the use of these anti-inflammatory plants in Brazilian folk medicine to treat joint and related pain (LIMA et al., 2020).

M. minutiflora leaf polyphenols rich-extract has shown anti-inflammatory activity and could reduce edema and the migration of leukocytes towards the site of inflammation, and was associated with suppressed concentrations of the pro-inflammatory cytokines such as TNF-α and IL-1β, while the antinociceptive actions involve central and peripheral mechanisms with the participation of α_2-adrenergic receptors. The anti-inflammatory mechanisms of *M. minutiflora* might be related to the decrease in the level of several inflammatory and pro-inflammatory mediators in the edema tissue via the suppression of pro-inflammatory cytokines concentrations (GATIS-CARRAZZONI et al., 2018).

Thus, the major compounds in the studied extracts (especially terpenoids and flavonoids) are known to act directly by mitigating the production and levels of circulating pro-inflammatory cytokines, influencing pathways related to oxidative stress, which are suggested to be responsible for the anti-inflammatory activities of several medicinal plants (GANDHI et al., 2018; QUINTANS et al., 2019). These therapeutic points of view would suggest using plant remedies to recommend these herb species used in traditional medicine to treat inflammation.

4.3.3 Anti-arthritic and anti-inflammatory activity

Studies have indicated that drugs that modulate or block the pro-inflammatory cytokine TNF-α and its related factors, including interleukins (IL)-1, IL-6, IL-8, and granulocyte macrophage-colony stimulating factor (GM-CSF) have been considered as one of the preferred treatments for the management of RA (BRENNAN et al., 1989; BRENNAN et al., 1992; MA; XU, 2013). *M. albicans* ethanolic leaf extract (MAEE), in doses of 50 and 100 mg/kg/body wt., was assessed for its analgesic and anti-inflammatory profiles in a carrageenan-induced arthritis-like model. The results showed that MAEE significantly lessened leukocyte migration in the pleurisy model and suppressed TNF-α and IL-1β in pleural lavage. Moreover, in the Complete Freund's Adjuvant (CFA) mice model, a primary animal model that mimics the signs and symptoms of RA in humans, MAEE administration in rats resulted in a significant decrease in nociceptive pain and hyperalgesic actions in the rearing test and decreased mechanical hyperalgesia. Moreover, MAEE significantly improved mobility in the open-field test and increased hind paw grip strength without any apparent damage to the liver. MAEE drastically reduced the volume of CFA-induced ipsilateral knee edema, the anti-inflammatory potency could be related to its positive effect on IL-6 and TNF-α in the knee joint (QUINTANS-JÚNIOR et al., 2020). Moreover, a dried extract of *M. albicans* (DEMA) reduced the carrageenan-induced edema of the paw. It ameliorated the inflammatory reactions by downregulating TNF-α and IL-1β and reducing antioxidant parameters, consequently reducing inflammatory nociception, important factors in reducing the inflammatory cascade (LIMA et al., 2020). Similarly, the *in vivo* anti-inflammatory effect of *M. albicans* fruits methanol extract, which is rich in phenolic compounds, flavonoids, hydroxybenzoic acids, terpenoids, ellagitannins, chlorogenic acid, and fatty acids was assessed in croton oil-induced ear edema in mice and was reported to have potential health benefits (CORREA et al., 2021).

An important aspect in these recent studies with different extracts of *M. albicans* (LIMA et al., 2020; QUINTANS-JÚNIOR et al., 2020; CORREA et al., 2021) is the possible ability of

the extracts to reduce RA symptoms, mitigate pro-inflammatory pathways, and reduce oxidative stress by acting as a potential anti-RA agent, similar to RA blockers which reduce TNF-α, IL-1β and IL-6 levels (ZANGERLE et al., 1992; TISSI et al., 1999). Moreover, TNF-α and IL-6 are cytokines at high levels in the synovial fluid of RA patients. The reduction of these cytokines has been directly related to improvements in the general condition of patients, especially in respect of joint pain that produces the greatest disability in these persons (WEI et al., 2015); thus the fact that the studied extracts of *M. albicans* seem to be able to act as TNF-α, IL-1β and IL-6 blockers is encouraging in respect of their potential use.

4.3.4 Osteoarthritis and joint pain

Interestingly, a clinical study conducted to assess the analgesic and immunomodulatory potential of *M. albicans* in knee osteoarthritis revealed its capability to lessen joint pain and inflammation and improve function (GOMES et al., 2021). This is probably the first clinical study using *M. albicans* for a common disease and provides further evidence for its use in traditional medicine. In the study, the oral administration of the extract at 1000 mg/day/body wt. for 30 days reduced the patients' pain, decreased Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Visual Analogue Scale of Pain (VASP) scores, and knee joint effusion resulting in functional improvement. This clinical study demonstrated the analgesic and anti-inflammatory effect of *M. albicans* on knee osteoarthritis, correlating with changes in the expression of inflammatory mediators in the synovial fluid. Moreover, the treatment decreased the expression of resist in and soluble TNF-α receptors (soluble tumour necrosis factor receptor (sTNFR)1 and sTNFR2 and increased the expression of adiponectin and leptin. In this study, the authors demonstrated the analgesic efficacy of *M. albicans* and their possible mechanisms concerning pain modulation, reduced inflammation,

and improved function in knee osteoarthritis. The study also corroborated the clinical safety of using this plant species and its therapeutic benefits (GOMES et al., 2021).

Although this study lacked a more careful analysis of possible toxicity and did not evaluate the inflammatory mediators involved (blood levels of cytokines, inflammatory mediators common in osteoarthritis, among others) more systemically, it is the first study in humans to provide evidence that supports the popular use of *M. albicans* and shows that it can be effective for diseases such as osteoarthritis and RA. This is, therefore, a fundamental study and provides a reasonable basis for further controlled and randomized clinical trials.

In summary, eight *in vivo* studies of the biological efficacy of the various extracts and their natural molecules from the *Miconia* genus were found to have potential health benefits, including analgesic, antioxidant, antinociceptive, and anti-osteoarthritis activities. Most importantly, *M. albicans* fruit extract has higher concentrations of flavonoids (quercetin, myricetin, kaempferol), terpenoids, and fatty acids such as palmitic, stearic, arachidic, behenic, elaidic, oleic, eicosenoic, and linoleic acids, as well as others, which might have contributed to the strong antioxidant and anti-inflammatory activities (CORREA et al., 2021). But there is still a lack of scientific evidence about some clinical aspects (molecular information, acute and chronic toxicity, effectiveness, etc.) and the possible effects of long-term use of pharmaceutical preparations that contain extracts from *Miconia* genus plants or their isolated compounds. This is an area that needs to be looked into more.

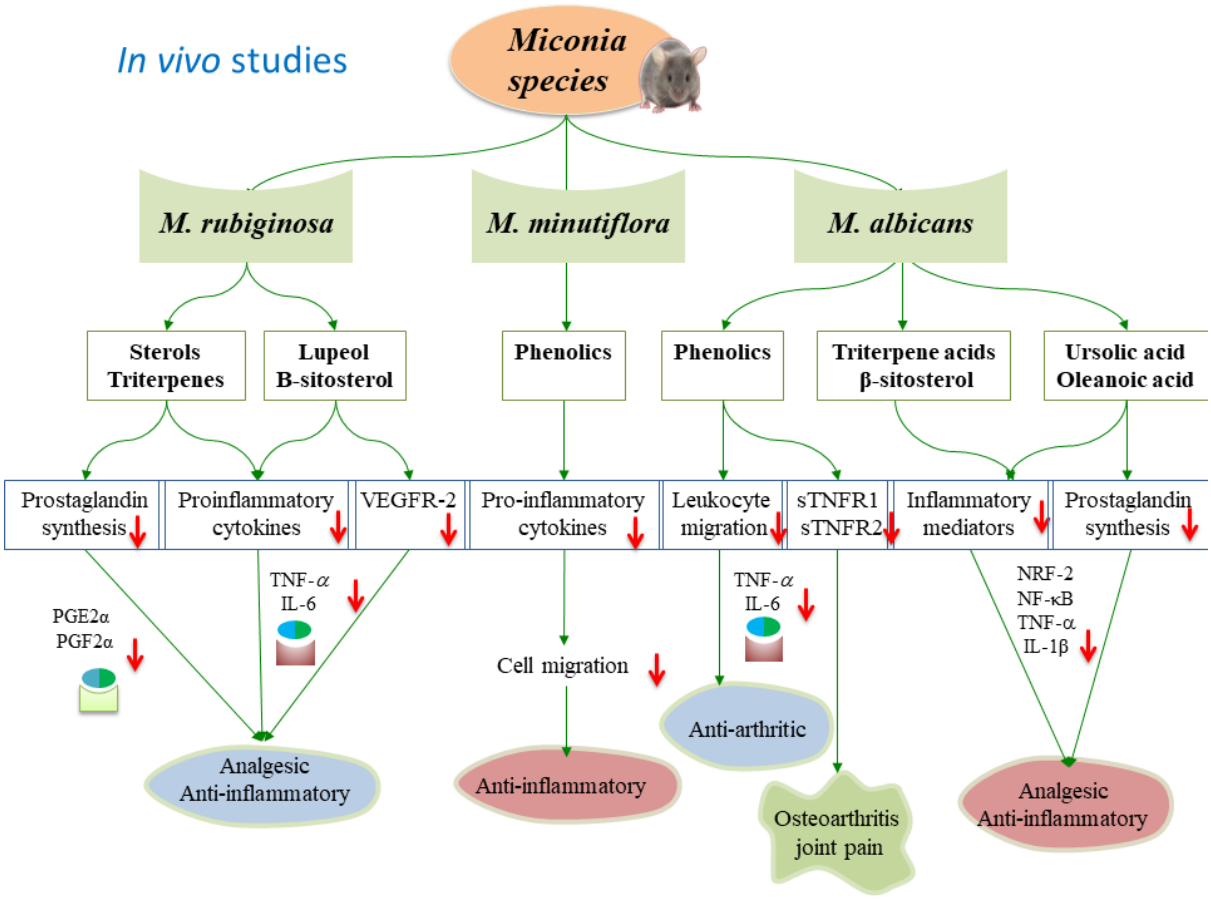


Figure 4. The *in vivo* biological studies evaluated the therapeutic potentials of the *Miconia* species, such as their analgesic, anti-inflammatory activity, anti-arthritis, joint pain, and anti-osteoarthritis activities The extracts and secondary metabolites from *Miconia* species, such as phenolic compounds, hydroxybenzoic acids, flavonoids, terpenoids, triterpenes, sterols, ellagitannins independently or synergistically, might have contributed to strong antioxidant and anti-inflammatory activities through cytokine-mediated responses. Pro-inflammatory and anti-inflammatory cytokine production might have modulated various ILs-mediated cellular responses to the tested diseases, especially for treating osteoarthritis, and joint pain.

4.3.5 *In vitro* studies

In our systematic search, we identified 18 *in vitro* studies of the pharmacological activities of the plant species from *Miconia* genus, such as the antioxidant property of *M. albicans* (CORREA et al., 2021), the cytotoxic activity of *M. burchellii* (CUNHA et al., 2021), the

antioxidative effect of *M. albicans* (LIMA et al., 2020), a chemo profile study of *M. albicans* (QUINTANS-JÚNIOR et al., 2020), the antioxidant, antibacterial and antimutagenic activity of *M. latecrenata* (GONTIJO et al., 2019), the phytochemistry, anti-inflammatory and antinociceptive properties of *M. minutiflora* (GATIS-CARRAZZONI et al., 2018), the schistosomicidal activity of *M. willdenowii* (VIEGAS et al., 2017), the phytochemistry of *M. rubiginosa* (RODRIGUES et al., 2011), the anti-leishmanial activity of *M. langsdorffii* (PEIXOTO et al., 2011), the cytotoxic and mutagenic activity of *M. albicans, M. cabucu*, and *M. stenostachya* (SERPELONI et al., 2011), the antibacterial activity of *M. salicifolia* (BUSSMANN et al., 2010), the antibacterial activity of *M. ligustroides* (CUNHA et al., 2010), and the phytochemical and antioxidant activity of *M. rubiginosa* (SPESSOTO et al., 2003).

4.3.6 Antibacterial and antifungal activities

A study conducted by BUSSMANN et al. (2010) reported that various solvent extracts prepared from 51 Peruvian medicinal plant species had antibiotic and inhibitory activity regarding microbial growth. The extracts inhibited *Escherichia coli* and *Staphylococcus aureus.* The ethanolic extracts exhibited higher inhibitory potential than the aqueous extracts against tested microorganisms. *M. salicifolia,* had the lowest minimum inhibitory concentrations (MIC) values, indicating its antibacterial activity. Several triterpene acids have great potential as antimicrobial compounds in treating infectious diseases (HORIUCHI et al., 2007). UA and OA from *M. ligustroides* have anti-microbial activity against some multi-resistant bacteria but not against many others (LIU, 1995; CUNHA et al., 2010). A synergetic effect was noted when different concentrations of various UA derivatives were evaluated to inhibit the growth of *Bacillus cereus*, *Vibrio cholerae*, *Salmonella choleraesuis*, *Klebsiella pneumoniae*, and *Streptococcus pneumonia,* and were found to have an antimicrobial effect against some of the microbes. The antimicrobial activity of several species from *Miconia*

against various microorganisms has already been reported in the literature (CELOTTO et al., 2003). The leaves of *M. latecrenata,* which contain copious amounts of ellagitannin, presented higher antibacterial effects against some gram-positive and negative strains. On the other hand, the phenolic-rich *M. latecrenata* show antimicrobial activity against antibiotic-resistant bacteria, which cause some significant infections to human health (GONTIJO et al., 2019).

The *M. willdenowii* leaves collected from the Brazilian Atlantic forest have antibacterial properties, and evidence would suggest that primin, the component with the highest concentration, is the main bioactive metabolite in the plant. Additionally, the findings reveal for the first time the extract's potent antibacterial and antifungal effects on *S. aureus*, *Candida krusei*, and *Candida glabrata* (VIEGAS et al., 2019). The phenolic compounds extracted from *M. latecrenata* leaves acquired from the Brazilian Atlantic forest showed the greatest potential for preventing the growth of *S. aureus* and *Pseudomonas aeruginosa*. Following bio-guided fractionation of the extract, the fraction demonstrated synergism with ampicillin and tetracycline for *S. aureus* and *P. aeruginosa,* respectively. These results imply that *M. latecrenata* leaf extracts and fractions may be employed as therapeutic antibacterial agents (RODRIGUES et al., 2020).

4.3.7 Anti-leishmanial and schistosomicidal activities

The extract was prepared from the aerial parts of *M. langsdorffii* was evaluated for their potential against promastigotes, mainly *Leishmania amazonensis*, the major parasite responsible for leishmaniasis in humans. Bioassay-guided fractionation of this extract revealed the presence of an extensive concentration of triterpenes. The compounds, UA and OA were observed to be the primary compounds in the plant extracts. Among the UA-derived substances, the C-28 methyl ester derivative exhibited the best activity. The study results showed that UA and OA are highly potent compounds of antileishmanial action and can be

effective agents against leishmania in the clinical location. An acute toxicity study of the molecules found they were safe even at high concentrations (ANDRADE et al., 2008), but further studies using animal models are warranted to screen these compounds for developing new antiprotozoal agents (PEIXOTO et al., 2011).

