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Antifungal and antioxidant activities and chemical constituents from *Pluchea sagittalis*

Atividades antifúngica e antioxidante e constituintes químicos de *Pluchea sagittalis*

Actividades antifúngicas y antioxidantes y constituyentes químicos de *Pluchea sagittalis*

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Abstract

Pluchea sagittalis (Asteraceae) is widely distributed in South America and used as a medicinal plant to treat various diseases. The objectives of the work were to evaluate the antioxidant and antifungal activities of the ethanol extract and fractions from the aerial parts of *P. sagittalis*, as well as to identify the chemical constituents of the most active sample by liquid chromatography coupled to mass spectrometry with electrospray ionization (LC-MS-ESI). The ethanolic extract obtained by maceration of aerial parts showed relevant activity in DPPH free radical scavenging (IC_{50} = 33.9 µg mL^{-1}) and presented an anti-*Candida* effect with MIC values of 93.75 µg mL^{-1} for *C. albicans* and 187

µg mL^{-1} for *C. glabrata* and *C. tropicalis*. However, the results found for the chloroform fraction prepared from the extract were more significant. This fraction showed the highest antioxidant activity with an IC_{50} of 12.5 µg mL^{-1} and inhibited the growth of *Candida* spp. with MIC values ranging from 46.87 to 93.75 µg mL^{-1}. The qualitative phytochemical screening of this fraction indicated the presence of phenolic compounds and flavonoids. Twenty-three metabolites were identified by LC-MS in the chloroform fraction including caffeoylquinic acids derivatives, flavones and fatty acids, which have recognized antioxidant and antimicrobial effects. The results of the activities, as well as the chemical constituents present in the chloroform fraction, reveal the potential of this fraction as a source of antioxidant and antifungal agents.
Keywords: *Pluchea sagittalis*; Caffeoylquinic acids; Flavonoids; Fatty acids; *Candida* spp.; LC-MS.

Resumo
Pluchea sagittalis (Asteraceae) é amplamente distribuída na América do Sul e utilizada como planta medicinal no tratamento de diversas doenças. Os objetivos do trabalho foram avaliar as atividades antioxidante e antifúngica do extrato etanólico e frações das partes aéreas de *P. sagittalis*, bem como identificar os constituintes químicos da amostra mais ativa por cromatografia líquida acoplada à espectrometria de massas com ionização por electrospray (CL-EM-IES). O extrato etanólico obtido por maceração das partes aéreas mostrou relevante atividade no sequestro de radicais livres DPPH (CI_{50} = 33,9 µg mL^{-1}) e apresentou efeito anti-*Candida* com valores de CIM de 93,75 µg mL^{-1} para *C. albicans* e 187 µg mL^{-1} para *C. glabrata* e *C. tropicalis*. No entanto, os resultados encontrados para a fração clorofórmio preparada a partir do extrato foram mais significativos. Esta fração apresentou a maior atividade antioxidante com um CI_{50} de 12,5 µg mL^{-1} e inibiu o crescimento de *Candida* spp. com valores de CIM entre 46,87 e 93,75 µg mL^{-1}. A triagem fitoquímica qualitativa desta fração indicou a presença de compostos fenólicos e flavonoides. Vinte e três metabólitos foram identificados por CL-EM na fração clorofórmio incluindo derivados de ácidos cafeoilquínicos, flavonas e ácidos graxos, que por sua vez possuem reconhecidos efeitos antioxidantes e antimicrobianos. Os resultados das atividades, bem como os constituintes químicos presentes na fração clorofórmio, revelam o potencial desta fração como fonte de agentes antioxidantes e antifúngicos.
Palavras-chave: *Pluchea sagittalis*; Ácidos cafeoilquínicos; Flavonoides; Ácidos graxos; *Candida* spp.; CL-EM.

Resumen
Pluchea sagittalis (Asteraceae) está ampliamente distribuida en América del Sur y se utiliza como planta medicinal en el tratamiento de diversas enfermedades. Los objetivos de este trabajo fueron evaluar las actividades antioxidante y antifúngica del extracto etanólico y fracciones de las partes aéreas de *P. sagittalis*, así como identificar los constituyentes químicos de la muestra más activa mediante cromatografía líquida acoplada a espectrometría de masas de ionización por electrospray (CL-EM-IES). El extracto etanólico obtenido por maceración de partes aéreas mostró una actividad relevante en la captación de radicales libres DPPH (CI_{50} = 33,9 µg mL^{-1}) y presentó un efecto anti-*Candida* con valores de CIM de 93,75 µg mL^{-1} para *C. albicans* y 187 µg mL^{-1} para *C. glabrata* y *C. tropicalis*. Sin embargo, los resultados encontrados para la fracción de cloroformo preparada a partir del extracto fueron más significativos. Esta fracción mostró la mayor actividad antioxidante con una CI_{50} de 12,5 µg mL^{-1} e inhibió el crecimiento de *Candida* spp. con valores de CIM entre 46,87 e 93,75 µg mL^{-1}. El cribado fitoquímico cualitativo de esta fracción indicó la presencia de compuestos fenólicos y flavonoides. Veintitrés metabolitos fueron identificados por CL-EM en la fracción de cloroformo, incluyendo derivados de ácidos cafeoilquínicos, flavonas y ácidos grasos, que a su vez tienen efectos antioxidantes y antimicrobianos reconocidos. Los resultados de las actividades, así como los constituyentes químicos presentes en la fracción cloroformo, revelan el potencial de esta fracción como fuente de agentes antioxidantes y antifúngicos.
Palabras clave: *Pluchea sagittalis*; Ácidos cafeoilquínicos; Flavonoides; Ácidos grasos; *Candida* spp.; CL-EM.

1. Introduction

Advances in the medical field in recent decades have allowed the development of new drugs and treatments that promote greater life expectancy for immunocompromised patients such as those with neutropenia, HIV, cancer, chronic immunosuppression, residential prostheses, burns and diabetes mellitus. However, the increase in the immunocompromised population has led to a greater number of cases of fungal infections in which it has become a global public health problem (Nicola et al., 2019; Sanguinetti et al., 2019).

Fungi are pathogens that cause superficial and cutaneous diseases or can cause systemic diseases such as invasive fungal infections that contribute to the mortality of immunocompromised individuals (Badiee & Hashemizadeh, 2014; Sanguinetti et al., 2019). Worldwide, more than 300 million people are affected by serious fungal infections resulting in 1.6 million deaths (Life, 2017; Beardsley et al., 2018). Among the invasive diseases caused by fungi, invasive candidiasis affects

more than 750,000 people per year with a mortality rate of 40 per cent (Gaffi, 2020). *Candida albicans* is the main cause of candidemia and it is the most virulent pathogen, followed by other species *C. glabrata, C. tropicallis, C. parapsolis, C. krusei* and *C. auris* (Guinea, 2014; Kullberg & Arendrup, 2015; Beardsley et al., 2018). Even with the high mortality rate, there are still few investments seeking new therapeutic options for the treatment of candidiasis and as consequence the number of available antifungals is limited (Almeida et al., 2019; Rodrigues & Nosanchuk, 2020) From 2011 to 2014 only two antifungal compounds were approved by the Food and Drug Administration (FDA), efinaconazole and tavaborole. From 2015 to 2019 two other antifungals were approved, isavuconazone sulfate and fosravuconazole. In addition, drugs such as amphotericin and griseofulvin, launched in 1958, are still used to treat fungal infections (Newman & Cragg, 2016; Newman & Cragg, 2020). It is also notorious the toxicity associated with current antifungals and the increase in infections caused by resistant strains (Nicola et al., 2018). *Candida* species have already shown resistance in all classes of antifungals: polyenes, azoles, fluoropyrimidine and echinocandins (Vandeputte et al., 2012; Campoy & Adrio, 2017; Oliveira & Rodrigues, 2020). So, one of the greatest current challenges is the development of better agents to combatting fungal infections.

In this context, studies have shown that plants are an alternative in the search for new antifungals. Extracts from several species have already been shown to be active against fungi, including *Candida* spp. and various natural products have been reported for antifungal activity. In addition, two antifungal compounds based on natural products are in phase III trials, ibrexafungerp inspired by the triterpene enfumafungin and rezafungin based on the structure of echinocandins (Newman & Cragg, 2020).

Pluchea sagittalis (Lam) Cabrera, studied in this work, belongs to the Asteraceae family (Anderberg, 1994). It is popularly known as chitoco and lucera, it is widely used in folk medicine as a purgative, digestive, antispasmodic, to treat skin diseases (Campos-Navarro & Scarpa, 2013; Bieski et al., 2015), amenorrhea, inflammation (De Albuquerque et al., 2007), liver disease, stomach pain, cough suppressant, antipyretic and antiseptic (Filipov, 1994). Studies with *P. sagittalis* have shown that the species has several biological properties such as wound healing (Alerico et al., 2015), antinociceptive (Figueredo et al., 2011) antifibrotic (Ouriques et al., 2018), antioxidant (Pérez-García et al., 2001; Parejo et al., 2003), activity on the respiratory burst and the stress protein synthesis (Pérez-García et al., 2001), antiviral (Simões et al., 1999; Visintini Jaime et al., 2013), citotoxic (Monks et al., 2002), anti-inflammatory (Pérez et al., 1995; Gorzalczany et al., 1996; Pérez-García et al., 1996; Pérez-García et al., 2005; Schmidt et al., 2009) and ability to change the absorptive characteristics of the gastrointestinal tract (Burger et al., 2000). The presence of compounds that inhibit photosynthesis (Carvalho et al., 2019) and with insecticidal activity (Vera et al., 2008; Sosa et al., 2017) has also been reported.