The crude ethanolic extract from *M. willdenowii* was assessed for its schistosomicidal activity. The extract showed greater schistosomicidal activity against *S. mansoni* worms than praziquantel. The ethanolic extract was further subjected to fractionation to identify its active lead molecule(s), with the hexane sub-fraction having considerably greater schistosomicidal activity against the adult worms. Moreover, chromatographic isolation of this active sub-fraction led to the isolation of 2-methoxy-6-pentyl-benzoquinone, also known as primin. This activity may be attributed to this significant bioactive metabolite. The authors reported that *M. willdenowii* extracts containing its active lead molecule primin showed important antischistosomal activity. Thus, these substances could be novel candidate for the treatment and management of microbes and could also act as a less toxic natural remedy against schistosomiasis (VIEGAS et al., 2017). The leishmanicidal activity of primin-containing ethanolic extract of *M. willdenowii* was also reported by VIEGAS et al. (2019), which demonstrated inhibition of 99.7% of the promastigote forms of *L. amazonensis* at a concentration of 80 μg/mL.

4.3.8 Cytotoxic and mutagenic effects

Knowledge of plant genotoxicity and potential mutagenic effects is necessary to develop plant-based phytochemical products and drugs. Extracts of plant species from *Miconia* were prepared and assessed for their cytotoxicity, mutagenicity, and protective effects on Chinese hamster lung fibroblast cell cultures (V79). The cytotoxicity study indicated a remarkable decline in cell viability at higher concentrations of plant extracts from *Miconia*, suggesting that

the mixtures of polyphenols present in these extracts could contribute to the dose-dependent cytotoxicity potential of this plant and should be further validated by the pharmaceutical industry due to its ever-growing number of nutraceutical properties (HALLIWELL, 2007).

The plant extracts found to be the most active as pro- or anti-oxidants revealed the presence of a copious amount of phenolic compounds. Hence, the reports of the study suggest that the plant extracts prepared from the *Miconia genus* containing large amounts of phenolics are responsible for the antioxidant activity *in vitro* due to their free radical scavenging properties. Phenolic components in fruits, vegetables, and medicinal herbs have been proposed to be active secondary metabolites and have antioxidant and anticancer properties (LIU, 2004). The interactions and potential synergistic properties of various plant extract polyphenols and their beneficial effects were reported in the study by FEINSTEIN et al. (1993), including regarding doxorubicin (XDR)-induced damage. In addition to XDR, the DNA-damaging potential of adriamycin has been reported (FEINSTEIN et al., 1993). Adriamycin is a substance that causes excessive production of free radicals, resulting in the generation of oxidative injuries to DNA and the production of oxidative stress-mediated lipid peroxidation (QUILES et al., 2002). The amelioration of DNA impairment reported in the study suggests that *Miconia* extracts containing phenolics may have protective effects on XDR-induced DNA damage by neutralizing the free radicals-mediated inflammatory reactions. Polyphenols have been shown as worthy quenchers of circulating free radicals, and therefore they inhibit DNA damage and act as vital antioxidant molecules (GALATI; O'BRIEN, 2004). The most outstanding feature of the present study is the indication of a therapeutic role of *Miconia* extracts in the recovery of XDR-induced DNA damage by enhancing DNA-repair efficiency in the damaged cells, which has been attributed to the presence of high levels of bioactive polyphenols (SERPELONI et al., 2011). Therefore, this study suggests *Miconia* species rich in polyphenols have anti-oxidant effects and high anti-mutagenic activity.

4.3.9 Anti-inflammatory and antioxidant properties

This study summarizes the recent investigations into the anti-inflammatory effect of *M. albicans* fruit extract (MAFRE). The chemical profile showed a high concentration of phenolic compounds, flavonoids, and fatty acids, that benefit the counter-inflammatory response with less toxicity on VERO cells. Flavonoids are the predominant substances in MAFRE and it has been shown that they are natural immunomodulators of pro and anti-inflammatory molecules (GANDHI et al., 2018). Nine fatty acids have been found in MAFRE, in addition to linoleic acid, which is one of its major constituents. The phytochemical contents of *M. albicans* fruit have been previously investigated and shown to contain untapped resources of phytochemicals with effective pharmacological actions beneficial for pharmaceutical and nutritional purposes (CORREA et al., 2021). *M. albicans* extract exhibited potent antioxidant activity, probably due to the high concentration of flavonoids, tannins, saponins, leucoanthocyanins, and, steroids. Significant levels of total phenolic (551.3 mg gallic acid equivalent/g of dried extract) and flavonoid contents (367.19 mg catechin equivalent/g of dried extract) have been identified. A study using HPLC-PDA revealed the presence of rutin and quercetin as two major flavonoids in the extract which act strongly to inhibit levels of nitric oxide, the intracellular reactive oxygen species (ROS) pathway, and pro-inflammatory cytokines, thus reducing, for example, the levels of TNF-α and IL-6, whilst also mitigating the oxidative imbalance common in the inflammatory process and tissue injury (SHANMUGASUNDARAM; ROZA, 2022). Regarding the antioxidant activity of this standardized extract, it has been shown that the anti-inflammatory phytochemicals rutin and quercetin present in *M. albicans* appears to exhibit profound activity in modulating the damaging effects of reactive oxygen species (LIMA et al., 2020).

The primary pharmacological activities of *M. latecrenata* support its therapeutic potential concerning ROS/reactive nitrogen species (RNS) related anti-inflammatory disorders.

Furthermore, the phenolic compounds from *M. latecrenata* significantly contribute to minimizing or inhibiting biological macromolecule damage, especially to DNA molecules. Phytochemical analysis of *M. latecrenata* revealed a high total phenolic content, especially ellagitannins, demonstrating a potential pharmacological activity. In addition, the extract's high antioxidant, antibacterial, and antimutagenic activities were observed in different tests. Therefore, this ellagitannin-rich extract can accelerate and reduce costs in the search for new therapeutic agents (GONTIJO et al., 2019).

4.3.10 Anticancer and antiproliferative activity

The *in vitro* anticancer potential of *Miconia chamissois* Naudin for treating glioblastomas was examined in the study by SILVA et al. (2020). The hydroethanolic extract of *M. chamissois* and its chloroform partition were tested for cytotoxicity in glioblastoma cell lines. A single molecule, matteucinol, was identified in the fraction. In the adult glioblastoma lines, matteucinol induced intrinsic apoptosis, which induced cell death. Additionally, matteucinol markedly decreased the tumour cells' invasion, migration, and clonogenicity. In an *in vitro* study, *M. burchellii* leaves were reported to have antiproliferative activity with the ethyl acetate fraction showing potent cytotoxic activity against four of the five tumor cell lines tested.

The cytotoxic activity was attributed to the strong presence of pheophorbide A ethyl ester in the respective fraction, which is projected to be effective against leukemia cell lines. The investigation showed that neither the fractions nor the compounds from *M. burchellii* contributed significantly to the antiproliferative potential (CUNHA et al., 2021). Additionally, *M. ferruginata*, a native Brazilian plant from the Cerrado biome known as "pixirica" or "babatena," which is rich in flavonoid derivatives from quercetin, catechins, and phenolic acids, showed potential cytotoxicity against tumor cells of 4T1, A549, and MDA-MB-231 in association with minimal cell toxicity against fibroblasts and should be taken into consideration

for further research against the treatment of cancer (BARROSO et al., 2021). Hydroethanolic extracts from the leaves of *M. albicans* and *M. chamissois;* reduced the viability of human cancer cell lines. They exhibited cytotoxic potential, leading to the discovery of novel chemotherapeutic agents for cervical cancer (ROSA et al., 2021).

To summarize, the *in vitro* studies reported that extracts from the *Miconia* genus and isolated compounds had anti-proliferative, anticancer, analgesic, antibacterial, cytotoxic, mutagenic, anti-leishmanial, antinociceptive, schistosomicidal and anti-osteoarthritic properties. The presence of phenolic compounds, hydroxybenzoic acids, flavonoids, terpenoids, ellagitannins and chlorogenic acids, and fatty acids such as palmitic, stearic, arachidic, behenic, elaidic, oleic, eicosenoic, linoleic acids in the *Miconia* genus may have contributed to the strong antioxidant, anti-cancer, and anti-inflammatory activities (CORREA et al., 2021).

4.3.11 Safety of *Miconia* species

Several *in vivo* experiments indicate that extracts from certain *Miconia* species and their isolated phytochemicals are safe for medical use, at least in the doses and routes assessed in our survey. However, there is a lack of studies demonstrating safety in controlled clinical trials. The consumption of *Miconia* extracts and biomolecules from the aerial parts of the plants has not been reported to cause any undesirable outcome. *M. albicans* methanol fruit extract has strong antioxidant and anti-inflammatory properties and contains phenolic compounds, flavonoids, terpenoids, ellagitannins, and chlorogenic acid, and is potentially non-toxic to VERO cells, with 95% cell viability (CORREA et al., 2021). Moreover, no untoward outcomes were reported in a recently published clinical trial in patients with knee osteoarthritis. Patients received *M. albicans* extracts (1000 mg/day/body wt. orally for 30 days) (GOMES et al., 2021). An acute toxicity study of *M. minutiflora* leaf methanolic extract (2000 mg/kg/body wt., orally)

in experimental models *in vivo* showed no significant changes in weight, or food and water intake, and did not produce any deaths or treatment-linked adverse reactions (GATIS-CARRAZZONI et al., 2018).

In general, *in vitro* studies reveal the antioxidant potential of plants from the *Miconia* species (GONTIJO et al., 2019; LIMA et al., 2020; CORREA et al., 2021). The n-butanol fraction and the isolated flavonoids from the methanolic extract of *M. albicans* leaves had significant antioxidant activities with a scavenging capacity against 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (PIERONI et al. 2011). The strong occurrence of phytochemicals, such as steroids, triterpenes, alkaloids, anthraquinones, glycosides, flavonoids, leucoanthocyanins, tannins, and saponins present in *Miconia* species, are known to have significant free radical scavenging capacity.

Moreover, GOMES et al (2021) highlighted the clinical safety of *M. albicans* usage. In a longitudinal examination involving knee osteoarthritis patients, *M. albicans* was administered orally at 1000 mg/day for 30 days, demonstrating no signs of toxicity or impairment in chronic usage, *M. albicans* showcases potential benefits for arthritis patients akin to those promoted by ibuprofen. This pioneering clinical study emphasizes *M. albicans'* positive impact on pain relief and functional improvement in knee osteoarthritis patients, reaffirming its potential as a safe alternative.

Therefore, the *in vitro* and *in vivo* studies suggested that the consumption of a limited number of the species from the *Miconia* genus is safe and can be used for clinical and therapeutic purposes; this is supported by the fact that it has been used in folk medicines around the globe for a long period without any adverse outcomes being reported.

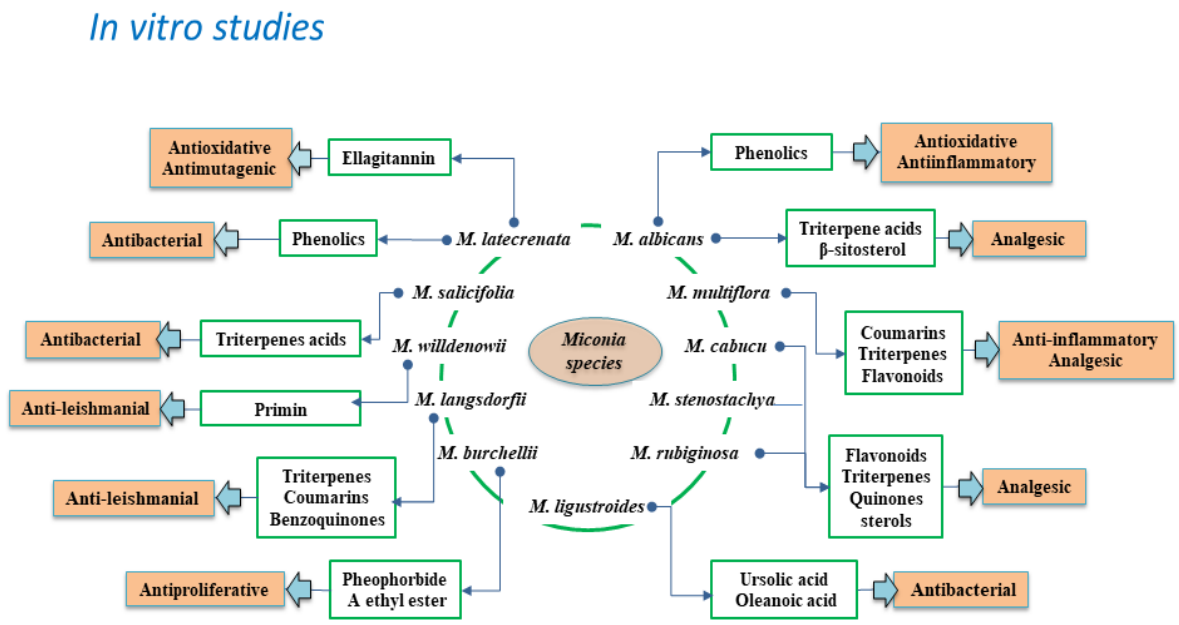


Figure 5. *In vitro* pharmacological studies of *Miconia* species showed several crucial health benefits, mainly focused on anti-inflammatory, antioxidant, analgesic, antibacterial, anti-leishmanial, antinociceptive, schistosomicidal, anti-osteoarthritis, cytotoxic, and mutagenic activities. Phenolic compounds, hydroxybenzoic acids, flavonoids, terpenoids, ellagitannins, chlorogenic acids, fatty acids, and many more, might have contributed to the aforementioned therapeutic potentials of *Miconia* species. Evidence indicates that the extracts and secondary metabolites from *Miconia* species are also safe for consumption and could be explored further for managing diseases.

4.4 Conclusion

In conclusion, we reviewed all current studies of the *Miconia* genus and its phytochemical and pharmacological properties to provide an up-to-date understanding of its therapeutic potential. *In vitro* pharmacological studies were mainly focused on its anti-inflammatory, analgesic, antibacterial, cytotoxic, mutagenic, antioxidant, anti-leishmanial, antinociceptive, schistosomicidal, and anti-osteoarthritic properties.

The *in vivo* biological studies mostly evaluated its therapeutic potential regarding its analgesic, antioxidant, antinociceptive, and anti-osteoarthritic properties. The identification and authentications of phenolic compounds; hydroxybenzoic acids; flavonoids; terpenoids; ellagitannins; chlorogenic acid; fatty acids such as palmitic, stearic, arachidic, behenic, elaidic, oleic, eicosenoic, and linoleic acids, among many others, has sparked increasing interest in these species as a strong antioxidant and anti-inflammatory agents.