Regarding the chemical profile of *P. sagittalis*, flavonoids, phenolic compounds (Martino et al., 1976; Martino & Debenedettii; Coussio, 1979; Carvalho et al., 2019), triterpenes (Pérez-García et al., 2005) and sesquiterpenes (Vera et al., 2008; Sosa et al., 2017) have already been isolated. Among these compounds, phenolics and flavonoids have a recognized ability to scavenge free radicals (Verma & Pratap, 2010), therefore, they can act by inhibiting oxidative stress within cells. Oxidative stress is the result of a disturbance in the balance between the formation of reactive radical species and elimination by antioxidant compounds. The accumulation of reactive species results in oxidation processes of cellular components and genetic material causing cardiovascular and neurodegenerative diseases, cancer, aging and other diseases. Thus, extracts rich in metabolites with antioxidant potential can act on oxidative stress preventing various diseases (Kaurinovic & Vastag, 2019).

The aims of the present work were to evaluate the antifungal and antioxidant activities of extract and fractions of *P. sagittalis*, as well as to propose the chemical composition of the most active fraction by high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS).

2. Methodology

2.1 Plant material

The aerial parts of *P. sagittalis* were collected in the garden of the Federal Institute of Triângulo Mineiro (Uberaba/Minas Gerais State, Brazil) under the coordinates 19° 39' 56.3"S 47° 58' 11.4"W in March 2018. The plant was identified by a specialist and registered on the National System of Genetic Resource Management and Associated Traditional Knowledge for Research (SISGEN) under the code A797D24.

2.2 Preparation of plant material, ethanol extraction and liquid-liquid extraction

The aerial parts were dried in an oven with circulating air (mark) at 40 °C for 7 days. Then, the plant material was ground in a knife mill (TEC MILL-TE633). The dry material from the aerial parts (0.2 kg) was extracted with ethanol (98% v v^{-1}) by maceration at room temperature for two days. The ethanol extract (EE) was filtered and the solvent removed on a rotary evaporator (LGI-Scientific-Student) at 40 °C under reduced pressure. This procedure was repeated 3 times and the dried ethanol extract was frozen and lyophilized.

The fractionation of the ethanol extract was carried out through the liquid-liquid extraction process. A mass of 17.0 g of EE was solubilized in 200 mL of methanol/water (9:1 v v^{-1}) and successively fractionated with equal volumes (4 x 200 mL) of solvents in increasing order of polarity (hexane and chloroform). The hexane (7.46 g) and chloroform (8.89 g) fractions were concentrated and dried under reduced pressure at 40 °C using rotary evaporator. Fractionation did not follow with other solvents of higher polarity since the addition of chloroform promoted the formation of a single phase with the remainder after extraction in hexane.

2.3 Phytochemical screening

Qualitative phytochemical analysis with ethanol extract and fractions of *P. sagittalis* was performed by thin layer chromatography (TLC) using TLC plates with silica gel 60 F 254 nm according to Wagner & Bladt (2009) and Waksmundzka-Hajnos et al. (2008). Two solvent systems were used as mobile phase: ethyl acetate/ formic acid/ acetic acid/ water (10.0 : 1.1 : 1.1 : 2.6 v v^{-1}), and chloroform/ metanol/ ammonium hydroxide (9.0 : 1.0 : 0.25 v v^{-1}).

The developers used were: aqueous ferric chloride solution (10% w v^{-1}) for detection of tannins and phenolic compounds; aluminum chloride methanolic solution (1% w v^{-1}) and NP-PEG reagent for flavonoids; Dragendorff and iodine-chloro-platin reagents for alkaloids; Potassium hydroxide ethanolic solution (10% w v^{-1}) for anthraquinones, anthrones and coumarins and Liebermann-Burchard for terpenes, sterols and saponins.

2.4 Total phenol content

The determination of the total phenols content of the extract and fractions of *P. sagittalis* was carried out using the Folin–Ciocalteau method (Sousa et al., 2007). Methanolic solutions of the ethanol extract and fractions at 400 μg mL^{-1} were prepared. In an aliquot (0.5 mL) of the methanolic solution, 2.5 mL of an aqueous solution of the Folin-Ciocalteau reagent (10% v v^{-1}) and, 2.0 mL of the sodium carbonate aqueous solution (7.5% w v^{-1}) were added. The mixture was kept in a heated bath at 50 °C for 5 minutes. After cooling the samples, absorbance measurements were recorded in a spectrophotometer (PG Instruments-T60) at 760 nm. The total phenol content of the samples was determined from the equation ($y = 0.0132x + 0.039$ and $r^2 = 0.9994$) obtained by the analytical curve using gallic acid as a standard (50, 40, 30, 25, 20, 15, 10 and 5 μg mL^{-1}). The results were expressed in mg of gallic acid equivalent per gram of dry extract (mg EAG g^{-1}). Analyzes were performed in triplicate.

2.5 Flavonoid content

Flavonoid content was determined according to Woisky and Salatino (1998). Methanolic solutions from extracts and fractions were prepared at 500 µg mL^{-1}. An aliquot of 2.0 mL of the methanolic solution from each sample was transferred to test tubes. Then, 1.0 mL of a solution of aluminum chloride ($AlCl_3$) in methanol (5% w v^{-1}) and 2.0 mL of methanol were added. The resulting solution was kept at room temperature for 30 minutes. Sample absorbances were measured in a spectrophotometer (PG Instruments-T60) at 425 nm. The flavonoid content was determined from the equation obtained by the analytical curve ($y = 0.0248x + 0.0006$ and $r^2 = 0.9996$) using quercetin as a standard (40, 30, 20, 10, 5 and 1 µg L^{-1}). The results were expressed in equivalent mg of quercetin per gram of dry extract (mg EQ g^{-1}). Analyzes were performed in triplicate.

2.6 Antioxidant activity

The antioxidant activity for the extract and fractions of *P. sagittalis* was determined using the 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) according to Sousa et al. (2007). The samples were solubilized in methanol at a concentration of 800 µg mL^{-1} (solution 100%), and subsequently, five dilutions were performed at 83, 66, 49, 32 and 15%. Then, a volume of 2.7 mL of DPPH methanolic solution (40 µg mL^{-1}) was added to an aliquot of 0.3 mL of each dilution extract or fraction. After one hour of reaction, the absorbance reduction values were immediately measured at a wavelength of 517 nm using a spectrophotometer (PG Instruments-T60). The percentage of sequestered DPPH was calculated by the expression: Antioxidant Activity (%) = $[Abs_{control} - (Abs_{sample} - Abs_{white} / Abs_{control})] \times 100$. Where: $Abs_{control}$ is the absorbance of methanol with the DPPH solution, Abs_{sample} is the initial absorbance of the mixture (DPPH methanolic solution + sample); Abs_{white} is the absorbance of the methanolic solution of the samples. The inhibitory concentration (IC_{50} - Concentration of sample necessary to scavenge 50% of DPPH radicals) was calculated from the equation of the calibration curve obtained from the percentage of scavenged DPPH with the concentrations tested for each sample.

2.7 High liquid performance chromatography coupled to electrospray ionization mass spectrometry (HPLC-MS-ESI)

The chloroform fraction showed better results in the tests of antifungal and antioxidant activities, then it was analyzed by liquid chromatography (HPLC-Agilent Infinity 1260) coupled to a high resolution mass spectrometer (Agilent® 6520 B) quadrupole time of flight (Q-TOF) with a source of ionization by electrospray (ESI).

The chromatographic parameters were: Agilent Poroshell C18 column (100 mm x 2.1 mm x 2.6 µm); mobile phase (A) composed of a mixture of ultrapure water with formic acid (0.1% v v^{-1}) and mobile phase (B) methanol. The gradient programming was 2% B (0 min), 98% B (0-15 min) and 100% B (15-17 min) with a flow rate of 0.7 mL min^{-1}. A volume of 1 µL of the sample at a concentration of 2.0 mg mL^{-1} was injected into the chromatograph. The high-resolution mass spectrometer operated with a nebulizer pressure at 58 PSI, drying gas flow at 8.0 L min^{-1}, temperature of 220 °C and capillary energy of 4.5 kV. High resolution ion masses were obtained (MS) in negative mode and sequential mass spectrometry (MS^2) analyzes were performed at different collision energies (5-30 eV). The proposed molecular formula for each compound was selected according to the MassHunter® Software following the lowest difference between the experimental mass and the theoretical mass (error in ppm), the equivalence of double bonds, and nitrogen rule. The proposals for the identification of compounds were carried out based on mass spectra libraries and works in the literature.

2.8 Antifungal activity

The antifungal activity was evaluated by the method of microdilution in broth considering the standards recommended by the Clinical and Laboratory Standards Institute (CLSI, 2008). The tested microorganisms were obtained from

the American Type Culture Collection (ATCC, Rockville MD, USA). The following yeasts were used in the evaluation of the antifungal activity: *Candida albicans* (ATCC 28366), *Candida tropicalis* (ATCC 13803) and *Candida glabrata* (ATCC 15126).