In addition, analysis of the phytochemical composition of this species yielded eight bioactive phytomolecules: pheophorbide an ethyl ester, kaempferol, kaempferol-3-O-β-glucopyranoside, kaempferol-3-O-β-galactopyranoside, OA, UA, lupeol, and β-sitosterol that added therapeutic value to each plant belonging to the *Miconia* species. Phytochemical and pharmacological studies focused on the aerial parts belonging to *M. albicans*, *M. rubiginosa, M. salicifolia, M. ligustroides, M. stenostachya, M. cabucu, M. langsdorffii, M. willdenowii, M. minutiflora, M. latecrenata,* and *M. burchellii*.

There is sufficient *in vitro* and *in vivo* evidence to suggest that the extracts and natural molecules from *Miconia* are safe for consumption for clinical and therapeutic purposes, which is supported by the fact that no adverse outcomes have been reported regarding their traditional use.

Given the variety of their effects and potential health benefits, future investigations are warranted, particularly studies that examine the most effective delivery mechanisms and more

randomized studies of more species and controlled clinical trials to further examine their potential beneficial effects.

5 STUDY 2: HPLC-DAD-ESI-/MS/MS analysis of *Miconia albicans* Sw. Triana (Melastomataceae), and assessment of its anti-hyperalgesic and anti-inflammatory profiles in a mice arthritis-like model

The study has been published in the journal *Journal of Ethnopharmacology* with an impact factor of 5.4 (Appendix E). https://doi.org/10.1016/j.jep.2020.112938

5.1 Methodology

5.1.1 Plant material and extraction

M. albicans leaves were collected in Areia Branca, Sergipe State, Brazil, a region that is a part of the Brazilian Mata Atlantic (Atlantic Forest) (Coordinates: 10° 46' 43" S; 37° 20' 53" O). The botanist Marta C.V. Farias gathered voucher specimens of *M. albicans* (ASE 38244) and SisGen A08F052, which were then identified by Dr. Ana Paula Prata and deposited at the Herbarium (UFS), Sao Cristóvão, Sergipe. Following collection, the leaves were ground in a knife mill before being dried at 40° C in an air-circulating oven. After being successfully extracted with ethanol extract for 72 hours, the dried, ground-up leaves were concentrated by evaporation to produce 100 g of dry powder.

5.1.2 HPLC-DAD analysis

EEMA was analyzed using a Shimadzu Prominence chromatograph equipped with a solvent pump: LC-20AT, a self-injector: SIL-20A, a degassing system: DGU-20A, an array detector of diodes: SPD-M20A, one oven: CTO-20A and one system controller: CBM-20A. The column used was a Kromasil® C18 (250 mm x 4.6 mm ID, 5.0 µm) and a Kromasil® C18 pre-column (4.6 mm ID x 3.0 mm, 5.0 µm). The analysis of the data obtained using HPLC-DAD was carried out using the software Lab Solutions® (Shimadzu). Samples were filtered on 0.45 µm nylon membranes (Tedia). The method developed comprised a mobile phase

consisting of the solvents: water (0.1% phosphoric acid) and acetonitrile, (77:13, v/v) in isocratic mode for 40 min, going to 100% acetonitrile in 42 min and thus remaining up to 62 min at a flow rate of 0.60 mL/min at a temperature of 40° C, the injection volume was 10 μL and detection was performed at 356 nm. In the extract the rutin was identified by the retention time, UV spectrum and co-injection using commercial standard (Sigma). It was quantified using a calibration curve obtained with five distinct concentrations of the standard, injected in triplicate. The results were expressed as mean ± standard deviation μg of rutin/5 mg of dry EEMA. The limits of detection (LOD), quantification (LOQ) and linearity were determined based on what is recommended by RDC Nº 166, dated June 24, 2017 (ANVISA).

Liquid chromatography-high-resolution mass spectrometry instrumentation and conditions

A Shimadzu® (Kyoto, Japan) High Performance Liquid Chromatography System, coupled with an Amazon X or micrOTOF II (Bruker Daltonics, Billerica, MA, USA) with an electrospray ion (ESI) source was used to perform the ESI-IT-MS/MS and ESI-TOF-MS analysis, respectively. The LC System consisted of a LC-20AD solvent pump unit (flow rate of 600 $\mu L.min^{-1}$); a DGU-$20A_5$ online degasser; a CBM-20A system controller and an SPD-M20A (190 - 800 nm) diode array detector. The LC separation was performed on a Kromasil C-18 5 mm 100Å, 250 x 4.6 mm (Kromasil, Bohus, Sweden) analytical column. Injections (20 μL) were performed using an autosampler (SIL-20A). The mobile phase consisted of 0.1% formic acid in water (solvent A) and methanol (solvent B). Exploratory gradient was performed to elution in 60 min. The analysis parameters are as follows: capillary 4.5 kV, ESI in positive mode, final plate offset 500 V, 40 psi nebulizer, dry nitrogen gas with a flow rate of 8 mL/min and a temperature of 300º C. CID fragmentation was achieved in auto MS/MS mode using

advanced resolution mode for MS and MS/MS mode. The spectra (m/z 50-1000) were recorded every two seconds.

5.1.3 Animals

The Federal University of Sergipe's (UFS) Bioterium provided male Swiss albino mice (25-30 g). The animals were kept in polypropylene cages with a 12-hour light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.) at a constant ambient temperature (21±2° C). Prior to the experiments, the mice were given free access to filtered water and feed for up to 60 min. The UFS Animal Care and Use Committee has previously given its approval to all animal experimentation protocols (CEUA/UFS-23/2017). The National Council for the Control of Animal Experimentation (CONCEA), the Brazilian Directive of Use for the Care and Utilization of Animals for Scientific and Educational Purposes (DBCA), the CONCEA Guidelines for Euthanasia Practice, and Normative Resolution 15/2013 are a set of ethical guidelines. The doses and route of administration of the EEMA used in the experimental protocols of this study were: 50 and 100 mg/kg/body wt., by *per os* (p.o.; orally by gavage).

5.1.4 Carrageenan-induced pleurisy in mice

Increasing oral dosages of EEMA (50 and 100 mg/kg/body wt.) dissolved in distilled water were administered to several mice groups, while a control group additionally received distilled water. One hour before the injection of carrageenan into the pleura, the control group received nothing except distilled water. The mice were given 100 µL of a 1% (w/v) carrageenan suspension in sterile saline solution intrapleurally to cause pleurisy (Oliveira et al., 2012). Sham group got saline. The carrageenan solution was injected using a specially designed 13 x 5 needle into the right side of the thoracic cavity.

The animals were put to death four hours after the pleurisy was induced, and 1 mL of phosphate-buffered saline (PBS) containing ethylenediaminetetraacetic acid (EDTA; 10 mM) was used to pleural lavage the pleura to collect the pleural inflammatory exudate. An aliquot of 50 μL of the pleural lavage was diluted with Turk's solution (1:20) after being centrifuged (1500 rpm for 10 minutes at room temperature). The precipitate was then resuspended in 1 mL of PBS. Using a light microscope and a Neubauer chamber, the total number of leukocytes was determined (VINEGAR et al., 1973). ELISA was used to measure the amounts of TNF-α and IL-1β in the centrifuged exudates' supernatant.

5.1.5 Intra-articular injection of CFA

By giving four intra-articular injections of 10 μL of CFA (1 mg/mL *Mycobacterium tuberculosis* in paraffin oil, Sigma, EUA) into the right knee joint at days 0, 7, 14, and 21, we were able to create a modified form of a previously validated model of arthritic inflammation. Intraperitoneally (i.p.) administered ketamine/xylazine (100/5 mg/kg/body wt.) was used to anaesthetize mice. Using the femoral condyles as a guide, CFA or saline was injected into the articular area through the patellar ligament (GAULDIE et al., 2004; GHILARDI et al., 2012).

5.1.6 Evaluation of pain-related behaviors

On days 0 and 18 following the initial intra-articular CFA injection, behavioural indicators of arthritic joint discomfort, such as rearing and horizontal exploration activity, were assessed. On days 0, 4, 11, 18, and 25, mechanical hyperalgesia and grip strength tests were also conducted.

Horizontal exploratory activity and rearing

Additionally, assessed were spontaneous behaviours. A modified open-field test was used to measure horizontal exploration activity (locomotor activity) (RODRIGUES et al., 2012; TADAIESKY et al., 2006). A rectangular chamber made of polypropylene measuring 40 X 30 X 17 cm was populated with mice. Sixteen equal-sized rectangles measuring 10 X 7.5 cm each were used to partition the room into a grid using lines painted on the chamber floor for visual measurement. The experimenter counted the total number of times that distinct lines were crossed during the course of two minutes. The number of total vertical rearings requiring both hind limbs simultaneously was quantified during a five-minute test session after the mice had been adapted in open plexiglass observation chambers (KOEWLER et al., 2007).

Mechanical Hyperalgesia

Using a digital analgesimeter (digital Von Frey; Insight®, Sao Paulo, Brazil), mechanical hyperalgesia was investigated in mice using the technique previously described by CUNHA et al. (2004). The mice were housed in cages (12 x 10 x 17 cm) with wire mesh floors in a calm area, where they were given an hour to acclimatize before the measurements began. A progressive increase in pressure was taught to the investigator to put the tip perpendicularly to the middle of the hind paw. The paw withdrew at the conclusion, and there were obvious flinching movements afterward. The pressure intensity was then automatically recorded after this response. Three measurements with short intervals were averaged to determine the stimulus intensity.

Measurement of grip strength

A grip strength meter (Model EFF 305, Insight®, Brazil) was used to evaluate grip strength. The device was made up of a force transducer and a T-shaped metal bar. The experimenter gently held the mouse by the base of the tail and allowed it to grasp the metal bar

with its hind paw in order to test the grip strength of the hind paws. The mice were first permitted to hold onto a wire mesh cylinder with their forepaws in order to prevent them from holding onto the metal bar during the recording (MONTILLA-GARCÍA et al., 2017). The experimenter pushed the mice backward by the tail until grip was lost as soon as they had their hind paws on the transducer metal bar. Each measurement's peak force was automatically recorded by the device in grams (g). Each mouse's hindlimb grip strength was evaluated in triplicate. Prior to the administration of CFA or saline (control group), the average of two measurements made on different days was used to calculate the basal grip strength values for each animal. This value served as a reference for later calculations and was taken into account as 100% of grip strength.

5.1.7 Anti-inflammatory effect

Determination of knee diameter

A digital electronic caliper (MTX®, TollsWord) was used to measure the knee joint's diameter. The anterior-posterior (AP) and latero-lateral (LL), ipsilateral, and contralateral locations of the knee were measured. The radius is equal to the mean of AP and LL divided by two, and the area was derived using the formula: $V = \frac{4*\pi*raio^3}{3}$. The difference between the contralateral and ipsilateral knee volumes, divided by the contralateral knee volume, multiplied by one hundred, was used to compute the percentage increase in joint edema.

TNF-α and IL-6 cytokines

At the end of the evaluations performed on the animals subjected to the arthritis model, anesthesia (ketamine/xylazine 100/5 mg/kg/body wt. i.p.) was performed and the intra-articular space was washed three times with 10 μL of 10 μM EDTA in PBS. After centrifugation (10000 rpm, 5 min), the supernatant was diluted and the concentrations of TNF-α and IL-6 were

measured using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience®) according to the manufacturer's instructions. The colorimetric measurements at 450 nm were made in a microplate reader (ASYS®) and the concentrations were obtained by interpolation from a standard curve. All results were expressed as picograms (pg) of cytokine per milliliter (mL).

5.1.8 Statistical analysis

The data obtained were evaluated by analyses of variance (ANOVA) by means of one- and two-way trials, followed by Bonferroni's post hoc test or Tukey's test. In all cases, differences were considered significant if p was < 0.05. For these analyses we used the Graph Pad Prism (5.0) software (San Diego, CA, USA).

5.2 Results

5.2.1 Analysis of the EEMA by HPLC-DAD

Rutin's existence was confirmed by HPLC-DAD analysis of the EEMA, allowing for its quantification. Rutin was present in the amount of 6.59±0.08 μg/5 mg dry EEMA. The relative LOD and LOQ values were 0.18 and 0.57 μg/mL (Figure 6). This finding supports information from the literature that *M. albicans* contains rutin (PIERONI et al., 2011). Because it has an antiarthritic action, inhibits the inflammatory process in both its acute and chronic stages, and lowers RA by suppressing oxygen radical overproduction, rutin was chosen as an analytical marker of EEMA (GUARDIA et al., 2001; OSTRAKHOVITCH; AFANAS'EV, 2001).

By analyzing the fragmentation patterns seen in their mass spectra (MS2 and MS3 tests), 23 compounds in *M. albicans* leaf extract were speculatively identified. For the thorough assessment of the samples, information from literature and reference standards was also used. In Table 3, substances identified by negative ionization are included together with their peak assignments, retention durations, and mass spectrum data. The analysis of the fragmentation pathways of ions in the negative modes, the observation of glycosidic residues (pentosyl (132 Da), rhamnosyl (146 Da), glucosyl (162 Da), and rutinosyl (308 Da) cleaved sequentially, and the comparison of the generated distinctive aglycone fragments to the existing literature, all assisted in the identification of flavonols. Among these substances, one was found to be kaempferol glycoside (19), eight were found to be glycosides of quercetin (9, 10, 11, 12, 14, 15, and 20), three were found to be isorhamnetin glycosides (17, 19, and 21), and two were found to be myricetin glycosides (7 and 8).

On the basis of the primary ion fragments generated during the MS2 experiments, derivatives of kaempferol, quercetin, isorhamnetin, and myricetin were also observed. These derivatives appeared at m/z 284 and 285 for kaempferol derivatives, m/z 300 and 301 for

quercetin derivatives, m/z 315 and 314 for isorhamnetin derivatives, and m/z 316 and 317 for myricetin derivatives; these pairs of ion fragments corresponded to the respective homolytic and heterolytic cleavage of the glycosidic bonds in these compounds (JAISWAL et al., 2012; WANG et al., 2018)

Additionally, the MS3 ion fragments were matched to data from the literature and were associated with the flavonol core (kaempferol, quercetin, isorhamnetin, or myricetin) (Table 3). After investigation of the parent ion m/z 631 [M-H] characterized by the sequential loss of 162 Da (hexose) and 152 Da (galloyl moiety) in keeping with its high solution MS data, compound 7 was identified as myricetin galloyl hexoside (JAISWAL et al., 2012). There were identified and provisionally ascribed five ellagitannins. They could be recognized by the distinctive fragment ion spectra they produced, which showed successive losses of the residues galloyl (m/z 152), gallate (m/z 170), and HHDP (m/z 301). Compound 13, showed the deprotonated molecular ion at *m/z* 447 [M-H] and daughter ion at *m/z* 301 corresponding to the neutral loss of 162 Da (hexose). MS^3 fragments of *m/z* 301 follow ions characteristic of ellagic acid.