Initially, stock solutions were prepared by dissolving the extract and fractions in DMSO at a concentration of 192,000 µg mL^{-1}. Then, dilutions were carried out in buffered RPMI 1640 culture medium (pH 7.2) with 165 mM of 3-N-morpholinopropanesulfonic acid (MOPS, Acros Organics) up to a concentration of 12,000 µg mL^{-1}. The inoculum was prepared using the spectrophotometric method (530 nm) and compared with the 0.5 McFarland scale to obtain a value of 6.0 x 10^6 CFU mL^{-1}. Subsequently, dilutions in RPMI broth were performed until obtaining 1.2 x 10^3 CFU mL^{-1}. The determination of minimum inhibitory concentration (MIC) was performed using 96-well microplate plates in which serial dilutions were made to test concentrations ranging from 1.43 to 3,000 µg mL^{-1}. In each well, 100 µL of the inoculum suspension was added and the final volume tested was 200 µL. Amphotericin B was tested as a positive control at concentrations ranging from 8.0 µg mL^{-1} to 0.031 µg mL^{-1}. Sterility controls of the culture medium, samples, inoculum and DMSO were performed. The reference strains *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019) were used as method control according to the M27-A3 protocol (CLSI, 2008). The microplates were incubated for 48 h at 37 ºC, and then the MIC was determined by adding 30 µL of resazurin developer (0.02% w v^{-1}).

3. Results and Discussion

3.1 Extraction yield and phytochemical screening

The ethanol extract of the aerial parts showed a yield of 21.0 g (10.5% w w^{-1}) from the dry mass of the aerial parts. The hexane and chloroform fractions had yields of 7.46 g (43.8% w w^{-1}) and 8.89 g (52.3% w w^{-1}) from the ethanol extract, respectively. According to the sequence of solvents used in the liquid-liquid extraction it was possible to separate the low and medium polarity constituents present in the ethanol extract. After the initial extraction of lipophilic compounds with hexane, chloroform was added to the remaining polar phase forming only one phase. For this reason, the liquid-liquid extraction did not follow with other solvents with higher polarity. This procedure is important since the components of similar polarity with the solvent are concentrated in the fraction and this can generate significant differences within biological tests. Table 1 shows the results for the phytochemical screening of the ethanol extract and fractions of *P. sagittalis*.

Table 1. Preliminary phytochemical screening of aerial parts of *P. sagittalis*.

Developers	Class of compounds	Samples		
		EE	FH	FC
Liebermann-Burchard	Terpenes and steroids	+	+	+
Ferric Chloride	Phenolics and tannins	+	+	+
Aluminum chloride	Flavonoids	+	-	+
NEP/PEG	Flavonoids	+	-	+
Dragendorff	Alkaloids	-	-	-

Note: ethanol extract (EE), hexane fraction (HF), chloroform fraction (CF). (-) not identified; (+) identified. Source: Authors.

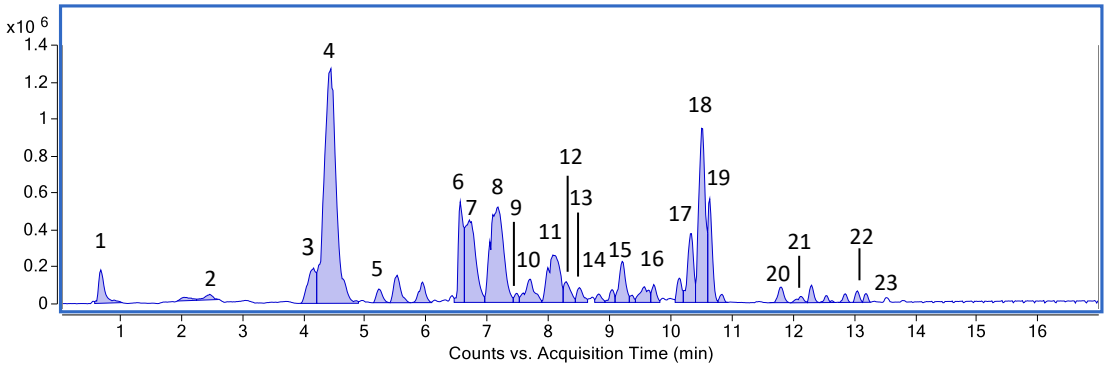
By qualitative phytochemical screening, it was possible to suggest the presence of flavonoids, phenolics, tannins, terpenes and steroids in the EE and CF fraction of *P. sagittalis* (Table 1), and in the HF fraction, the presence of terpenes, steroids and phenolic compounds was evidenced. These classes of compounds were also found in other works with *P.*

sagittalis (Figueredo et al., 2011; Rodrigues, 2011; Grandini et al. 2017; Martino et al., 1979; Martino et al., 1984). Alkaloids were not revealed in the extract and in any of the investigated fractions.

3.2 Identification of compounds by HPLC-MS-ESI

The chloroform fraction was analyzed by HPLC-MS-ESI for having presented relevant results for antioxidant and antifungal activities that will be discussed later. In Figure 1, the base peak chromatogram (BPC) obtained in negative mode for the chloroform fraction is represented, and in Table 2 is the proposed identification of compounds present in this sample.

Figure 1. Chromatogram of the chloroform fraction of *P. sagittalis* obtained by HPLC-MS-ESI in negative mode.



Source: Authors.

According to the identification proposal (Table 2), it was possible to confirm the presence of phenolic compounds and flavonoids in the chloroform fraction in which they were evidenced in the preliminary phytochemical screening. Twenty-three compounds were identified and their structures are shown in Figure 2. The molecular ion $[M-H]^-$ at *m/z* 191 was identified as quinic acid (1) and reports of the presence of this compound in *P. sagittalis* were not found. However, some caffeoylquinic acids (derived from quinic acid) have been identified and the presence of these compounds in the species is well known. Compounds 2, 3 and 4 had molecular ion at *m/z* 353, and according to Masiki et al. (2017) and Lin & Harnly (2010) correspond to the isomers 3-caffeoylquinic acid (2), 5-caffeoylquinic acid (3) and 4-caffeoylquinic acid (4). The three acids showed as main ion fragment the $[M-H\text{-caffeoyl}]^-$ at *m/z* 191. The 3-caffeoylquinic acid (2) has already been isolated from the ethyl acetate fraction, while the 5-caffeoylquinic acid (3) has been isolated from the ethyl ether fraction of the aerial parts of *P. sagittalis* (Martino et al., 1984). Compounds 3 and 4 were also identified in the ethanol extract of *P. indica* leaves (Ruan et al., 2019).

Compounds 6, 7 and 8 with *m/z* 515 were related to three dicaffeoylquinic acids already isolated from the ethyl ether fraction of aerial parts of *P. sagittalis* (Martino et al., 1979; Martino et al., 1984). These isomers were also isolated from the ethyl acetate fraction of the leaves, methanol extract of the roots of *P. sagittalis* (Stüker, 2012), ethyl acetate fraction of *P. symphytfolia* leaves (Scholz et al., 1994), and identified in *P. indica* (Ruan et al., 2019). Both isomers showed a main fragment at *m/z* 353 $[M-H\text{-caffeoyl}]^-$ for the loss of 162 u. The fragmentation spectra (MS^2) of the three compounds showed the characteristic fragments at *m/z* 191 $[M-H\text{-2caffeoyl}]^-$, 179 $[M-H\text{-caffeic-quinic}]^-$, and 173 $[M-H\text{-2caffeoyl-}H_2O]^-$. Compound 10 identified as feruloyl-caffeoylquinic acid with *m/z* 529 was found in *P. indica* (Ruan et al., 2019). Compound 11 with *m/z* 677 (3,4,5-Tricaffeoyl-quinic acid) was isolated from methanol extract of *P. sagittalis* roots (Stüker, 2012) and together with

compound 12 of *m/z* 839 were isolated from *P. symphytfolia* and identified in *P. indica* (Scholz et al., 1994; Ruan et al., 2019). A high content of caffeoylquinic acids in *P. sagittalis* has been previously reported (Martino et al., 1989).

The flavonoids were another class of compounds found in *P. sagittalis.* The molecular ion $[M-H]^-$ at *m/z* 477 was identified as isorhamnetin 3-*O*-glucoside (9), the aglycone has already been isolated in *P. symphytfolia* (Scholz et al., 1994), from ethyl acetate extract of *P. carolinensis* leaves (Córdova et al., 2006), and identified in *P. indica* (Ruan et al., 2019). This flavonoid showed a loss of 162 u from the cleavage between hexose and aglycone resulting in the fragment $[M-H-Hexose]^-$ at *m/z* 287.

Compounds 13 and 18 showed molecular ions $[M-H]^-$ at *m/z* 345 and 343, respectively, and corresponded to methoxylated flavones. Compounds 14 and 17 with molecular ions $[M-H]^-$ at *m/z* 315 and 329 respectively, were identified as methoxylated quercetins. Although not reported in *P. sagittalis*, other flavonoids with a similar profile have already been identified in the species (Martino et al., 1976; Martino et al., 1984; Carvalho et al., 2019). Compound 15 with molecular ion $[M-H]^-$ at *m/z* 359 was related to a trihydroxy-trimethoxy flavone. This compound can correspond to 5,7,3'-trihydroxy-3,6,4'-trimethoxyflavone or 5.3',4'-trihydroxy-3,6,7-trimethoxyflavone already isolated from aerial parts of *P. sagittalis* (Martino et al., 1984).

Compounds 19, 20, 21, 22 and 23 are derivatives of fatty acids that present hydroxylations and unsaturations in the chair. This class of compound has already been isolated from *P. indica* with similar structures (Ruan et al., 2018). Compound 5 of *m/z* 387 has not yet been reported in *P. sagittalis*.

When studies involving the chemical constituents of *P. sagittalis* are compared, qualitative and quantitative variations can be observed (Parejo et al., 2003; Martino et al., 1984; Martino et al., 1979; Rodrigues, 2011; Stüker, 2012; Martino et al., 1976, Ahemd & Kamel, 2013; Pérez-García et al., 1996; Grandini et al., 2017; Carvalho et al., 2019). Even within the same species, several factors can affect the chemical composition of the plant, such as plant part, climatic conditions, soil type, growth stage, seasonality, in addition to the extraction techniques and analytical methods employed (Dhifi et al., 2016; Moreira et al., 2021).