By analyzing the parent ion m/z 503 [M-H], which revealed fragment MS2 with m/z 315 corresponding to the loss of acetyl-deoxyhexoside unit (188 Da), compound 22 was identified as methyl ellagic acid-O-acetate-deoxyhexoside. The fragment of methyl ellagic acid was detected in an MS3 spectrum at m/z 315 (NEVES et al., 2018). According to MENA et al. (2012), the fragments of isomers 1 and 2 corresponded to HHDP-hexoside, and peak 3 was speculatively identified as the pedunculagin isomer (Table 3) (NEVES et al., 2018).

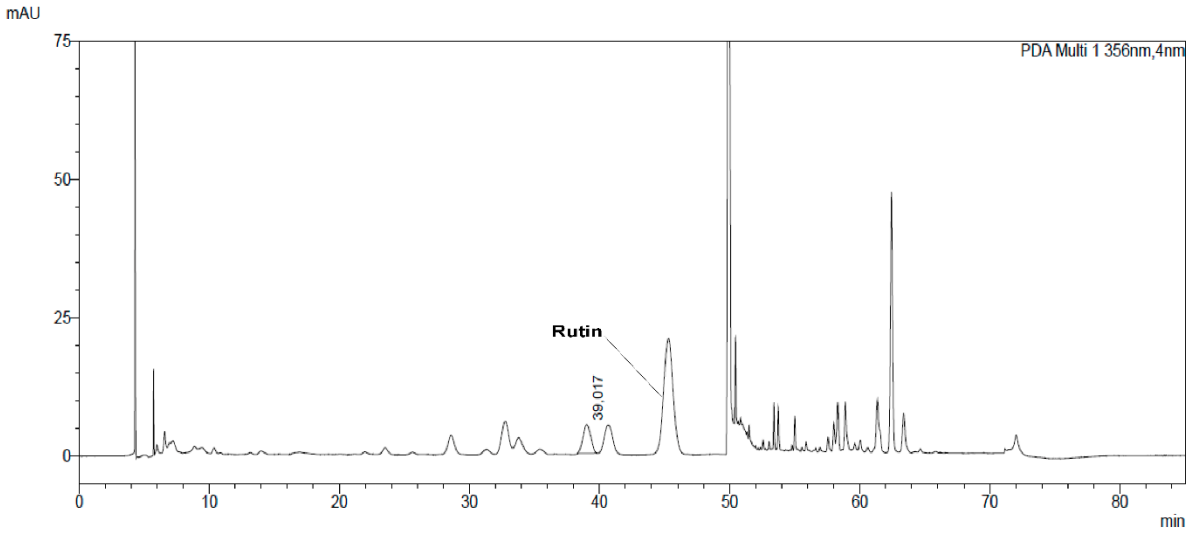


Figure 6. RP-HPLC chromatogram of ethanolic *Miconia albicans* extract at 356 nm for identification and quantification of rutin (Retention time: 39.0 min).

5.2.2 Effect of M. albicans on carrageenan induced pleurisy in mice

As predicted, when compared to the sham group, the leukocytes in the pleural cavity considerably ($p<0.001$) increased following carrageenan administration. We showed that EEMA pretreatment (50 and 100 mg/kg/body wt., p.o.) significantly decreased the number of leukocytes ($p<0.001$) when compared to the vehicle group, and this effect appeared to be dose-dependent given the significant difference ($p<0.01$) between the groups treated (Figure 7A). These findings supported, at least in part, earlier research that found *M. albicans* aerial parts crude methylene chloride extract and its two main compounds, ursolic acid and oleanoic acid, displayed a striking anti-inflammatory profile. However, this research used a non-specific test (the formalin test) and had only begun to analyze its results. Additionally, after HPLC-DAD-ESI-MS/MS analysis the EEMA demonstrated a different than expected phytochemical profile

of its main compounds due to being different types of extracts (more polar than methylene chloride extract).

We looked at the levels of cytokines in the pleural exudates of pleurisy induced by the carrageenan model, an important screening animal model for anti-inflammatory drugs, to understand how some of the key cytokines for the management of chronic painful diseases, such as RA, could be affected by the EEMA pretreatment (MOORE, 2003). TNF-α and IL-1β levels were measured using an ELISA test in the supernatant of centrifuged exudates. After injecting carrageenan, we discovered that TNF-α and IL-1β levels considerably ($p<0.001$) rose in the supernatant of centrifuged exudates. However, compared to the vehicle group, pretreatment with EEMA at both doses was able to significantly lower ($p<0.001$) TNF-α and IL-1β levels in pleural exudates (Figure 7B and 7C).

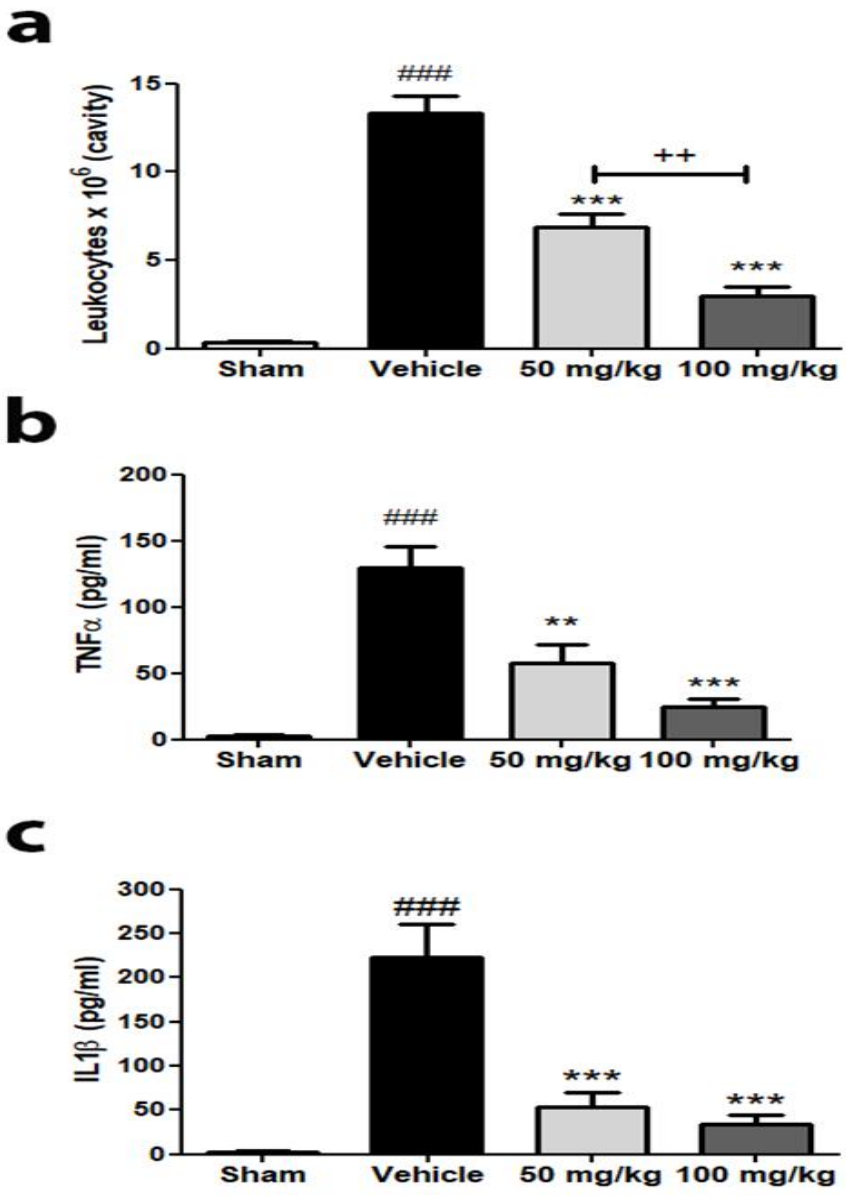


Figure 7. Effect of *M. albicans* on carrageenan induced pleurisy in mice. Distilled water (vehicle and Sham) or *M. albicans* (50 and 100 mg/kg/body wt., p.o.) were administered 1 h before carrageenan injection. The analyses were performed 4 h after carrageenan injection to evaluate the recruitment of total leukocytes (a) and the levels of TNF-α (b), IL-1β (c). Data were expressed as mean ± SEM (n = 10 animals per group). **p<0.01 and ***p<0.001 vs vehicle groups; $^{###}$p<0.001 vs sham groups; $^{++}$ p<0.01 vs MIC groups (One-way ANOVA, followed by Tukey's test).

5.2.3 Anti-hyperalgesic effect of M. albicans on mechanical hyperalgesia in a model of CFA-induced arthritis

Figure 8A depicts the assessment of mechanical hyperalgesia in the CFA-induced arthritic mouse model. Pretreatment with EEMA (50 and 100 mg/kg/body wt., p.o.) significantly reversed the hyperalgesic behaviour on all the days assessed when compared with the vehicle group ($p<0.001$), demonstrating its antihyperalgesic profile. The vehicle group showed a significant reduction in nociceptive threshold compared to the sham group ($p<0.001$). At 11, 18, and 25 days, we found that the mice that had been given the vehicle had considerably lower hind paw grip strength compared to the sham group ($p<0.001$), while the mice that had been given the EEMA had significantly higher grip strength (Figure 8B).

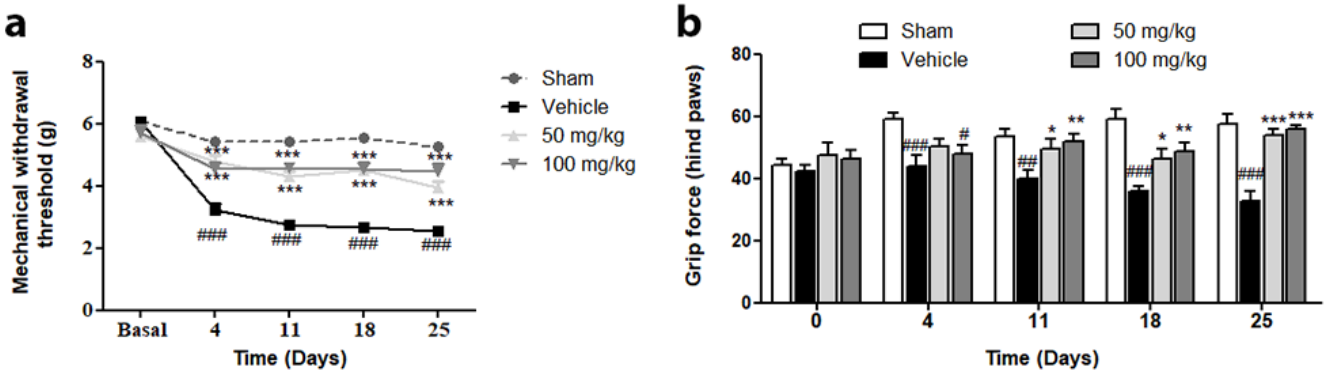


Figure 8. a) Anti-hyperalgesic effect of *M. albicans* (50 and 100 mg/kg body wt., p.o.) on mechanical hyperalgesia in a model of CFA-induced arthritic. b) Effect of ´ *M. albicans* (50 and 100 mg/kg/body wt., p.o.) on muscle strength of hind paws in CFA-induced arthritis model assessed by Grip Strength Meter. Data were expressed as mean ± SEM (n = 10 animals per group). *p<0.05, **p<0.01 and ***p<0.001 vs vehicle groups; $^{\#\#}$p<0.01; $\#^{\#\#}$p<0.001 vs sham groups (two-way ANOVA followed by Bonferroni's post hoc test).

5.2.4 Anti-inflammatory effect of M. albicans on joint swelling and pro-inflammatory cytokines levels induced by CFA intra-articular injection.

In line with expectations, repeated injections of CFA led to a considerable increase in knee edema in the ipsilateral knee joint as compared to the contralateral knee joint or in the knee joints of mice that had saline injections (Figure 9A). In mice receiving CFA treatment, knee edema was limited to the ipsilateral joint and absent in the contralateral hind limb. This edema persisted for 25 days from the start of the CFA provocation. In order to track the progression of the arthritis-like condition or maybe reduce this inflammatory state, oral administration of EEMA or a vehicle was started on day 1 and continued until day 25. At days 4, 11, 18, and 25 after commencing CFA provocation, the anti-inflammatory profile of chronic EEMA administration at dosages of 50 and 100 mg/kg/body wt. (by oral route) was assessed (Figure 9A). EEMA considerably ($p<0.001$) decreased the width of the ipsilateral knee edema brought on by CFA, seemingly in a dose-dependent way. In this study, we showed that pretreatment with EEMA, at both doses, significantly ($p<0.001$) inhibited the rise in IL-6 and TNF-α levels in the knee joint fluid of mice as compared to the vehicle group (Figure 9B and 9C).

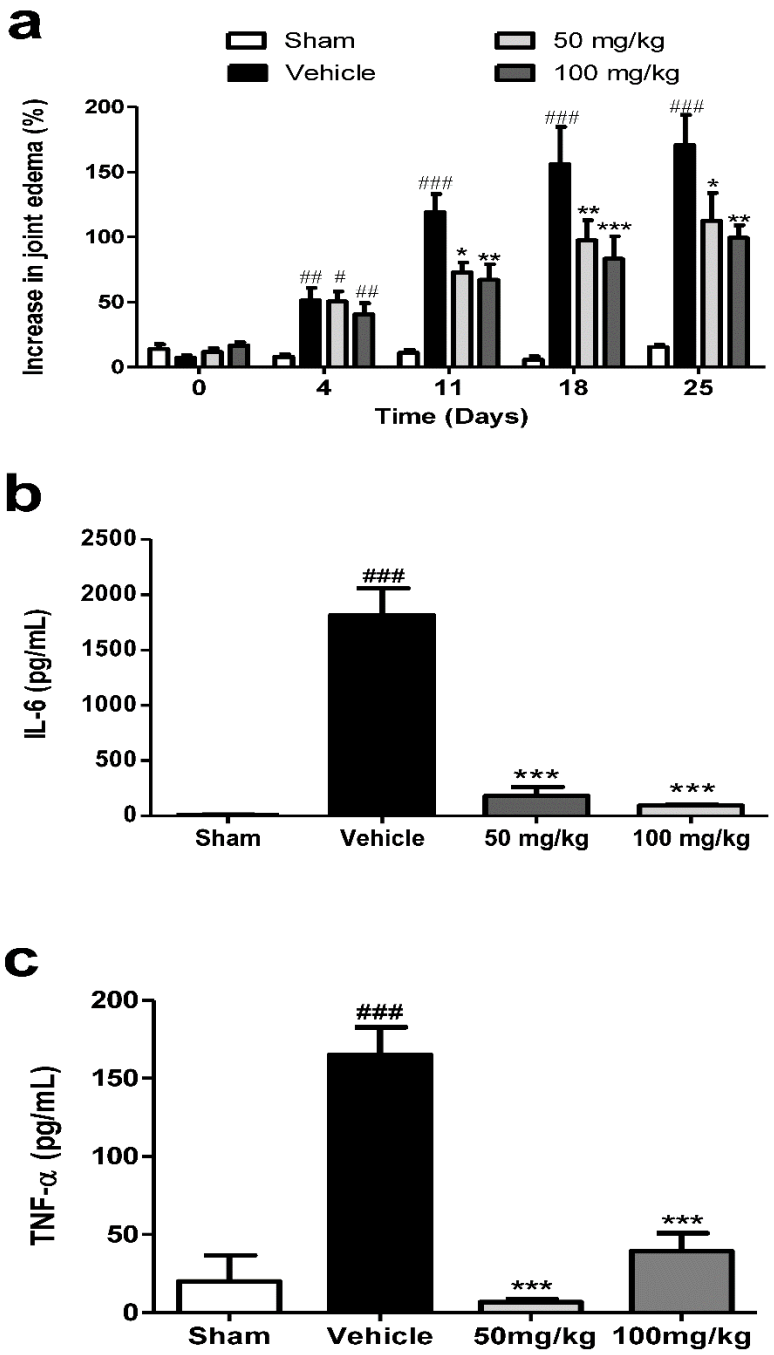


Figure 9. Evaluation of the anti-inflammatory effect of *M. albicans* (50 and 100 mg/kg/body wt.) on joint swelling (a) and pro-inflammatory cytokines levels (b, c) induced by CFA intra-articular injection. a) Edema inhibition (n=11). b) Synovial fluid IL-6 levels (n=6). c) Synovial fluid TNF-α levels (n=5). **p<0.01 and ***p<0.001 vs vehicle groups; $^{\#}$p<0.05; $^{\#\#}$p<0.01,

###p<0.001 vs sham groups (two-way ANOVA followed by Bonferroni's post hoc test or One-way ANOVA followed by Tukey's test).