It is noteworthy here that the extraction sequence used in this study promoted the concentration in the chloroform fraction of the main phenolic compounds and flavonoids already isolated or identified in *P. sagittalis*.

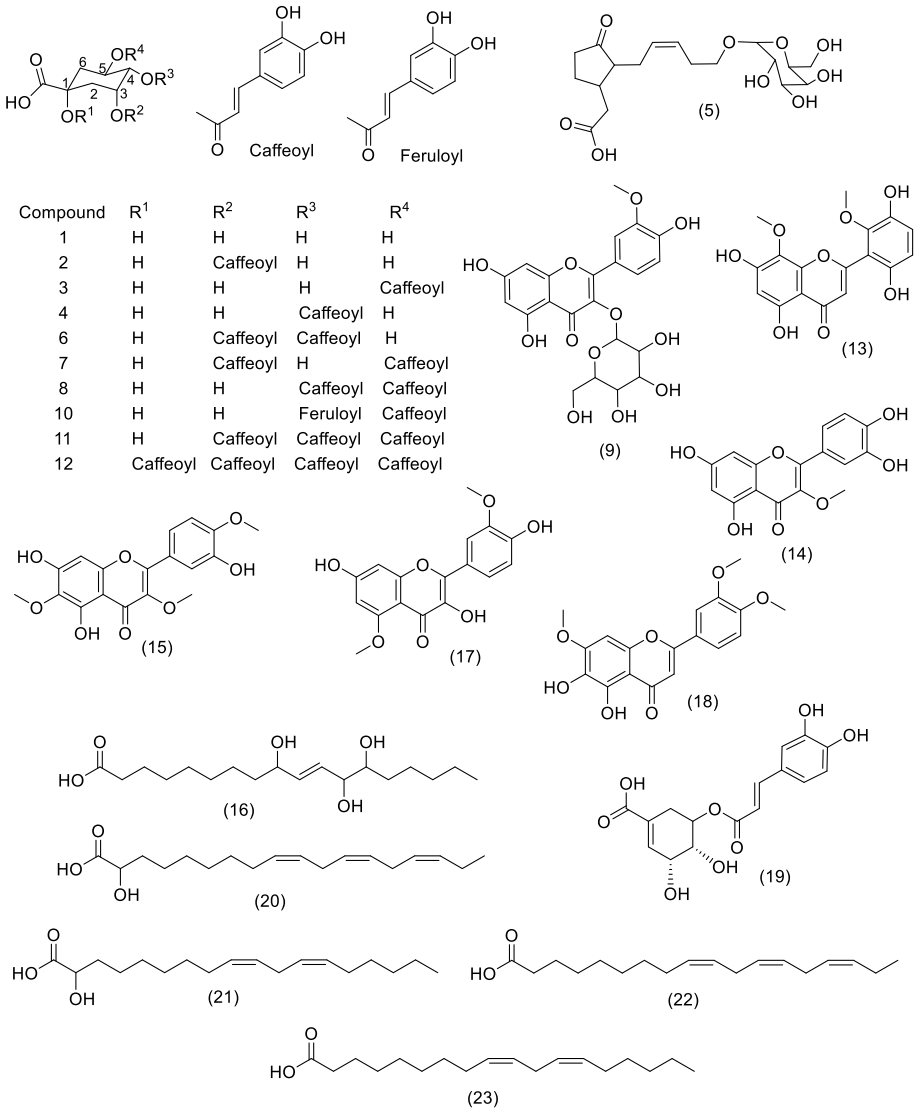
Table 2. Proposed identification of compounds present in the chloroform fraction of *P. sagittalis* by HPLC-MS-ESI.

N.	R_t (min)	$[M - H]^-$ MS	Error (ppm)	Fragments MS^2	Molecular formula	Tentative identity	Reference
1	0.68	191.0561	0.0	10 eV: **191**, 173, 127, 109	$C_7H_{12}O_6$	Quinic acid	Metlin; Abu-Reidah et al., 2015
2	2.48	353.0877	-0.28	20 eV: 257, **191**, 179, 135	$C_{16}H_{18}O_9$	3-Caffeoyl-quinic acid	Masiki et al., 2017; Matsui et al., 2007; Lin and Harnly, 2010; Martino et al., 1984
3	4.20	353.0878	0.00	10 eV: **191**, 179, 135	$C_{16}H_{18}O_9$	5-Caffeoyl-quinic acid	Masiki et al., 2017; Lin & Harnly, 2010; Martino et al., 1984
4	4.50	353.0879	0.28	10 eV: **191**, 179, 173, 135	$C_{16}H_{18}O_9$	4-Caffeoyl-quinic acid	Masiki et al., 2017; Lin & Harnly, 2010
5	5.22	387.1660	-0.25	20 eV: 370, 369, 225, **207**, 163	$C_{18}H_{28}O_9$	Hydroxyjasmonic acid -*O*-hexoside	Pereira et al., 2013
6	6.55	515.1192	-0.58	20 eV: **353**, 191, 179, 173, 161, 135	$C_{25}H_{24}O_{12}$	3,4-Dicaffeoyl-quinic acid	Han et al., 2008; Lin & Harnly, 2010; Stüker, 2012; Martino et al., 1984
7	6.74	515.1194	-0.19	20 eV: **353**, 191, 179, 161, 135	$C_{25}H_{24}O_{12}$	3,5-Dicaffeoyl-quinic acid	Han et al., 2008; Stüker, 2012; Martino et al., 1984
8	7.15	515.1194	-0.19	20 eV: **353**, 191, 179, 173, 135	$C_{25}H_{24}O_{12}$	4,5-Dicaffeoyl-quinic acid	Han et al., 2008; Lin & Harnly, 2010; Stüker, 2012; Martino et al., 1984
9	7.50	477.1038	0.00	20 eV: 431, **315**, 301, 300, 271, 243, 214, 161	$C_{22}H_{22}O_{12}$	Isorhamnetin 3-*O*-glucoside	Han et al., 2008; Lin & Harnly, 2007
10	7.70	529.1349	-0.37	20 eV: 367, **353**, 191, 179, 173	$C_{26}H_{26}O_{12}$	Feruloyl-caffeoyl-quinic acid	Han et al., 2008; Lin & Harnly, 2010
11	8.10	677.1516	0.59	20 eV: **515**, 353, 335, 191, 179, 173, 161, 155, 135, 111	$C_{34}H_{30}O_{15}$	3,4,5-Tricaffeoyl-quinic acid	Han et al., 2008; Lin & Harnly, 2010; Stüker, 2012
12	8.30	839.1831	0.23	20 eV: **839**, 677, 631, 515, 353, 191, 179, 135	$C_{43}H_{36}O_{18}$	1,3,4,5-Tetracaffeoyl-quinic acid	Scholz et al., 1994; Ruan et al., 2019
13	8.50	345.0619	0.87	20 eV: 330, **315**, 287, 271, 243	$C_{17}H_{14}O_8$	Viscidulin III	Wang et al., 2008
14	8.80	315.0510	0.00	20 eV: **300**, 271, 255, 243, 227	$C_{16}H_{12}O_7$	Quercetin 3-methyl ether	Metlin; Iwashina et al., 2012

N.	R_t (min)	$[M - H]^-$ MS	Error (ppm)	Fragments MS^2	Molecular formula	Tentative identity	Reference
15	9.20	359.0769	-0.83	20 eV: 344, **329**, 314, 301, 286, 258, 230	$C_{18}H_{16}O_8$	5,7,3`-trihydroxy-3,6,4`-trimethoxy-flavone or 5,3`,4`-trihydroxy-3,6,7-trimethoxy-flavone	Falcão et al., 2013; Han et al., 2008; Martino et al., 1984
16	9.87	329.2334	7.28	20 eV: 229, 211, 193, 183, 171, 139,	$C_{18}H_{34}O_5$	Trihydroxy-octadecenoic acid	Agalar et al., 2018
17	10.32	329.0666	-0.30	20 eV: 314, 299, **271**	$C_{17}H_{14}O_7$	Quercetin dimethyl-ether	Falcão et al., 2013
18	10.52	343.0822	-0.29	20eV: **328**, 313, 298, 285, 270, 257	$C_{18}H_{16}O_7$	Dihydroxy-trimethoxy-flavone	Salih et al., 2017
19	10.60	251.1652	-0.39	20 eV: **251**, 233, 191, 123	$C_{15}H_{24}O_3$	Ilicic acid	Metlin; Mamoci et al., 2011; Nguyen et al., 2010
20	11.80	293.2121	-0.34	20 eV: **275**, 235, 223, 195, 183, 171, 155, 121	$C_{18}H_{30}O_3$	Hydroxy-octadecatrienoic acid	Gomez-Romero et al., 2010
21	12.13	295.2279	0.00	20 eV: **295**, 277, 251, 211, 171	$C_{18}H_{32}O_3$	Hydroxy-octadecadienoic acid	Metlin; Gomez-Romero et al., 2010,
22	13.18	277.2174	0.36	20 eV: 259, 150, 127	$C_{18}H_{30}O_2$	Octadecatrienoic acid (Linolenic acid)	Gomez-Romero et al., 2010
23	13.50	279.2328	-0.71	20 eV: 279, 261	$C_{16}H_{32}O_2$	Octadecadienoic acid (Linoleic acid)	Metlin

Source: Authors.

Figure 2. Compounds identified in chloroform fraction of *P. sagittalis.*



Compound	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	Caffeoyl	H	H
3	H	H	H	Caffeoyl
4	H	H	Caffeoyl	H
6	H	Caffeoyl	Caffeoyl	H
7	H	Caffeoyl	H	Caffeoyl
8	H	H	Caffeoyl	Caffeoyl
10	H	H	Feruloyl	Caffeoyl
11	H	Caffeoyl	Caffeoyl	Caffeoyl
12	Caffeoyl	Caffeoyl	Caffeoyl	Caffeoyl

Source: Authors.

3.3 Total phenol content, flavonoid content and antioxidant activity

The results for the content of total phenols, total flavonoids and antioxidant activity are shown in Table 3.

Table 3. Phenolics and flavonoids content and antioxidant activity from *P. sagittalis*

Samples	Total phenols (mg GAE/g extract)	Flavonoids (mg QE/g extract)	Antioxidant activity IC_{50} (µg mL^{-1})
EE	281.4	25.9	33.9
HF	19.4	–	53.4
CF	354.0	12.9	12.5
BHT	--	--	7.8

Note: (EE) ethanol extract, (HF) hexane fraction, (CF) chloroform fraction. –: not obtained. Control (Butylated hydroxytoluene - BHT). Source: Authors.

Of the analyzed samples, the chloroform fraction had the highest content of phenolic compounds, indicating that the liquid-liquid extraction from the ethanolic extract promoted the concentration of phenolic compounds in this fraction. Unlike total phenols, the ethanol extract had a higher content of flavonoids (25.9 mg QE/g extract). The total phenol content for EE and FC was higher than the flavonoid content; this condition can be justified by the methodology used, which measures the total concentration of phenolic hydroxyl groups present in the extract covering a larger amount of compounds (Blainski et al., 2013). The method for total flavonoids using $AlCl_3$ is more specific, stable complexes are formed with the carbonyl and hydroxyl groups of the flavonoids (Sepahpour et al., 2018). The hexane fraction had the lowest content of phenolic compounds and no flavonoids were determined, which is expected due to the fact that this fraction is non-polar.

The antioxidant activity of *P. sagittalis* was performed by the DPPH radical scavenging method; the results for the extract and fractions are shown in Table 2. The ethanol extract, hexane fraction and chloroform fraction presented IC_{50} values of 33.99, 53.39 and 12.51 μg mL^{-1}, respectively. IC_{50} values below 50 μg mL^{-1} indicate that the extract has high antioxidant activity, 50-100 μg mL^{-1}, moderately active, 100-200 μg mL^{-1}, low activity and above 200 μg mL^{-1}, the extract is considered inactive (Reynertson et al., 2005).

EE and CF showed high antioxidant activity, with emphasis on FC which exhibited IC_{50} of 12.50 μg mL^{-1}. These results followed a clear correlation with phenols content, CF (354.0 mg GAE/g extract) and EE (281.4 mg GAE/g extract) had the highest values of total phenols, and consequently the lowest IC_{50} values. The HF (lowest polarity) had the lowest phenol content (19.4 mg GAE/g extract) and the highest IC_{50} value (53.4 μg mL^{-1}), indicating moderate antioxidant activity. The high free radical scavenging capacity of phenols is well known. These compounds are able to stabilize free radicals by hydrogen atom donation or by electron transfer mechanisms (Chen et al., 2020).

The proposed identification by HPLC-MS-ESI revealed the presence of several phenolic compounds within the chloroform fraction. Caffeoylquinic acids and their derivatives can be important contributors to the antioxidant activity of this sample. The antioxidant activity of some caffeoylquinic acids has already been evaluated by DPPH method, compounds 4 and 5 showed IC_{50} of 32.76 and 116.4 μg mL^{-1}, respectively (Ganzon et al., 2018). Compounds 7, 8 and 9 were also evaluated and had an IC_{50} of 18.2, 13.4 and 10.4 μg mL^{-1}, respectively (Manh et al., 2006). The increase in caffeoyl quinic acid content has already been related to the increase in the antioxidant activity intensity of sweet potato leaves (Jeng et al., 2015).

Flavonoids were another class of compounds found within the chloroform fraction reported for antioxidant action. Flavonoid-rich extracts are linked to strong antioxidant activity (Wang et al., 2020). In particular, quercetin and quercetin derivatives have significant free radical scavenging capabilities (Oh et al., 2019; Ozgen et al., 2016). Thus, the high antioxidant activity observed with CF may be related to the synergism between flavonoids and phenolic compounds present in this fraction.

The total phenol content and the antioxidant activity of aerial parts of *P. sagittalis* cultivated in Bolivia (Bulo Bulo, Carrasco, Bolivia) were evaluated. The methanolic and aqueous extracts, and the hexane, dichloromethane, ethyl acetate and aqueous fractions had phenol content 76.6, 134.4, 2.9, 85.9, 237.9 and 128.0 GAE/ mg extract, while the antioxidant activity values in terms of IC_{50} were 124.0, 119.4, not determined, 156.5, 237.9 and 71.0 μg mL^{-1}, respectively (Parejo et al., 2003). As in this work, the fraction with the highest content of phenols was found to have the highest activity in DPPH free radical scavenging. The IC_{50} values found for EE and CF are lower when compared to extracts and fractions analyzed by Parejo et al. (2003), suggesting greater antioxidant potential.

The antioxidant activity of the aqueous extract of the whole plant of *P. sagittalis* was evaluated in blood leukocytes showing strong inhibition of free radicals *in vivo* and *in vitro*, however, the activity observed was not related to any class of metabolite (Pérez-García et al., 1996). In the study by Grandini et al. (2017), extracts of flowers and leaves with stalks of *P.*

sagittalis obtained by supercritical extraction with CO_2 under different pressure conditions were evaluated by the DPPH radical test. Flower extracts showed IC_{50} between 31.56 and 77.02 g extract/g DPPH, while extracts from leaves together with stems showed IC_{50} between 58.48 and 97.03 g extract/ g DPPH. In this study, the antioxidant effect was attributed to the presence of phenolic compounds present in the extracts.

It is important to consider that although the conditions of the works with *P. sagittalis* mentioned above are different from this one, the results of antioxidant activity found here corroborate these studies, showing the high antioxidant potential of *P. sagittalis*.

3.4 Antifungal activity

The antifungal activity was evaluated by the broth microdilution method. The FC fraction showed better inhibition values for the three evaluated yeasts (Table 4).

Table 4. Antifungal activity of the extract and fractions of *P. sagittalis.*

Samples	Minimum Inhibitory Concentration - MIC (µg mL^{-1})		
	C. albicans **(ATCC 28366)**	***C. tropicalis*** **(ATCC 13803)**	***C. glabrata*** **(ATCC 28366)**
EE	93.75	187.5	187.5
HF	3000	3000	1500
CF	46.87	93.75	93.75
Positive control	0.25	0.12	0.25

Note: (EE) ethanol extract, (HF) hexane fraction, (CF) chloroform fraction. Positive control: Amphotericin B; Yeast control by protocol M27-A3 CLSI (2008): *C. krusei* (MIC = 1.0 µg mL^{-1}) and *C. parapsilosis* (MIC = 0.5 µg mL^{-1}). Source: Authors.

According to Kuete (2010), the antimicrobial activity can be considered significant for MIC < 100 µg mL^{-1}, moderate for MIC between 100 and 625 µg mL^{-1} and weak for MIC > 625 µg mL^{-1}. Thus, it can be considered that the EE of *P. sagittalis* had a significant activity for *C. albicans* and moderate for other yeasts. The FC fraction had a significant effect for *C. albicans*, *C. tropicalis* and *C. glabrata* with MIC values between 93.75 and 46.87 µg mL^{-1}, while the hexane fraction showed weak activity for all tested yeasts (3000-1500 µg mL^{-1}).

The antimicrobial activity of the methanol extract of aerial parts of *P. sagittalis* was evaluated against some bacteria and fungi by disk diffusion assay, *C. albicans* and *Saccharomyces cerevisiae* were considered resistant with a halo of inhibition (8-11 mm). Among the bacteria tested, only *Micrococcus luteus* was sensitive to the extract with an inhibition halo of 11.1-16 mm (De Souza et al., 2004). The aqueous/ethyl acetate and aqueous/hexane fractions of *P. sagittalis* leaves and the methanol extract of the roots had a MIC of 500 µg mL^{-1} for different Gram negative and Gram positive bacteria and some fungi, including *C. albicans* (Stüker, 2012). In this work, the FC fraction and EE were more active for *C. albicans* with MIC of 46.87 and 93.75 µg mL^{-1}, respectively. Other studies of *P. sagittalis* against *C. glabrata* and *C. tropicalis* were not found in the literature. The hydroalcoholic extract of the aerial parts of *P. sagittalis* also showed antibacterial activity against several microorganisms that cause bovine mastitis (Olanda et al., 2019). The preparation of the ethanol extract followed by fractionation may have contributed to evidence the antifungal activity of CF, and these results corroborate with previous investigations in relation to *P. sagittalis*.

The relevant activity of FC against the tested yeasts may be related to its chemical composition, phenolic acids and flavonoids have been shown to be strong anti-*Candida* agents (Martins et al., 2015; Oliveira et al., 2018). Extracts from other plants rich in compounds derived from caffeoyl acid and flavonoids have already shown antifungal activity (Džamić et al., 2013; Faustino et al., 2018; Oliveira et al., 2018). Furthermore, studies have also indicated antifungal activity of some monohydroxylated and unsaturated fatty acids (Liang et al., 2020) and, the extract rich in fatty acids such as compounds 34 and 35 may have action against *Candida* species (Rhimi et al., 2018).