5.3 Discussion

Arthritis, a chronic joint inflammation, is challenging to treat due to the dysregulation of pro-inflammatory cytokines and enzymes (HADAVI; SHENKER, 2019; WALSH; MCWILLIAMS, 2014; CHU et al., 2018). RA is a frequent autoimmune disease, with chronic pain being a difficult symptom to treat. Treatment aims to eliminate symptoms, slow disease progression, and optimize patients' quality-of-life. Current therapeutic options lack specificity, efficacy, and adverse events (KHANNA et al., 2007; NGOC et al., 2005). Chronic RA is often associated with low adherence and treatment abandonment due to side-effects or ineffectiveness (HER; KAVANAUGH, 2015; MÜLLER et al., 2015). New therapeutic proposals aim to manage specific cytokines and cytokine receptors, neutralizing their function and preventing their binding to their cognate receptors (VENKATESHA et al., 2014).

Plant-derived molecules and medicinal plants exhibit anti-inflammatory activity by inhibiting cytokines, chemokines, nitric oxide production, and modulating Th1/Th2 cells and the arachidonic acid pathway (CHOUDHARY et al., 2015; GANDHI et al., 2018; RUTTEN et al., 2014; QUINTANS et al., 2014; 2019). Extracts enriched with flavonoids and terpenes are effective in managing pro-inflammatory cells, producing both anti-inflammatory and analgesic effects (GANDHI et al., 2018; LEYVA-LÓPEZ et al., 2016; QUINTANS et al., 2019). Natural products (NPs) are promising for developing innovative therapeutic proposals for RA management, as they can target the main targets of the autoimmune disease (CARVALHO et al., 2019; CHOUDHARY et al., 2015; KOLASINSKI, 2012). Drugs modulating proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are also promising

for RA treatment, as they reduce pain and slow disease progression (CARVALHO et al., 2019; FELDMANN et al., 1996; WALSH; MCWILLIAMS, 2014).

Swelling, pain, and stiffness of the synovial joints are symptoms of RA, which is closely related to an inflammatory response driven on by many environmental and hereditary causes (ARAÚJO-FILHO et al., 2018; CHOUDHARY et al., 2015; TAPPE-THEODOR; KUNER, 2014; KOLASINSKI, 2012; SIQUEIRA-LIMA et al., 2017; FRANK-BERTONCELJ et al., 2017). Despite the undeniable improvements in these patients' quality of life, current medication treatments do not totally alleviate the primary symptoms of RA or provide patients with long-lasting benefits (HER; KAVANAUGH, 2015; MÜLLER et al., 2015). Advances in analytical techniques enable complex mixture identification, plant metabolic profiles, and avoiding dereplication of known compounds. Hyphenated techniques, like LC-MS/MS, combine separation and structural identification for NP identification, mainly using collision-induced dissociation (CID) activation (ERNST et al., 2014; DEMARQUE et al., 2016).

Folk medicine practitioners have, with or without the knowledge of the primary physician, used a sizable number of herbal products in the treatment of autoimmune illnesses, including RA. Many of these compounds found in medicinal plants are now being researched for their chemical properties and therapeutic potential. *M. albicans* is one of the medicinal species that is frequently used to treat rheumatic pain, but nothing is known about its chemistry, molecular makeup, or potential mode of action. In order to determine the potential anti-inflammatory mechanism, we assessed *in vivo* the anti-arthritic activity of EEMA in a mouse arthritis model and carried out phytochemical analyses utilizing HPLC-DAD-ESI-MS/MS methods. By analyzing the fragmentation patterns seen in their mass spectra (MS2 and MS3 tests), 23 chemicals in *M. albicans* leaf extract were speculatively identified. The chemical profile of EEMA as observed by HPLC-ESI-MS/MS is consistent with information from the literature

and other investigations of the genus and species (PIERONI et al, 2011; LIMA et al, 2018; CUNHA et al, 2019).

The proinflammatory cytokines TNF-α and IL-1β are crucial in RA pathogenesis, with their levels in synovial tissue, fluid, and serum (GOODMAN et al., 1993; MATSON et al., 2006; LIU et al., 2009). To characterize the anti-inflammatory effect of *M. albicans*, a mouse model of pleurisy induced by carrageenan was used. The results showed that pretreatment with *M. albicans* significantly reduced leukocyte infiltration compared to the vehicle group, with a dose-dependent effect. This finding supports previous studies showing the anti-inflammatory properties of crude methylene chloride extracts from aerial parts from *M. albicans*. The study investigated the impact of EEMA pretreatment on TNF-α and IL-1β levels in pleural exudates from the carrageenan model, an important animal model for anti-inflammatory drugs (MOORE, 2003). Results showed that carrageenan injection increased TNF-α and IL-1β levels, but pretreatment with EEMA significantly reduced them. TNF-α, IL-1β, and IL-6 are abundant in RA patients, and drugs modulating these cytokines may be promising for managing chronic painful diseases like RA (SIEBERT et al., 2015; BRENNAN, 1989; BRENNAN et al., 1992; MA; XU, 2013).

The study investigated the effect of chronic oral administration of EEMA on pain-related behavior and anti-inflammatory effects in the CFA-induced knee arthritis model. Results showed that CFA-induced arthritis in mice produced a lower number of line crosses and vertical rearing compared to the sham group. Pretreatment with EEMA significantly recovered these behaviors, with a significant difference in vertical rearing parameters compared to the vehicle group. In the open-field test, higher doses showed a significant difference compared to the vehicle group. This finding is consistent with previous studies on knee inflammation in rats. The test evaluates animal exploration, anxiety, and analgesic effects, with rearings being more

sensitive to intra-articular inflammation caused by CFA. An increase in vertical movement may be related to analgesia and not just anxiety parameters, explaining the study's results.

The study evaluated mechanical hyperalgesia in a CFA-induced arthritis mouse model. The vehicle group showed a significant reduction in nociceptive threshold, but pretreatment with EEMA significantly reversed hyperalgesic behavior. Quercetin, a flavonoid in EEMA, reduced titanium dioxide-induced knee joint mechanical hyperalgesia in rats and zymosan-induced knee joint mechanical hyperalgesia in another arthritis model (BORGHI et al., 2018; GUAZELLI et al., 2018). Flavonoids like kaempferol, quercetin, isorhamnetin, and myricetin play a crucial role in modulating inflammatory mediators. Repeated injections of CFA significantly increased knee edema in the ipsilateral knee joint, limiting it to the ipsilateral joint. To follow the arthritis-like condition or mitigate it, oral administration of EEMA or vehicle was initiated at day 1 and maintained until day 25. EEMA significantly reduced the diameter of CFA-induced ipsilateral knee edema in a dose-dependent manner. Mitigating proinflammatory cytokines produced by EMMA, mainly TNF-α and IL-1β, contributes to the reduction of edema formation at the knee joint (KANNAN et al., 2005).

IL-6 is a crucial cytokine in the development of RA, modulating T-cell and B cells involved in the inflammatory cascade (SRIRANGAN; CHOY, 2010). Tocilizumab, a humanized anti-IL-6R monoclonal antibody, supports its role in RA symptoms. TNF-α plays a role in RA physiopathology, forming other cytokines, amplification, endothelial activation, joint destruction, and nociceptive sensitization (BRENNAN; MCINNES, 2008; SCOTT, 2017; FELDMANN; MAINI, 2003, 2001). A pretreatment with EEMA, significantly blocked the increase of IL-6 and TNF-α levels in knee joint fluid in mice. This suggests EEMA's potential for use in folk medicine and suggests a more focused mechanism of action than commonly available drugs in the pharmaceutical market. Synthetic and biologic anti-rheumatic drugs targeting inflammatory cytokines like TNF-α and IL-6 are promising for controlling disease

activity in RA patients (TANAKA; MOLA, 2014; MIZOKAMI et al., 2012). However, only 20-50% of patients achieve clinical remission within 6 months. Drugs blocking IL-6/IL-6R have been considered beneficial, but monotherapy has produced contradictory effects. EEMA, an efficient blocker of high TNF-α and IL-6 levels, could be a promising clinical use for RA patients (PISETSKY; WARD, 2012).

5.4 Conclusion

This study's phytochemical profile, which includes 23 components with the majority being polyphenolic compounds, may be contributing to the synergistic description of the anti-inflammatory and anti-hyperalgesic effects seen in tests on animals. The flavonoid-rich profile of the *M. albicans* extract, which was attributed to the ability to reduce pro-inflammatory cytokines, IL-1β and TNF-α, and to produce an antioxidative stress (LIMA et al., 2020), was also reported in a recent article by our group. Rutin and quercetin were the main flavonoids found in the extract. Our findings imply that EEMA consistently and significantly improved pain behaviour reduction in RA-induced mice without causing any obvious liver damage and to cause a favorable regulation of key cytokines associated with arthritis (TNF-α, IL-1β and Il-6). These results provide unprecedented support for *M. albicans'* anti-arthritic profile and further the ethnopharmacological usage of this species of medicinal plant.

6 GENERAL CONCLUSION AND FINAL CONSIDERATIONS

In the initial investigation, we methodically locate and assess the results of literature on *in vitro* and *in vivo* investigations that highlight the medicinal benefits and related molecular functions of the *Miconia* genus. The review considered the safety concerns associated with the genus while assessing the pharmacological properties of phytochemicals and their impact on health. All the information needed for the genus was gathered. The analgesic, antioxidant, antinociceptive, and anti-osteoarthritis activities of 14 species in the genus *Miconia* were assessed in eight *in vivo* experiments, as well as their potential for anti-inflammatory, anticancer, cytotoxic, mutagenic, and antioxidant effects. The therapeutic potential and mechanisms of action of these species were also investigated in eighteen *in vitro* experiments. *Miconia* species that are commonly available are safe for human consumption, according to the limited number of studies. Furthermore, *M. albicans* was the most widely utilized medicinal species, or at least the one that was most frequently documented in ethnopharmacological usage. Research findings support its clinical safety, including potential anti-inflammatory properties modulating pro- and anti-inflammatory cytokines and its related signaling markers. Therefore, we used experimental techniques to investigate the anti-inflammatory and anti-arthritic effects of the ethanolic leaf extract of *M. albicans* (EEMA), with special emphasis on modulation of cytokines, as well as dereplication and quantification using HPLC-DAD-ESI-/MS/MS. The study found 23 compounds in *M. albicans*, with glycoside flavonoids mainly derived from quercetin and rutin being the main compounds. EEMA significantly reduced leukocyte migration, TNF-α, and IL-1β levels in pleurisy and CFA animal models, moreover, reduced levels of IL-6 and TNF-α in the joint knee in animals provoked by algogens in an experimental model of arthritis. It also improved mobility, hindpaw grip strength, and reduced CFA-induced ipsilateral knee edema. These findings support the medicinal plant's potential in folk medicine.

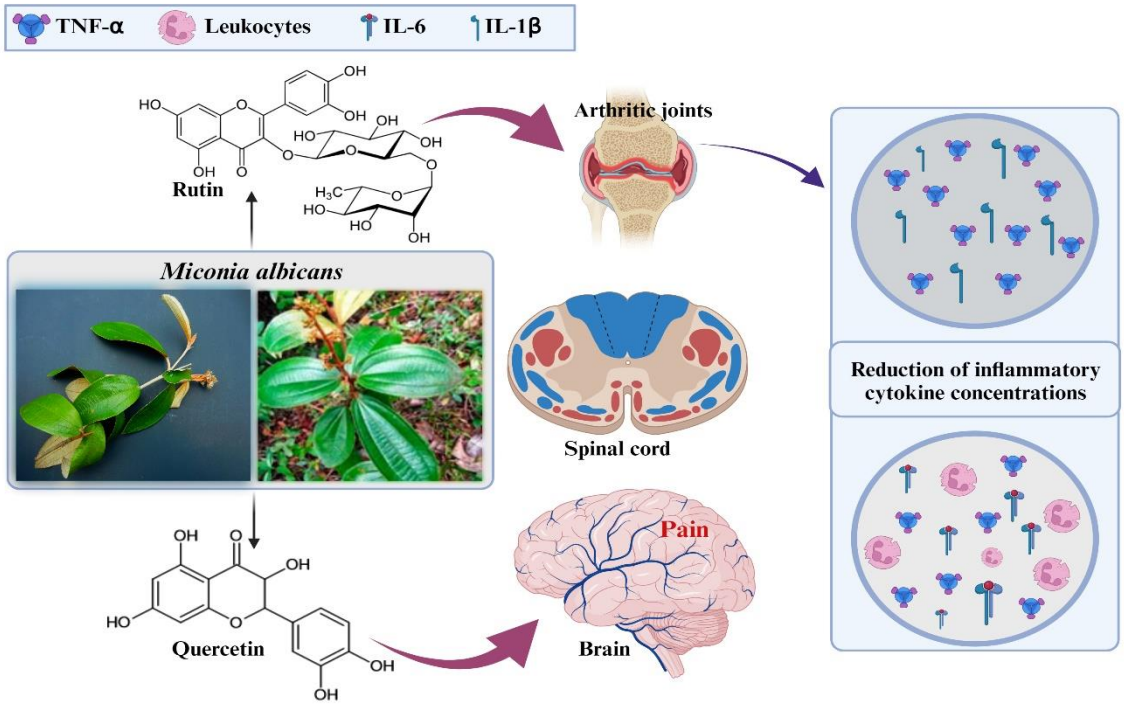


Figure 10. Antiarthritic and antihyperalgesic properties of *M. albicans* and its active constituents on reduction of inflammatory cytokines (TNF-α, IL-6 and IL-1β).