C. albicans and other *Candida* species have been associated with several opportunistic fungal infections (Martins et al., 2015). In this context, the chloroform fraction can be considered a promising source of anti-*Candida* agents since it showed significant activity against all yeasts tested with MIC values lower than 100 µg mL^{-1} and lower than other studies with extracts and fractions of *P. sagittalis* (Stuker, 2012). Furthermore, the results found with the chloroform fraction can be considered even more promising, since the observed MIC values are lower than several other extracts from different plants and some compounds isolated against these microorganisms (Martins et al., 2015).

4. Conclusion

P. sagittalis is a medicinal plant with great biological potential and several studies have proven its popular use. The present work allowed to evidence the antioxidant and antifungal action of the aerial parts of *P. sagittalis*. From the liquid-liquid extraction of the ethanolic extract, it was possible to obtain the chloroform fraction enriched with phenolic compounds and flavonoids, and with high antioxidant and anti-*Candida* potential. The compounds identified in the chloroform fraction by HPLC-MS have recognized antifungal and antioxidant activities. Caffeoyl quinic acids and flavonoids were the main special metabolites identified in the most active fraction. Thus, this study contributes to the chemical and biological knowledge of *P. sagittalis* which can be considered a source of antioxidant and antifungal compounds. Further studies with the chloroform fraction evaluating its effect against other *Candida* species and its cytotoxic activity will be carried out.

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References

Abu-Reidah, I. M., Ali-Shtayeh, M. S., Jamous, R. M., Arráez-Román, D., & Segura-Carretero, A. (2015). HPLC–DAD–ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. *Food Chemistry*, 166(1), 179-191. https://doi.org/10.1016/j.foodchem.2014.06.011.

Ağalar, H. G., Çiftçi, G. A., Göger, F., & Kırımer, N. (2017). Activity guided fractionation of *Arum italicum* miller tubers and the LC/MS-MS profiles. *Records of Natural Products*, 12, 64-75.

Ahemd, S. A., & Kamel, E. M. (2013). Phenolic constituents and biological activity of the genus Pluchea. Der Pharma Chemica, 5(5), 109-114.

Aldholmi, M., Marchand, P., Ourliac-Garnier, I., Le Pape, P., & Ganesan, A. (2019). A Decade of Antifungal Leads from Natural Products: 2010-2019. *Pharmaceuticals*, 12(4), 1-22. https://doi.org/10.3390/ph12040182.

Alerico, G. C., Beckenkamp, A., Vignoli-Silva, M., Buffon, A., & Von Poser, G. L. (2015). Proliferative effect of plants used for wound healing in Rio Grande do Sul state, Brazil. *Journal of Ethnopharmacology*, 176, 305-310. https://doi.org/10.1016/j.jep.2015.11.001.

Almeida, F., Rodrigues, M. L., & Coelho, C. (2015). The still underestimated problem of fungal diseases worldwide. *Frontiers in microbiology*, 10(214), 1-5. https://10.3389/fmicb.2019.00214.

Bremer, K. R. (1994). Asteraceae: Cladistics & classification. Portland: Timber Press.

Badiee, P., & Hashemizadeh, Z. (2014). Opportunistic invasive fungal infections: diagnosis & clinical management. *The Indian Journal of Medical Research*, 139(2), 195-204. https://pubmed.ncbi.nlm.nih.gov/24718393.

Beardsley, J., Halliday, C. L., Chen, S. C. A., & Sorrell, T. C. (2018). Responding to the emergence of antifungal drug resistance: perspectives from the bench and the bedside. *Future Microbiology*, 13(10), 1175-1191. https://10.2217/fmb-2018-0059.

Bieski, I. G. C., Leonti, M., Arnason, J. T., Ferrier, J., Rapinski, M., Violante, I. M. P., Balogun, S. O., Pereira, J. F. C. A., Figueiredo, R. D. C. F., Lopes, C. R. A. S., Da Silva, D. R., Pacini, A., Albuquerque, U. P., & Martins, D. T. O. (2015). Ethnobotanical study of medicinal plants by population of Valley of Juruena Region, Legal Amazon, Mato Grosso, Brazil. *Journal of Ethnopharmacology*, 173, 383-423. https://doi.org/10.1016/j.jep.2015.07.025.

Blainski, A., Lopes, G. C., & De Mello, J. C. (2013). Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. *Molecules*, 18(6), 6852-6865. https://10.3390/molecules18066852.

Burger, M. E., Baldisserotto, B., Teixeira, E. P., & Soares, J. (2000). Action of the extracts of *Pluchea sagittalis* on the absorptive characteristics of the gastrointestinal tract. *Brazilian Archives of Biology and Technology*, 43(1). http://dx.doi.org/10.1590/S1516-89132000000100012.

Campos-Navarro, R., & Scarpa, G. F. (2013). The cultural-bound disease "empacho" in Argentina. A comprehensive botanico-historical and ethnopharmacological review. *Journal of Ethnopharmacology*, 148(2), 349-360. https://doi.org/10.1016/j.jep.2013.05.002.

Campoy, S., & Adrio, J. L. (2017). Antifungals. *Biochemical Pharmacology*, 133, 86-96. https://doi.org/10.1016/j.bcp.2016.11.019.

Carvalho, A. C., Lira, J. C. S., Pereira, T. M., Silva, S. C., Simote-Silva, S. Y., Oliveira, F. K. D., King-Diaz, B., Lotina-Hennsen, B., & Veiga, T. A. M. (2019). Natural products from Pluchea sagittalis act as inhibitors of photosynthesis in vitro. *Natural Product Research*, 33(4), 557-562. https://10.1080/14786419.2017.1396587.

Chen, J., Yang, J., Ma, L., Li, J., Shahzad., N., & Kim, C. K. Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids. *Scientific Reports*, 10, 2611 (2020). https://doi.org/10.1038/s41598-020-59451-z.

CLSI - Clinical and Laboratory Standards Institute . (2008). Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard, (3rd edition), CLSI document m27-A3.

De Souza, G. C., Haas, A. P. S., Von Poser, G. L., Schapoval, E. E. S., & Elisabetsky, E. (2004). Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. *Journal of Ethnopharmacology*, 90(1), 135-143. https://doi.org/10.1016/j.jep.2003.09.039.

Córdova, W. H. P., Mesa, L. G., Hill, A. L. P., Lima, C. N., Lamas, G. D., Suáreza, M. O., & Domínguezа, R. S. (2006). Antimicrobial activity of crude extracts and flavonoids from leaves of *Pluchea carolinensis* (Jacq.) G. Don. *Pharmacologyonline*, 3, 757-761.

De Albuquerque, U. P., De Medeiros, P. M., De Almeida, A. L. S., Monteiro, J. M., De Freitas Lins Neto, E. M., De Melo, J. G., & Dos Santos, J. P. (2007). Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach. *Journal of Ethnopharmacology*, 114(3), 325-354. https://doi.org/10.1016/j.jep.2007.08.017.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities. *A Critical Review Medicines*, *3*(4), 1-16. https://doi.org/10.3390/medicines3040025.

Džamić, A. M., Soković, M. D., Novaković, M., Jadranin, M., Ristić, M. S., Tešević, V., & Marin, P. D. (2013). Composition, antifungal and antioxidant properties of *Hyssopus officinalis* L. subsp. pilifer (Pant.) Murb. essential oil and deodorized extracts. *Industrial Crops and Products*, 51, 401-407. https://doi.org/10.1016/j.indcrop.2013.09.038.

Falcão, S. I., Vale. N., Gomes, P., Domingues, M. R., Freire, C., Cardoso, S. M., & Vilas-Boas, M. (2013). Phenolic profiling of Portuguese propolis by LC-MS spectrometry: uncommon propolis rich in flavonoid glycosides. *Phytochemical Analysis*, 24(4), 309-318. https://doi.org/10.1002/pca.2412.

Faustino, M. V., Pinto, D. C. G. A., Gonçalves, M. J., Salgueiro, L., Silveira, P., & Silva, A. M. S. (2018). *Calendula* L. species polyphenolic profile and in vitro antifungal activity. *Journal of Functional Foods*, 45, 254-267. https://doi.org/10.1016/j.jff.2018.04.013.

Figueredo, S. M., Do Nascimento, F. P., Freitas, C. S., Baggio, C. H., Soldi, C., Pizzolatti, M. G., De Ibarrola, M. D. C. C., De Arrua, R. L. D., & Santos, A. R. S. (2011). Antinociceptive and gastroprotective actions of ethanolic extract from *Pluchea sagittalis* (Lam.) Cabrera. *Journal of Ethnopharmacology*, 135(3), 603-609. https://doi.org/10.1016/j.jep.2011.03.006.

Filipov, A. (1994). Medicinal plants of the Pilagá of Central Chaco. *Journal of Ethnopharmacology*, 44(3), 181-193. https://doi.org/10.1016/0378-8741(94)01185-0.

GAFFI - Global Action Found for Fungal Infections. (2020). Disponível em: <https://www.gaffi.org/>. Acesso em: maio de 2020.

Ganzon, J. G., Chen, L. G., & Wang, C. C. (2018). 4-O-Caffeoylquinic acid as an antioxidant marker for mulberry leaves rich in phenolic compounds. *Journal of Food and Drug Analysis*, 26(3), 985-993. https://doi.org/10.1016/j.jfda.2017.11.011.

Giada, M. (2013). Food phenolic compounds: Main classes, sources and their antioxidant power. In: Morales-Gonzále, J. A. Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants, Rijeka: InTech, cap. 4, 87–112.

Gómez-Romero, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry*, 71,(16), 1848-1864. https://doi.org/10.1016/j.phytochem.2010.08.002.

Gorzalczany, S., Acevedo, C., Muschietti, L., Martino, V., & Ferraro, G. (1996). Search for antiinflammatory activity in Argentine Medicinal Plants. *Phytomedicine*, 3(2), 181-184. https://doi.org/10.1016/S0944-7113(96)80033-X.

Grandini, C. P. (2017). Obtenção de extratos voláteis e não voláteis de Pluchea sagittalis (Lam.) Cabrera (quitoco): 39571 Richer Costa Camargo et al., Potencial antimicrobiano de extratos etanólicos de plantas contra staphylococcus aureus multirresistentes isoladas de mastite bovina processos e análises. Dissertação (Mestrado em Engenharia e Tecnologia de Materiais). Pontifícia Universidade Católica do Rio Grande do Sul, Brasil.

Guinea, J. Global trends in the distribution of *Candida* species causing candidemia. *Clinical Microbiology and Infection*, 20, 5-10. https://doi.org/10.1111/1469-0691.12539.

Han, J., Ye, M., Qiao, X., Xu, M., Wang, B. R., & Guo, D. A. (2008). Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. Journal of *Pharmaceutical and Biomedical Analysis*, 47(3), 516-525.

Iwashina1, T., Smirnov, S. V., Damdinsuren, O., & Kondo, K. (2012). Flavonoids from Reaumuria soongarica (Tamaricaceae) in Mongolia. *Bulletin of the National Museum of Nature and Scienca*, 38(4), 189–195.

Jeng, T. L., Lai, C. C., Liao, T. C., Lin, S. Y., & Sung, J. M. (2015). Effects of drying on caffeoylquinic acid derivative content and antioxidant capacity of sweet potato leaves. *Journal of Food and Drug Analysis*, 23(4), 701-708. https://doi.org/10.1016/j.jfda.2014.07.002.

Kaurinovic, B., & Vastag, G. (2019). Flavonoids and Phenolic Acids as Potential Natural Antioxidants. In: Shalaby, E., & Azzam, G. M. Antioxidants in Foods and Its Applications, IntechOpen, cap. 7, 178.

Kuete, V. Potential of cameroonian plants and derived products against microbial infections: A review. *Planta Medica*, 76(14), 1479-1491. https://10.1055/s-0030-1250027.

Kullberg, B. J., & Arendrup, M. C. (2015). Invasive Candidiasis. *New England Journal of Medicine*, 373(15), 1445-1456. https://10.1056/NEJMra1315399.

Liang, N., Dacko, A., Tan, A. K., Xiang, S., Curtis, J. M., & Gänzle, M. G. (2020). Structure-function relationships of antifungal monohydroxy unsaturated fatty acids (HUFA) of plant and bacterial origin. *Food Research International*, 134, 109237. https://doi.org/10.1016/j.foodres.2020.109237.

LIFE - Leading International Fungal Education. (2017). The burden of fungal disease: New evidence to show the scale of the problem across the globe. https://gaffi.org/the-burden-of-fungal-disease-new-evidence-to-show-the-scale-of-the-problem-across-the-globe/.

Lin, L. Z., & Harnly, J. M. (2007). A screening method for the identification of glycosylated flavonoids and other phenolic compounds using a standard analytical approach for all plant materials. *Journal of Agricultural and Food Chemistry*, 55(4), 1084-1096, 2007. https://doi.org/10.1021/jf062431s.

Mamoci, E., Cavoski, I., Simeone, V., Mondelli, D., Al-Bitar, L., & Caboni, P. (2011). Chemical composition and in vitro activity of plant extracts from *Ferula communis* and *Dittrichia viscosa* against postharvest fungi. *Molecules*. 16(3), 2609-2625. https://doi.org/10.3390/molecules16032609.

Mandeel, Q., & Taha, A. Assessment of in vitro. Antifungal activities of various extracts of indigenous bahraini medicinal plants. *Pharmaceutical Biology*, 43(4), 340-348. https://10.1080/13880200590951766.

Manh, H. T., Na, M., Thuong, P. T., Su, N. D., Sok, D., Song, K. S., Seong, Y. H., & Bae, K. (2006). Antioxidant activity of caffeoyl quinic acid derivatives from the roots of Dipsacus asper Wall. *Journal of Ethnopharmacology*, 108, 188-92. https://10.1016/j.jep.2006.04.029.

Martino, V. S., Debenedettii, S. L., & Coussio, J. D. (1979). Caffeoylquinic Acids from *Pterocaulon virgatum* and *Pluchea sagittalis*. *Phythochemistry*, 18, 2052.

Martino, V. S., Ferraro, G. E., & Coussro, J. D. (1976). A new flavonoid from *Pluchea sagittalis*. *Phythochemistry*, 15, 1086-1087.

Martino, V. S., Ferraro, G. E., Debenedetti, S. J., & Coussio, J. D. (1989). Determinación espectrofotométrica del contenido de ácidos cafeoilquínicos en especies de Compuestas argentinas usadas en medicina popular. *Acta Farmacéutica Bonaerense*, 8(1), 3-9.

Martino, V. S., Ferraro, G. E., Debenedetti, S. L., & Coussio, J. D. (1984). Compuestos Polifenólicos aislados de *Pluchea sagittalis* (Compuestas). *Acta Farmacéutica Bonaerense*, 3(2), 141-146.

Martins, N., Barros, L., Henriques, M., Silva, S., & Ferreira, I. C. F. R. (2015). Activity of phenolic compounds from plant origin against Candida species. *Industrial Crops and Products*, 74, 648-670. https://doi.org/10.1016/j.indcrop.2015.05.067.

Masike, K., Mhlongo, M. I., Mudau, S. P., Nobela, O., Ncube, E. N., Tugizimana, F., George, M. J., & Madala, N. E. (2017). Highlighting mass spectrometric fragmentation diferences and similarities between hydroxycinnamoyl-quinic acids and hydroxycinnamoyl-isocitric acids. *Chemistry Central Journal*, 11(29), 1-7. https://doi.org/10.1186/s13065-017-0262-8.

Matsui, Y., Nakamura, S., Kondou, N., Takasu, Y., Ochiai, R., & Masukawa, Y. Liquid chromatography-electrospray ionization-tandem mass spectrometry for simultaneous analysis of chlorogenic acids and their metabolites in human plasma. *Journal of Chromatography B*, 858(1-2), 96-105. https://doi.org/10.1016/j.jchromb.2007.08.013.

Metlin Database. [Online] Available: https://metlin.scripps.edu/landing_page.php?pgcontent=mainPag. (Oct 18, 2020).

Monks, N. R., Ferraz, A., Bordignon, S., Machado, K. R., Lima, M. F. S., Rocha, A. B., & Schwartsmann, G. (2002). In vitro Cytotoxicity of Extracts from Brazilian Asteraceae. *Pharmaceutical Biology*, 40(7), 494-500. https://10.1076/phbi.40.7.494.14681.

Moreira, M. A. B., Souza, A. D., Jardim, F. B. B., Cunha, L. C. S., Martins, M. M., Goulart, L. R., Morais, S. A. L., Aquino, F. J. T., Cruz, W. O., Costa, L. G., & Borges Neto, W. (2021). Chemical profile and chemometric analysis of genetically modified soybeans produced in the Triângulo Mineiro Region (MG), Brazil. *Journal of Agricultural Studies*, 9(2), 73-95. http://dx.doi.org/10.5296/jas.v9i2.18139.

Naveed, S., Ibrar, M., & Khan, I. (2016). In vitro evaluation of medicinal, antioxidant activities and phytochemical screening of Iphiona grantioides and Pluchea arguta subsp. Glabra Qaiser. *Pakistan Journal of Botany*, 48(6), 2505-2511.

Negri, M., Salci, T. P., Shinobu-Mesquita, C. S., Capoci, I. R. G., Svidzinski, T. I. E., & Kioshima, E. S. (2014). Early State Research on Antifungal Natural Products. *Molecules*, 19(3), 2925-2956. https://doi.org/10.3390/molecules19032925.

Nessma, A. E. Z., & Elsayed, E. H. (2017). Efficacy of *Pluchea dioscoridis* leaf extract against pathogenic *Candida albicans*. *The Journal of Infection in Developing Countries*, 11(4), 334-342. https://10.3855/jidc.8447.

Newman, D. J., & Cragg, G. M. (2016). Natural Products as Sources of New Drugs from 1981 to 2014. *Journal of Natural Products*, 79(3), 629-61. https://10.1021/acs.jnatprod.5b01055.

Newman, D. J., & Cragg, G. M. (2020). Natural Products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770-803. https://10.1021/acs.jnatprod.9b01285.

Nguyen, D. T., Göpfert, J. C., Ikezawa, N., Macnevin, G., Kathiresan, M., Conrad, J., Spring, O., & Ro, D. K. (2010). Biochemical conservation and evolution of germacrene A oxidase in Asteraceae. *Journal of Biological Chemistry*, 285(22), 16588-16598. 10.1074/jbc.M110.111757.

Nicola, A. M., Albuquerque, P., Paes, H. C., Fernandes, L., Costa, F. F., Kioshima, E. S., Abadio, A. K. R., Bocca, A. L., & Felipe, M. S. (2019). Antifungal drugs: New insights in research & development. *Pharmacology & Therapeutics*, 195, 21-38. https://doi.org/10.1016/j.pharmthera.2018.10.008.

Oh, W. Y., Ambigaipalan, P., & Shahidi, F. (2019). Preparation of quercetin esters and their antioxidant activity. *Journal of Agricultural Food Chemistry*, 67(38), 10653-10659. https://doi.org/10.1021/acs.jafc.9b04154.