7 PERSPECTIVES

To provide a current understanding of the therapeutic potential of the *Miconia* genus and its phytochemical and pharmacological qualities, we evaluated all recent publications on the *Miconia* genus. Its anti-inflammatory, analgesic, antibacterial, cytotoxic, mutagenic, antioxidant, anti-leishmanial, antinociceptive, schistosomicidal, and anti-osteoarthritic activities were the main topics of its pharmacological investigations. Our findings also suggest that *M. albicans* improved pain behaviour reduction in RA-induced mice consistently and significantly, without causing any apparent liver damage, and created a positive modulation of important cytokines associated with arthritis (TNF-α, IL-1β, and IL-6). This discovery lends unheard-of support to *M. albicans'* anti-arthritic profile and furthers the ethnopharmacological usage of this species of medicinal plant.

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APPENDIX A (STUDY 1)

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Review article

Health functions and related molecular mechanisms of *Miconia* genus: A systematic review

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ABSTRACT

The *Miconia* genus is traditionally used in folk medicine in Brazil and other tropical American countries and is represented by 282 species in this region. It is a multifaceted genus of medicinal plants widely used to treat rheumatoid arthritis (RA), pain, inflammatory diseases, and many more therapeutic applications. In the present study, we systematically identify and discuss the literature on *in vivo* and *in vitro* studies focusing on the therapeutic potentials and related molecular mechanisms of the *Miconia* genus. The review also assessed phytochemicals and their pharmacological properties and considered safety concerns related to the genus. Literature searches to identify studies on the *Miconia* genus were carried out through four main electronic databases, namely PubMed, Embase, Scopus, and Web of Science limited to Medical Subjects Headings (MeSH) and Descriptores en Ciencias de la Salud (DCS) (Health Sciences Descriptors) to identify studies published up to December 2022. The relevant information about the genus was gathered using the keywords '*Miconia*', 'biological activities', 'therapeutic mechanisms', 'animal model, 'cell-line model', 'antinociceptive', 'hyperalgesia', 'anti-inflammatory', and 'inflammation'. The therapeutic potentials and mechanisms of action of 14 species from genus *Miconia* were

Abbreviations: AAPH, 2,2'-azobis (2-amidinopropane) dihydrochloride; CFA, Complete Freund's Adjuvant; DEMA, a dried extract of *M. albicans*; DPPH, 1,1-diphenyl-2-picrylhydrazyl; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukins; MAEE, *M. albicans* ethanolic leaf extract; MAFRE, *M. albicans* fruits extract; MeSH, Medical Subjects Headings; NFκB, nuclear factor-κB; Nrf2, nuclear factor erythroid-2-related factor 2; OA, oleanolic acid; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RA, rheumatoid arthritis; RNS, reactive nitrogen species; ROS, reactive oxygen species; sTNFR, soluble tumour necrosis factor receptor; TNF-α, tumor necrosis factor-α; UA, ursolic acid; VASP, Visual Analogue Scale of Pain; VEGFR-2, vascular endothelial growth factor receptor-2; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

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APPENDIX B

Table 1. Description of the main characteristics of *in vivo* studies using *Miconia genus*

Name of the plant	**Animal/Strains**	**Dose/Route**	**Effects and molecular mechanism**	**Authors, year**
Miconia rubiginosa (Bonpl.) DC.	Swiss mice and Wistar rats	100, 200 and 300 mg/kg *intraperitoneal*	↑central and peripheral analgesic; antinociceptive effect	**Spessoto et al., (2003)**
Miconia albicans Sw. Triana	Swiss albino mice and Wistar rats	200 mg/kg *intraperitoneal*	↓$PGE_2\alpha$ and $PGF_2\alpha$; ↑peripheral-mediated analgesic activity	**Vasconcelos et al., (2003)**
M. albicans	Swiss mice and Wistar rats	40 mg/kg *intraperitoneal*	↓inflammatory mediators and inhibition of synthesis of prostaglandins and also the blockage of their receptor sites; ↑peripheral mediated analgesic effect	**Vasconcelos et al., (2006)**

Miconia minutiflora (Bonpl.) DC.	Wistar rat and Swiss mice	50, 100 and 200 mg/kg *oral*	reduced inflammatory activity by decrease in cell migration; ↓pro-inflammatory cytokines TNF-α and IL-1β; ↑central and peripheral analgesic effect	**Gatis-Carrazzoni et al., (2018)**
M. albicans	Swiss mice	50 and 100 mg/kg *oral*	↓leukocyte migration; ↓TNF-α, IL-1β, and IL-6; ↓nociceptive and hyperalgesic behaviors; ↓ipsilateral knee edema; ↑mobility and hindpaw grip strength	**Quintans-Júnior et al., (2020)**
M. albicans	Swiss mice	25, 50 and 100 mg/kg *oral*	↓TNF-α and IL-1β; reduced inflammatory nociception and edema; exhibit antioxidant and anti-inflammatory properties	**Lima et al., (2020)**

M. albicans and *Curcuma longa* Linn	Human knee's osteoarthritis	1000 mg/kg *oral*	↓score of WOMAC, VASP; ↑analgesic and exhibit anti-inflammatory effect on knee osteoarthritis	**Gomes et al., 2021**
M. albicans	Swiss mice	2.5 mg/kg on ear edema	↓ear edema and MPO activity; revealed antioxidant and anti-inflammatory effects	**Correa et al., 2021**

PGE2α, prostaglandin E2α; PGF2α, prostaglandin F2α; TNF-α, tumor necrosis factor alpha; IL-1β, IL-1 beta; IL, interleukin; WOMAC, western Ontario and McMaster universities arthritis index; VASP, vasodilator stimulated phosphoprotein; MPO, myeloperoxidase.

APPENDIX C

Table 2. Description of the main characteristics of *in vitro* studies using *Miconia species* and its chemical composition

Plant Used			**Plant extract**	**Chemical composition**	**Therapeutic properties**	**Effects and Molecular Mechanism**	**Authors, year**
Genus	**Species**	**Plant part**					
M.	*rubiginosa*	Aerial parts	Hexane, methylene chloride and ethanol extract	Triterpenes and sterols	Analgesic activity	↑central and peripheral analgesic activity, antinociceptive effect; ↓prostaglandin synthesis	**Spessoto et al., (2003)**
M.	*albicans*	Aerial parts	Methylene chloride extract	Ursolic acid and oleanoic acid	Analgesic and anti-inflammatory activity	↑analgesic and anti-inflammatory activity; ↓ inflammatory mediators	**Vasconcelos et al., (2006)**
Miconia	*salicifolia* (Bonpl. ex Naudin)	Leaves and bark	Ethanolic and aqueous extracts	N/A	Antibacterial activity	ethanol extract has strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*	**Bussmann et al., (2010)**

Miconia	*ligustroides* (DC.) Naudin and isolated triterpene acids	Aerial parts	Methylene chloride extract	Ursolic acid and oleanolic acid	Antimicrobial activity	*M. ligustroides* showed appreciable inhibition against *Bacillus cereus*; ursolic acid displayed efficient activity against *B. cereus*; oleanolic acid exhibited growth inhibitory activity against *B. cereus* and *Streptococcus pneumoniae*	**Cunha et al., (2010)**
M.	*albicans,*	Aerial parts	Methanol extract	Flavonoids and tannins	Mutagenicity and antimutagenicity of the extracts	↑cytotoxic, antimutagenic activity; demonstrated the protective effects against DXR-induced DNA damage	**Serpeloni et al., (2011)**
Miconia	*cabucu* Hoehne,	Aerial parts					
Miconia	*stenostachya* DC.,	Aerial parts					
M.	*rubiginosa*	Aerial parts					

Miconia	*langsdorffii* Cogn.	Aerial parts	Hydroalcoholic extract	Triterpenes	Antileishmanial activity	↑*in vitro* antileishmanial activity against the promastigote forms of *Leishmania amazonensis*	**Peixoto et al., (2011)**
M.	*rubiginosa*	Leaves	Aerial parts	Triterpenes, flavonoids and quinones	N/A	N/A	**Rodrigues et al., (2011)**
Miconia	*willdenowii* Klotzsch ex Naudin.	Leaves	Ethanol extract	Benzoquinone	Schistosomicidal activity	↑ crude ethanolic extract of *M. willdenowii* showed the promising results, killing 65% of the *Schistossoma mansoni* worms. Primin as the active metabolite responsible for the observed schistosomicidal effect	**Viegas et al., (2017)**

M.	*minutiflora*	Leaves	Methanol extract	Ellagic acid, gallotannin and terpenes	Anti-inflammatory and antinociceptive	↑antioxidant, anti-inflammatory, and antinociceptive activity induced by hydrolyzable tannins; ↓proinflammatory cytokines TNF and IL-1β, decrease edema in both phases of inflammation	**Gatis-Carrazzoni et al., (2018)**
M.	*willdenowii*	Leaves	Ethanol extract	2-methoxy-6-pentyl-benzoquinone	Leishmanicidal and antimicrobial activities	inhibits promastigote forms of *L. amazonensis*; exhibited antimicrobial activity against pathogenic fungi, gram-positive and negative bacteria	**Viegas et al., (2019)**

Miconia	*latecrenata* Naudin.	Leaves	Aqueous extract	Tannins	Antioxidant, antibacterial, antimutagenic and antigenotoxic activities	↑antioxidant property due to high total phenolic content; antibacterial activity to gram-positive and negative strains; and antimutagenic property by decreasing the ROS	**Gontijo et al., (2019)**
Miconia	*chamissois* Naudin	Leaves	Hydroethanolic extract	Matteucinol	Cytotoxicity and anticancer potential	*M. chamissois* and matteucinol showed cytotoxicity and antitumor potential in glioblastoma cell lines	**Silva et al., (2020)**
M.	*albicans*	Leaves	Ethanol extract	Quercetin and rutin	Antihyperalgesic and anti-inflammatory profile	↓ levels of TNF-α and IL-1β in the joint; ↓ nociceptive and hyperalgesic behaviors	**Quintans-Júnior et al., et al., (2020)**

M.	*albicans*	Leaves	Ethanol extract	Polyphenols, leucoanthocyanins, tannins, steroids and saponins.	*In vitro* antioxidant activity	↑ exhibited strong antioxidant profiles due to enhanced content of polyphenols	**Lima et al., (2020)**
M.	*latecrenata*	Leaves	Organic extract	Phenolic compounds	Antibacterial activity	demonstrated the promising for inhibiting the growth of *S. aureus* and *Pseudomonas aeruginosa*	**Rodrigues et al., (2020)**
Miconia	*burchellii* Triana.	Leaves	Ethanol extract	triterpenes, flavonoids, steroids and pheophorbide	Cytotoxic activity	↑ antiproliferative and demonstrated cytotoxic against leukemia cell lines	**Cunha et al., (2021)**
M.	*abicans*	Fruit	Methanol extract	Flavonoids, organic acids, tannins and triterpenes	*In vitro* antioxidant and antiproliferative properties	↑antioxidant, ferrous chelating capacities; non-toxic to VERO cells, ensures cell viability, absence of antiproliferative effect against human tumor cell lines	**Correa et al., (2021)**

Miconia	*ferruginata* DC.	Leaves, stem and flowers	Ethanol extract	flavonoids derivatives of quercetin, catechins, and phenolic acids	anticancer	↑ anticancer; exhibits cytotoxicity against tumor cells of 4T1, A549, and MDA-MB-231	**Barroso et al., (2021)**
M.	*albicans*	leaves	Hydroethanolic extract	N/A	anticancer	mixture of plant extracts exhibited cytotoxic potential; ↓ viability of human cancer cell lines	**Rosa et al., (2021)**
M.	*chamissois*	leaves					

N/A, Not applicable; TNF-α, tumor necrosis factor alpha; IL-1β, IL-1 beta; IL, interleukin; ROS, reactive oxygen species; DXR, doxorubicin.

APPENDIX D

Table 3. Characterization of the compounds tentatively identified by HPLC-ESI-MS^n in *M. albicans*

Peak No.	t_R (min.)	*m/z* $[M-H]^-$	Molecular Formula	MS^2/MS^3	Tentative assignment
1	5.3	481.0661	$C_{20}H_{18}O_{14}$	MS^2 [481]: 301; 275/ MS^3 [481 → 301]: 257; 229; 185	HHDP-Hexoside
2	8.0	481.0634	$C_{20}H_{18}O_{14}$	MS^2 [481]: 301; 275/ MS^3 [481 → 301]: 283; 257; 229; 185	HHDP-Hexoside
3	13.2	783.0658	$C_{34}H_{24}O_{22}$	MS^2 [783]: 481; 301/ MS^3 [783 → 301]: 257; 229; 185	Pedunculagin
4	14.1	169.0125	$C_7H_6O_5$	MS^2 [169]: 125	Gallic acid
5	28.3	647.1201	$C_{36}H_{24}O_{12}$	MS^2 [647]:301 / MS^3 [647 → 301]: 257; 229; 185	Ellagic acid derivatives
6	30.3	197.0451	$C_9H_{10}O_5$	MS^2 [197]:169; 125	Ethyl gallate
7	31.4	631.0968	$C_{28}H_{24}O_{17}$	MS^2 [631]: 479; 317/ MS^3 [537 → 479]: 317; 316; 271	Myricetin-O-galloyl-hexoside
8	33.2	479.0837	$C_{21}H_{20}O_{13}$	MS^2 [479]: 317; 316/ MS^3 [479 → 316]: 287; 271; 179	Myricetin-O-hexoside
9	34.2	615.1000	$C_{28}H_{24}O_{16}$	MS^2 [615]: 463; 301/ MS^3 [615 → 463]: 301; 271; 179	Quercetin-O-Galloyl-hexoside

10	36.2	609.1451	$C_{27}H_{30}O_{16}$	MS^2 [609]:343; 301; 271 / MS^3 [609 → 301]: 271; 255; 179; 151	Rutin
11	36.2	463.0891	$C_{21}H_{20}O_{12}$	MS^2 [463]: 301; 271/ MS^3 [463 → 301]: 271; 255; 179; 151	Isoquercitrin
12	37.0	433.0765	$C_{20}H_{18}O_{11}$	MS^2 [433]: 301; 179/ MS^3 [433 → 301]: 271; 255; 179; 151	Quercetin-O-pentoside
13	37.2	447.0570	$C_{20}H_{16}O_{12}$	MS^2 [447]: 301; 300/ MS^3 [447 → 300]: 283; 257; 229; 185	Ellagic acid-O-deoxyhexoside
14	37.5	433.0770	$C_{20}H_{18}O_{11}$	MS^2 [433]: 301; 179/ MS^3 [433 → 301]: 271; 255; 179; 151	Quercetin-O-pentoside
15	37.5	585.0883	$C_{27}H_{22}O_{15}$	MS^2 [585]: 433; 301/ MS^3 [585 → 301]: 179; 151	Quercetin-O-Galloyl-pentoside
16	38.4	579.1319	$C_{26}H_{28}O_{15}$	MS^2 [579]: 355; 301; 300; 271; 255/ MS^3 [579 → 300]: 271; 255; 179; 151	Quercetin-O-deoxyhexose-pentoside
17	38.9	477.1041	$C_{22}H_{22}O_{12}$	MS^2 [477]: 357; 315; 314/ MS^3 [477 → 314]: 300; 285; 271; 243	Isorhamnetin-O-hexoside
18	39.7	417.0816	$C_{20}H_{18}O_{10}$	MS^2 [417]: 327; 285; 284/ MS^3 [417 → 284]: 255; 227; 151	Kaempferol-O-pentoside
19	40.2	461.1077	$C_{22}H_{22}O_{11}$	MS^2 [461]: 315/ MS^3 [461 → 315]: 300	Isorhamnetin-O-deoxyhexoside
20	40.2	599.1016	$C_{28}H_{24}O_{15}$	MS^2 [599]: 461; 447; 301/ MS^3 [599 → 301]: 255; 179; 151	Quercetin-O-galloyl-deoxyhexose

21	40.8	477.1043	$C_{22}H_{22}O_{12}$	MS^2 [477]: 357; 315; 314/ MS^3 [477 → 314]: 300; 285; 271; 243	Isorhamnetin-O-hexoside
22	42.9	503.0802	$C_{23}H_{20}O_{13}$	MS^2 [503]: 485; 443; 315; 300/ MS^3 [503 → 315]: 300	Methyl Ellagic acid-O-acetate-deoxyhexoside
23	43.2	301.0365	$C_{15}H_{10}O_7$	MS^2 [301]: 273; 255; 179; 151	Quercetin

APPENDIX E (STUDY 2)

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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

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ABSTRACT

Ethnopharmacological relevance: Miconia albicans (Sw) Triana (Melastomataceae), a medicinal plant widely used by practitioners of folk medicine in the northeast of Brazil, has been used to treat chronic inflammatory disorders, such as rheumatoid arthritis (RA) and other joint conditions. Oddly, there is little research on the species.

Aim of the study: We aimed to evaluate the anti-arthritic and anti-inflammatory profile of the ethanolic leaf extract of M. albicans (EEMA), as well as to perform dereplication and quantification by HPLC-DAD-ESI-/MS/MS.

Materials and methods: The compounds present in the extracts were identified by HPLC-DAD-ESI-MS/MS. The possible anti-inflammatory effect of EEMA (50 and 100 mg/kg, p.o) was evaluated using the pleurisy model induced by carrageenan and its action on IL-1β and TNF-α levels was also evaluated. The RA model was induced through the intra-articular injection of complete Freund's adjuvant (CFA).

Results: HPLC-DAD-ESI-MS/MS analysis identified 23 compounds, with glycoside flavonoids mainly derived from quercetin, and rutin being the main compounds. EEMA significantly reduced ($p < 0.001$) leukocyte migration in the pleurisy model and reduced TNF-α and IL-1β levels in pleural lavage ($p < 0.001$). In the CFA animal model, EEMA significantly reduced the nociceptive and hyperalgesic behaviors demonstrated by the rearing test ($p < 0.01$ or $p < 0.05$) and decreased mechanical hyperalgesia ($p < 0.001$). EEMA produced a significant improvement in mobility in the open-field test (only at the higher dose, $p < 0.05$). EEMA significantly ($p < 0.01$) increased hindpaw grip strength. The diameter of CFA-induced ipsilateral knee edema was significantly reduced ($p < 0.001$) by EEMA, which was related to reduced levels of IL-6 and TNF-α in the joint

Abbreviations: EEMA, Ethanolic leaf extract of *M. albicans*; RA, Rheumatoid arthritis; NSAIDs, Non-steroidal anti-inflammatory drugs; DMARDs, disease-modifying anti-rheumatic drugs; CID, collision-induced dissociation; TNF-α, tumor necrosis factor alpha

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APPENDIX F (OTHER PUBLICATIONS)

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REVIEW

The use of cyclodextrin inclusion complexes to improve anticancer drug profiles: a systematic review

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ABSTRACT

Introduction: Cyclodextrins (CDs) have been used extensively in inclusion complexes to improve the biological efficacy of complexed substances as well as to provide increased solubility and stability. We reviewed in vivo experimental studies of drug molecules complexed in cyclodextrins to evaluate whether these complexes improved bioavailability and enhanced the treatment of cancer, the second leading cause of death globally.

Area covered: The search terms cyclodextrins, anti-cancer, and cancer treatment were used to identify peer-reviewed publications limited to the English language in the PubMed, EMBASE, Scopus, and Web of Science electronic databases published from inception until July 2019. A total of 2760 studies were identified, of which 12 met the inclusion criteria. The review showed that cyclodextrin/anticancer drug complexes enhance solubility, reduce toxicity, and improve therapeutic efficacy in in vivo tumor models in the pharmacokinetic studies detailed and described below.

Expert opinion: The use of cyclodextrins combined with anticancer agents can provide better encapsulation and effective delivery of drugs to optimize their efficacy. Cyclodextrin inclusion complexes might also be a promising tool to lower the therapeutic dosage levels and thereby increase the safety and curative potential of the chemotherapeutic molecules.

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KEYWORDS
Cancer; cyclodextrins; cancer therapy; experimental systems; pharmacokinetics

1. Introduction

Cancer is characterized by uncontrolled cell growth and the development of tumors, which spread to the surrounding tissue, metastasizing in almost any part of the body. Among noncommunicable diseases (NCDs), cancer ranks the highest in worldwide mortality rate and is the greatest single cause of shortened life expectancy globally in the twenty-first century [1,2]. It is estimated that about 1 in every 6 deaths in the world is due to cancer, mostly in low- and middle-income countries [3]. A recent report showed that cancer was responsible for an estimated 9.6 million death in 2018 [3]. Currently, available cancer treatment policies are based on reducing or eliminating tumors, mainly through surgery and inhibiting the rapid growth of malignant cells by chemical and physical treatments such as chemo- and radiotherapies [4]. These clinical interventions can often be harmful to the patients, since surgery and radiotherapies destroy or inhibit normal cell growth and metabolism [5]. This has meant that the use of some cancer drugs has been limited due to their high cytotoxicity toward normal cells, which can affect quality of life [6]. In addition, aqueous solubility is considered as one of the major concerns of conventional chemotherapy due to the mostly nonpolar nature of chemotherapeutic agents [7]. As a result, various delivery vehicles have been used to try to enhance certain physicochemical properties of the conventional cancer therapy, increase their availability and better target them toward cancer cells [8].

The basics of cyclodextrin interactions were discovered over 100 years ago by Villiers and Schardinger, and a patent was recorded on the chemistry of cyclodextrins (CDs) and their complexes in 1953 [9]. CDs have a lipophilic inner cavity and a hydrophilic outer surface and possess substantial capabilities to form inclusion complexes due to the nature of their core-shell structure. The naturally occurring CDs are α-, β-, and γ-CDs having six, seven, or eight glucopyranose units in the cyclic structure. CDs are water-soluble macrocyclic oligosaccharides used extensively to combine with active drugs to produce inclusion complexes that can enhance the water solubility of poorly soluble compounds, improve physicochemical stability, or the release rates of drugs in pharmaceutical formulations in relation to the function of biologically active substances [10]. The key property of solubility enhancement in the formation of water-soluble inclusion complexes is due to some prominent features of CDs such as their rigid, truncated cone-shaped cavities, capable of encapsulating hydrophobic drug molecules in an apolar interior section [11], while the hydrophilic region of the CDs increases the drug's solubilization capacity through binding with water molecules [12]. The interactions with drug molecules influence

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ORIGINAL ARTICLE

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Myrtenol complexed with β-cyclodextrin ameliorates behavioural deficits and reduces oxidative stress in the reserpine-induced animal model of Parkinsonism

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Abstract

Current pharmacological approaches to treat Parkinson's disease have low long-term efficacy and important adverse side effects. The development of new pharmacological therapies has focused on novel plant-derived phytochemicals. The alcoholic monoterpene myrtenol has been isolated from several plant species, and has anxiolytic, analgesic, anti-inflammatory and antioxidant actions. Our study evaluated the neuroprotective potential of myrtenol complexed with β-cyclodextrin (MYR) on a progressive parkinsonism model induced by reserpine (RES) in mice. The complexation with cyclodextrins enhances the pharmacological action of monoterpenes. Male Swiss mice were treated daily with MYR (5 mg/kg, p.o.) and with RES (0.1 mg/kg, s.c.) every other day during 28 days. Behavioural evaluations were conducted across treatment. At the end of the treatment, immunohistochemistry for tyrosine hydroxylase (TH) and oxidative stress parameters were evaluated. Chronic MYR-treatment protected against olfactory sensibility loss, restored short-term memory and decreased RES-induced motor impairments. Moreover, this treatment prevented dopaminergic depletion and reduced the oxidative status index in the dorsal striatum. Therefore, MYR ameliorated motor and non-motor impairments in the progressive animal model of parkinsonism, possibly by an antioxidant action. Additional research is needed to investigate the mechanisms involved in this neuroprotective effect.

KEYWORDS
antioxidant, dopamine depletion, monoterpenes, natural product, neuroprotection, parkinsonism

1 | INTRODUCTION

Parkinson's disease (PD) is the most prevalent movement disorder.[1] This aging-related disease affects 1% to 2% of individuals worldwide.[2] This pathology may become a global pandemic, resulting in the most prevalent neurodegenerative disorder, surpassing Alzheimer's disease.[3] Men and Caucasians are more affected than women and African-Americans and Asians, respectively.[4-6]

The aetiology of PD remains unclear. Evidence suggests that the disease originates from genetic predisposition and exposure to environmental agents.[7] The core pathophysiological marks of PD are the loss of dopamine in the nigrostriatal pathway (mainly in the

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Involvement of the PKA pathway and inhibition of voltage gated Ca2 + channels in antihyperalgesic activity of Lippia grata/β-cyclodextrin

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ABSTRACT

Neuropathic pain (NP) is a difficult condition to treat because of the modest efficacy of available drugs. New treatments are required. In the study we aimed to investigate the effects of the essential oil from *Lippia grata* alone or complexed in β-cyclodextrin (LG or LG-βCD) on persistent inflammatory and neuropathic pain in a mouse model. We also investigated Ca^{2+} currents in rat dorsal root ganglion (DRG) neurons. Male Swiss mice were treated with LG or LG/β-CD (24 mg/kg, *i.g.*) and their effect was evaluated using an acute inflammatory pleurisy model and nociception triggered by intraplantar injection of an agonist of the TRPs channels. We also tested their effect in chronic pain models: injection of Freund's Complete Adjuvant and partial sciatic nerve ligation (PSNL). In the pleurisy model, LG reduced the number of leukocytes and the levels of TNF-α and IL-1β. It also inhibited cinnamaldehyde and menthol-induced nociceptive behavior. The pain threshold in mechanical and thermal hyperalgesia was increased and paw edema was decreased in models of inflammatory and neuropathic pain. PSNL increased inflammatory protein contents and LG and LG-βCD restored the protein contents of TNF-α, NF-κB, and PKA, but not IL-1β and IL-10. LG inhibited voltage gated Ca^{2+} channels from DRG neurons. Our results suggested that LG or LG-βCD produce anti-hyperalgesic effect in chronic pain models through reductions in TNF-α levels and PKA, and inhibited voltage-gated calcium channels and may be innovative therapeutic agents for the management of NP.

1. Introduction

According to the World Health Organization (WHO), about 20% of the population live with some degree of chronic pain, which is more common in women, older individuals and people with relative deprivation [1]. Neuropathic pain (NP) is one of the most important types of chronic pain. It is triggered by lesions to the somatosensory nervous system that alter its structure and function [2], causing hyperalgesia,

Abbreviations: β-cyclodextrin, β-CD; Dorsal root ganglion, DGR; Essencial Oil, EO; Freund's Complete Adjuvant, FCA; Interleukin-10, IL-10; Interleukin-1β, IL-1β; *Lippia grata*, LG; *Lippia grata* complexed with β-cyclodextrin, LG/β-CD; factor nuclear kappa B, NF-kB; Neurophatic pain, NP; protein kinase A, PKA; partial sciatic nerve ligation, PSNL; tumor necrosis factor alpha, TNF-α; transient receptor potential Ankyrin 1, TRPA1; transient receptor potential melastatin 8, TRPM8; transient receptor potential vanilloid 1, TRPV 1; voltage gated calcium channel, VGCC

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Review

HPLC-DAD-UV analysis, anti-inflammatory and anti-neuropathic effects of methanolic extract of *Sideritis bilgeriana* (lamiaceae) by NF-κB, TNF-α, IL-1β and IL-6 involvement

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ABSTRACT

Medicinal plants remain an invaluable source for therapeutics of diseases that affect humanity. *Sideritis bilgeriana* (Lamiaceae) is medicinal plant used in Turkey folk medicine to reduce inflammation and pain, but few studies scientific corroborates its medicinal use so creating a gap between popular use and scientific evidence. Thus, we aimed to evaluate the pharmacological effects of the methanolic extract of *S. bilgeriana* (MESB) in rodents nociception models and also performed its phytochemical analysis. Firstly, a screening was carried out that enabled the identification of the presence of phenolic compounds and flavonoids. In view of this, a chromatographic method by HPLC-DAD-UV was developed that made it possible to identify chlorogenic acid and its quantification in MESB. MESB-treated mice (MESB 50, 100 and 200 mg/kg, p.o.) reduced mechanical hyperalgesia and myeloperoxidase activity ($p < 0.01$), and also showed a reduced pain behavior in capsaicin test. In the carrageenan-induced pleurisy test, MESB (100 mg/kg p.o.) significantly reduced the leukocyte (polymorphonuclear) count in the pleural cavity and equally decreased the TNF-α and IL-1β levels ($p < 0.001$). In the PSNL model, mechanical hyperalgesia was reduced on the first evaluation day and during the 7 days of evaluation compared to the vehicle group ($p < 0.001$). Thermal hyperalgesia was also reduced 1 h after treatment compared to the vehicle group ($p < 0.001$) and reversed the loss of force initially displayed by the animals, thus inferring an analgesic effect in the muscle strength test. Analysis of the marrow of these animals showed a decrease in the level of pro-inflammatory cytokine IL-6 ($p < 0.001$) and factor NF-κB, in relation to the control group ($p < 0.05$). Moreover, the MESB treatment produced no noticeable side effects, no disturb in motor performance and no signs of gastric or hepatic injury. Together, the results suggests that MESB could be useful to management of inflammation and neuropathic pain mainly by the management of pro-inflammatory mediators (NF-κB, TNF-α, IL-1β and IL-6), so reinforcing its use in popular medicine and corroborating the need for further chemical and pharmacological studies for the species.

Abbreviations: ALT, alanine transferase; AST, aspartate aminotransferase; CNS, central nervous system; EDTA, Ethylenediamine tetraacetic acid; ELISA, Enzyme-Linked Immunosorbent Assay; HPLC-DAD-UV, High-performance Liquid Chromatography coupled to Diode-Array Ultraviolet Detector; HTAB, hexadecyltrimethylammonium bromide; MESB, methanolic extract of *Sideritis bilgeriana*; MPO, myeloperoxidase; NP, neuropathic pain; RIPA, Radio-Immunoprecipitation Assay; PNS, peripheral nervous system; PSNL, partial sciatic nerve ligation; PCDF, Polyvinylidene Difluoride.

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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)

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ABSTRACT

Ethnopharmacological relevance: *Miconia albicans* (Melastomataceae), commonly known in Brazil as "canela-de-velho", is used in folk medicine for treating rheumatoid arthritis and reducing pain and inflammation.

The aim of the current work was: to provide data on physicochemical characterization of the drug plant and dried extract from *M. albicans* leaves, as well as investigate the anti-inflammatory effect and antioxidant stress profile from the standardized dried extract of this species employing different model systems.

Materials and methods: plant material (dried crushed leaves) was extracted by turboextraction using 50% ethanol (v/v). Different pharmacological techniques were performed to establish quality control parameters of the plant drug, and dried extract of *M. albicans* (DEMA) was chemically characterized by HPLC-PDA to selection of the chemical marker. Total phenolic and flavonoid contents were determined by the Folin-Ciocalteu and $AlCl_3$ colorimetric methods, respectively. Antioxidant potential of the DEMA was investigated by employing different *in vitro* antioxidant assays, including DPPH and ABTS radical scavenging assays, ferric reducing antioxidant assay, NO scavenging assay, metal ion (Fe^{2+}) chelating activity and antioxidant capacity by inhibition of lipid peroxidation (TBARS). Finally, anti-inflammatory activity of the DEMA was evaluated using two models of acute inflammation: carrageenan induced inflammation and mechanical hyperalgesia.

Results and discussion: *M. albicans* leaves, after drying in forced air circulation chamber at ± 40 °C for 48 h and crushing in knife mill, presented a moisture content below the maximum allowed for plant drugs (6.4%). The powder of *M. albicans* was classified as moderately coarse and total ash content was found to be 6.27%. Preliminary phytochemical screening of DEMA revealed the presence of flavonoids, tannins, saponins, leucoanthocyanins and steroids. DEMA had significant higher total phenolic (551.3 mg gallic acid equivalent/g of dried extract) and flavonoid contents (367.19 mg catechin equivalent/g of dried extract). Two major compounds (λ = 340 nm) were identified in DEMA by HPLC-PDA: the flavonoids rutin and quercetin. Rutin content, selected as chemical marker, was determined and found to be 1.16 mg/g dried extract (r = 0.9941). Regarding to antioxidant activity, our results revealed the DEMA exhibited good antioxidant activity on different models. *M. albicans* treatment also reduced the levels of TNF-α e IL-1β and consequently inflammatory nociception and edema caused by carrageenan injection. Based on previous studies and our results, is possible to suggest a positive correlation between the flavonoids rutin and quercetin and the antioxidant and anti-inflammatory capacities.

Conclusion: Together, these data suggest that *M. albicans* has the possibility of use in conditions such as arthritis or other joint pain, even needing other work to better consolidate this profile.

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3 The Modulation of IL-6 Levels by Natural Products in Arthritis-Like Animal Models

A Systematic Review of Preclinical Evidence

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Multitargeted molecular docking and dynamics simulation studies of flavonoids and volatile components from the peel of *Citrus sinensis* L. (Osbeck) against specific tumor protein markers

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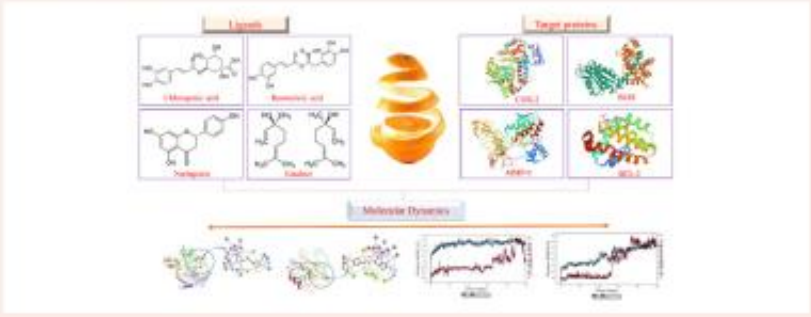
ABSTRACT

Citrus sinensis (L.) Osbeck (Rutaceae), commonly known as the sweet orange, is a popular and widely consumed fruit with several medicinal properties. The present study aimed to perform the *in silico* screening of 18 flavonoids and eight volatile components from the peel of *C. sinensis* against apoptotic and inflammatory proteins, metalloprotease, and tumor suppressor markers. Flavonoids obtained higher probabilities than volatile components against selected anti-cancer drug targets. Hence, the data from the binding energies against the essential apoptotic and cell proliferation proteins substantiate that they may be promising compounds in developing effective candidates to block cell growth, proliferation, and induced cell death by activating the apoptotic pathway. Further, the binding stability of the selected targets and the corresponding molecules were analyzed by 100 ns molecular dynamics (MD) simulations. Chlorogenic acid has the most binding affinity against the important anti-cancer targets iNOS, MMP-9, and p53. The congruent binding mode to different drug targets focused on cancer shown by chlorogenic acid suggests that it may be a compound with significant therapeutic potential. Moreover, the binding energy predictions indicated that the compound had stable electrostatic and van der Waal energies. Thus, our data reinforce the medicinal importance of flavonoids from *C. sinensis* and expand the need for more studies, seeking to optimize results and amplify the impacts of further *in vitro* and *in vivo* studies.



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Flavonoids as Th1/Th2 cytokines immunomodulators: A systematic review of studies on animal models

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ABSTRACT

Background: Flavonoids are naturally occurring compounds, extensively distributed in plants. T helper (Th)1 and Th2 cytokines balance plays an essential role in the reaction of inflammatory, allergic and infectious processes and transplantation rejection.

Purpose: This systematic review focuses on various classes of flavonoids with a view to evaluate whether Th1/Th2 cytokine-mediated pathways of immunoenhancement could reduce immune overwhelming reactions.

Methods: Articles in English published from inception to December 2017 reporting flavonoids with immunomodulatory activity for the management of immune-mediated disorders were acquired from PubMed, EMBASE, Scopus and Web of Science and a animal experiments where Th1 and Th2 cytokines were investigated to assess the outcome of immunoregulatory therapy were included.

Chapters: 1809 publications were identified and 26 were included in this review. Ten articles described the effect of flavonoids on allergic inflammation in an animal model of asthma; eleven *in vivo* studies evaluated the immunomodulating and immunosuppressive effects of flavonoids on Th1/Th2 cytokines production and five reports described the regulatory role of flavonoids for Th1/Th2 cytokine responses to experimental arthritis and myocarditis. Modulation of Th1/Th2 cytokine balance, inhibition of eosinophil accumulation and remodeling of the airways and lungs, downregulation of Notch and PI3K signaling pathways, regulation of CD4 + / CD8 + lymphocytes ratio and decreasing inflammatory mediator expressions levels are among the most important immunopharmacological mechanisms for the retrieved flavonoids.

Conclusion: Naturally occurring flavonoids discussed in the present article have optimal immunomodulation to prevent immune-mediated disorders through management of Th1/Th2 cytokine balance.

Introduction

Flavonoids are the most prevalent dietary phytochemicals in human diet, representing nearly two-third of the phytonutrients obtained from plant-based foods (Glässer et al., 2002; Maksimovic´ et al., 2005). Flavonoids have a C6-C3-C6 carbon skeleton consisting of two aromatic rings enclosing a heterocyclic six-membered ring with oxygen, characterized by a two common benzo-gamma-pyrone structure (Kumar and Pandey, 2013) and are divided into six subclasses that include flavones, flavonols, flavanones, flavanonols, isoflavones and anthocyanins (Narayana et al., 2001). These subclasses differ in structure and functional characteristics (Middleton, 1984). Flavonoids are said to contain antioxidant, anti-diabetic, anti-viral, anti-allergic, anti-inflammatory and anti-carcinogenic (Kumar and Pandey, 2013) and

Abbreviations: BALF, Bronchoalveolar Lavage Fluid; CIA, Collagen induced-Rheumatoid Arthritis; EEE, Epicatechin, (2β-O-7, 4β-8)-ent-epicatechin; ELISA, Enzyme-Linked Immunosorbent Assay; EAM, Experimental Autoimmune Myocarditis; GAD, Graft Arterial Diseases; PBS, Phosphate Buffered Saline, PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RPA, RNase Protection Assay; RT-PCR, Reverse Transcription-Polymerase Chain Reaction

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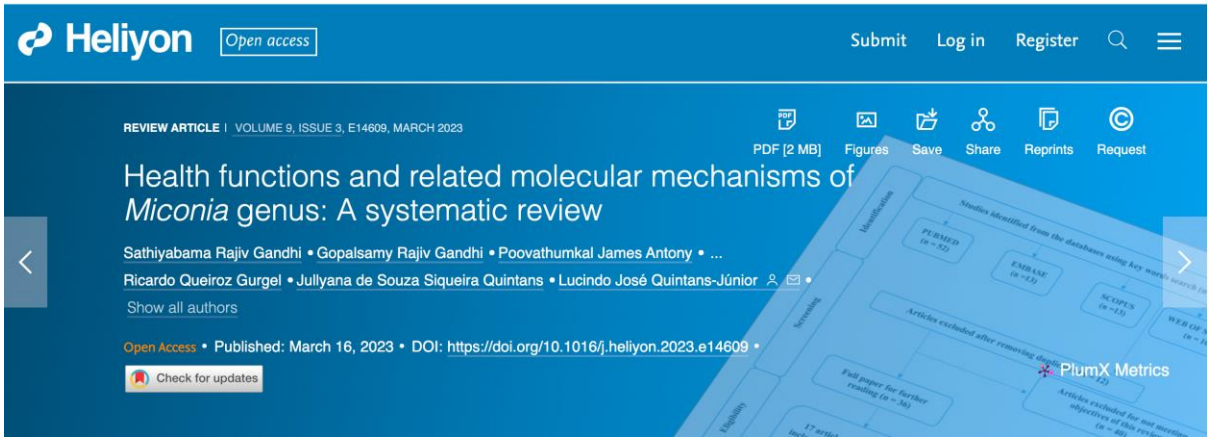
ANNEXURE A (RIGHTS AND CONTENTS OBTAINED TO INCLUDE DATA FROM ARTICLES IN THESIS AUTHORIZATION)



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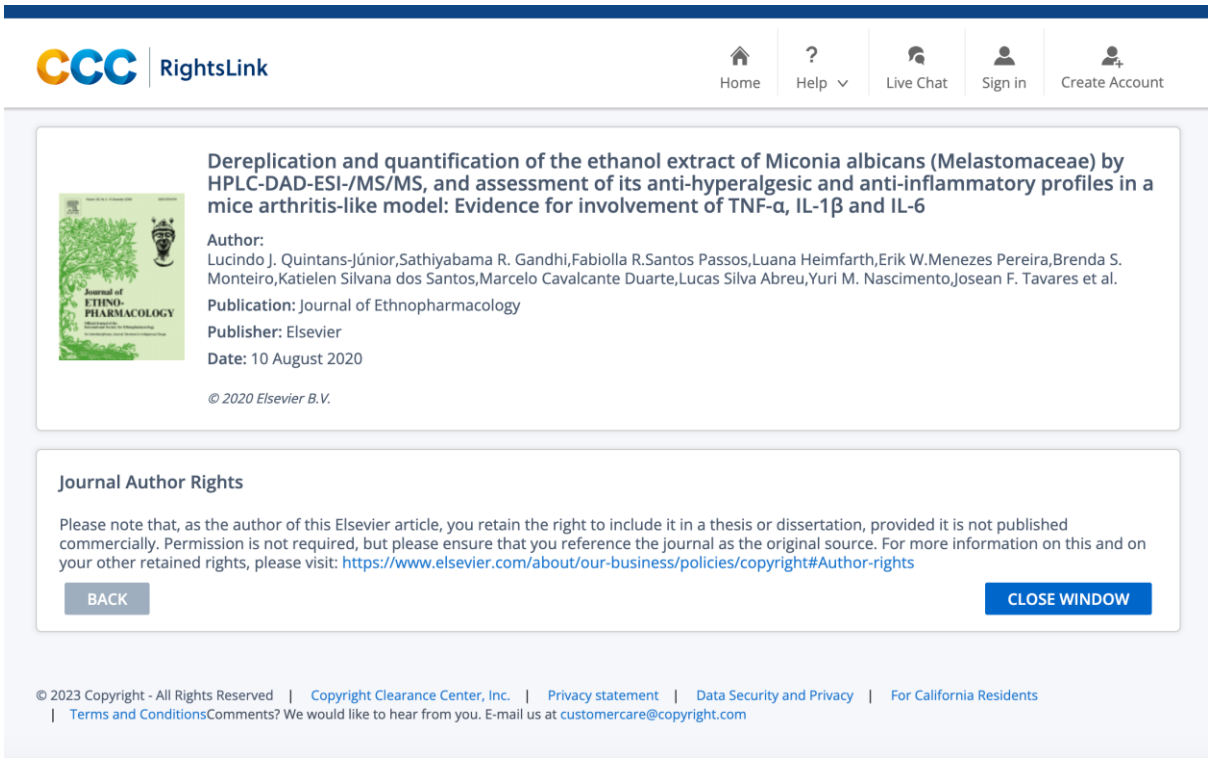

ANNEXURE B (ANIMAL ETHICS COMMITTEE)

UNIVERSIDADE FEDERAL DE SERGIPE
PRÓ-REITORIA DE PÓS-GRADUAÇÃO E PESQUISA
COORDENAÇÃO DE PESQUISA
COMITÊ DE ÉTICA EM PESQUISA COM ANIMAIS (CEPA)

CERTIFICADO

Certificamos que a proposta intitulada "**AVALIAÇÃO *IN VITRO E IN VIVO* DA ATIVIDADE ANTI-INFLAMATÓRIA DE EXTRATO ETANÓLICO DE MICONIA ALBICANS**", registrada com o **nº 23/2017**, sob a responsabilidade do **Prof. Dr. Lucindo José Quintans Júnior** que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Universidade Federal de Sergipe, em reunião de **26/09/2017**.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	Início: 01/10/2017, Término: 01/10/2018
Espécie/linhagem/raca	Camundongo Heterogênico Swiss
Nº de animais	192
Peso/Idade	25-30g / 2 meses
Sexo	M
Origem	Biotério Setorial do Departamento de Fisiologia da UFS.

Josemar Sena Batista
Prof. Dr. JOSEMAR SENA BATISTA
Coordenador do CEPA/UFS

Cidade Universitária "Prof. Aloísio de Campos"
Jardim Rosa Elze – São Cristóvão – SE
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ANNEXURE C (SISGEN)

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

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

Comprovante de Cadastro de Acesso

Cadastro nº AEB9A61

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

Número do cadastro:	**AEB9A61**
Usuário:	**LUCINDO JOSE QUINTANS JUNIOR**
CPF/CNPJ:	**930.961.434-04**
Objeto do Acesso:	**Conhecimento Tradicional Associado**
Finalidade do Acesso:	**Pesquisa**

Espécie

PLANTA INTEIRA

Fonte do CTA

CTA de origem não identificável