Olanda, G. B., Bevilaqua, G. A. P., Schuch, L. F. D., Prestes, L. S., & Job, R. B. (2019). Estabilidade da atividade antibacteriana do extrato de *Pluchea sagittalis* (LAM.) Cabrera frente a microrganismos causadores da mastite bovina. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR*, 22(1), 21-25. https://doi.org/10.25110/arqvet.v22i1.2019.6483.

Oliveira, D. M., Silva, T. F. R., Martins, M. M., Morais, S.A.L., Chang, R., Aquino, F. J. T., Silva, C. V., Teixeira, T. L., Martins, C. H. G., Moraes, T. S., Cunha, L. C. S., Pivatto, M., & Oliveira, A. (2018). Antifungal and cytotoxicity activities of *Banisteriopsis gyrophylla* leaves. *Journal of Pharmacy and Pharmacology*, 70(11), 1541-1552. https://doi.org/10.1111/jphp.12996.

Oliveira, S. C. D., & Rodrigues, A. G. (2020). *Candida albicans* antifungal resistance and tolerance in bloodstream infections: The triad yeast-host-antifungal. *Microorganisms*, 8(2), 1-19. https://doi.org/10.3390/microorganisms8020154.

Ouriques, F. G. D. O. M., Caruso, P. B., Silva, G. V. D., Dias, H., Marques, J. R., Nunes, F. B., Ferreira, P. M. A., & Oliveira, J. R. D. (2018). Antifibrotic effect of *Pluchea sagittalis* (Lam.) cabrera aqueous extract in grx cell lineage. *International Journal of Phytomedicine*, 10(1), 30-38. https://10.5138/09750185.1832.

Ozgen, S., Kilinc, O. K., & Selamoglu, Z. (2016). Antioxidant Activity of quercetin: A mechanistic review. *Turkish Journal of Agriculture - Food Science and Technology*, 4(12), 1134-1138. https://doi.org/10.24925/turjaf.v4i12.1134-1138.1069.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Saavedra, G., Murcia, M. A., Jiménez, A. M., & Codina, C. (2003). Investigation of Bolivian plant extracts for their radical scavenging activity and antioxidant activity. *Life Sciences*, 73(13), 1667-1681. https://doi.org/10.1016/S0024-3205(03)00488-0.

Pérez-García, F., Marín, E., Adzet, T., & Cañigueral, S. (2001). Activity of plant extracts on the respiratory burst and the stress protein synthesis. *Phytomedicine*, 8(1), 31-38. https://doi.org/10.1078/0944-7113-00018.

Pérez-García, F., Marín, E., Cañigueral, S., & Adzet, T. (1996). Anti-inflammatory action of *Pluchea sagittalis*: Involvement of an antioxidant mechanism. *Life Sciences*, 59(24), 2033-2040. https://doi.org/10.1016/S0024-3205(96)00556-5.

Pérez-García, F., Marín, E., Parella, T., Adzet, T., & Cañigueral, S. (2005). Activity of taraxasteryl acetate on inflammation and heat shock protein synthesis. *Phytomedicine*, 12(4), 278-284. https://doi.org/10.1016/j.phymed.2004.03.008.

Pérez, F., Marín, E., & Adzet, T. (1995). The antiinflammatory effect of several composite from South America. Extracts in rats. *Phytotherapy Research*, 9, 145-146. https://10.1002/ptr.2650090214.

Pereira, O. R., Peres, A. M., Silva, A. M. S., Domingues, M. R. M., & Cardoso, S. M. (2013). Simultaneous characterization and quantification of phenolic compounds in *Thymusx citriodorususing* a validated HPLC–UV and ESI–MS combined method. *Food Research International*, 54, 1773-1780. https://doi.org/10.1016/j.foodres.2013.09.016.

Reynertson, K. A., Basile, M. J., & Kennelly, E. J. (2005). Antioxidant potential of seven Myrtaceous fruits. *Ethnobotany Research and Applications*. 3, 25–36. https://ethnobotanyjournal.org/index.php/era/article/view/49.

Rhimi, W., Ben Salem, I., Iatta, R., Chaabane, H., Saidi, M., Boulila, A., & Cafarchia, C. (2018). *Dittrichia viscosa* L. leaves lipid extract: An unexploited source of essential fatty acids and tocopherols with antifungal and anti-inflammatory properties. *Industrial Crops and Products*, 113, 196-201. https://doi.org/10.1016/j.indcrop.2018.01.032.

Rodrigues, M. L., & Nosanchuk, J. D. (2020). Fungal diseases as neglected pathogens: A wake-up call to public health officials. *PLOS Neglected Tropical Diseases*, 14(2), 1-9. https://10.1371/journal.pntd.0007964.

Rodrigues, S. A. Efeito ansiolítico do extrato etanólico de Pluchea sagittalis (Lam.) Cabrera, Asteraceae, em modelos comportamentais. 2011. 105 f. Tese - Pós-Graduação em Biotecnologia, Univevrsidade Federal de Sergipe São Cristovão, Sergipe.

Ruan, J., Li, Z., Yan, J., Huang, P., Yu, H., Han, L., Zhang, Y., & Wang, T. (2018). Bioactive Constituents from the Aerial Parts of *Pluchea indica* Less. *Molecules*, 23(9), 2104. https://doi.org/10.3390/molecules23092104.

Ruan, J., Yan, J., Zheng, D., Sun, F., Wang, J., Han, L., Zhang, Y., Wang, T. (2019). Comprehensive chemical profiling in the ethanol extract of *Pluchea indica* aerial parts by liquid chromatography/mass spectrometry analysis of its silica gel column chromatography fractions. *Molecules*, 24(15), 2784. https://10.3390/molecules24152784.

Salih, E. Y. A., Fyhrquist, P., Abdalla, A. M. A., Abdelgadir, A. Y., Kanninen, M., Sipi, M., Luukkanen, O., Fahmi, M. K. M., Elamin, M. H., & Ali, H. A. (2017). LC-MS/MS tandem mass spectrometry for analysis of phenolic compounds and pentacyclic triterpenes in antifungal extracts of *Terminalia brownii* (Fresen). *Antibiotics*, 6(4), 37. https://doi.org/10.3390/antibiotics6040037.

Sanguinetti, M., Posteraro, B., Beigelman-Aubry, C., Lamoth, F., Dunet, V., Slavin, M., & Richardson, M. D. (2019). Diagnosis and treatment of invasive fungal infections: looking ahead. *Journal of Antimicrobial Chemotherapy*, 74(2), 27-37. https://10.1093/jac/dkz041.

Schmidt, C., Fronza, M., Goettert, M., Geller, F., Luik, S., Flores, E. M. M., Bittencourt, C. F., Zanetti, G. D., Heinzmann, B. M., Laufer, S., & Merfort, I. (2009). Biological studies on Brazilian plants used in wound healing. *Journal of Ethnopharmacology*, 122(3), 523-532. https://doi.org/10.1016/j.jep.2009.01.022.

Scholz, E., Heinrich, M., & Hunkler, D. (1994). Caffeoylquinic acids and some biological activities of *Pluchea symphytifolia*. *Planta Medica*, 60(4), 360-364. https://10.1055/s-2006-959501.

Sepahpour, S., Selamat, J., Manap, M. Y. A., Khatib, A., & Razis, A. F. A. (2018). Comparative analysis of chemical composition, antioxidant activity and quantitative characterization of some phenolic compounds in selected herbs and spices in different solvent extraction systems. *Molecules*, 23(2), 402. https://10.3390/molecules23020402.

Simões, C. M. O., Falkenberg, M., Mentz, L. A., Schenkel, E. P., Amoros, M., & Girre, L. (1999). Antiviral activity of South Brazilian medicinal plant extracts. *Phytomedicine*, 6(3), 205-214. https://doi.org/10.1016/S0944-7113(99)80010-5.

Sosa, A., Costa, M., Salvatore, A., Bardon, A., Borkosky, S., & Vera, N. (2017). Insecticidal effects of eudesmanes from *Pluchea sagittalis* (Asteraceae) on *Spodoptera frugiperda* and *Ceratitis capitata*. *International Journal of Environment, Agriculture and Biotechnology*, 2(1), 361-369. http://dx.doi.org/10.22161/ijeab/2.1.45.

Sousa, C. M. M., Silva, H. R., Vieira Jr, G. M., Ayres, M. C. C., Da Costa C. L. S., Araújo, D. S., Cavalcante, L. C. D., Barros, E. D. S., Araújo, P. B. M., Brandão, M. S., & Chaves, M. H. (2007). Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química Nova*, 30(2), 351-355. https://doi.org/10.1590/S0100-40422007000200021.

Stüker, C. Z. (2012). Prospecção fitoquímica s atividade biológica de plantas medicinais da família Asteraceae no Bioma Pampa. 2012. 224 f. Tese - Programa de Pós-Graduação em Química, Universidade Federal de Santa Maria, Santa Maria, 2012.

Vandeputte, P., Ferrari, S., & Coste, A. T. (2012). Antifungal resistance and new strategies to control fungal infections. *International Journal of Microbiology*, 2012, 1-26. https://10.1155/2012/713687.

Vera, N., Misico, R., Sierra, M. G., Asakawa, Y., & Bardón, A. (2008). Eudesmanes from *Pluchea sagittalis*. Their antifeedant activity on *Spodoptera frugiperda*. *Phytochemistry*, 69(8), 1689-1694. https://doi.org/10.1016/j.phytochem.2008.02.020.

Verma, A. K., & Pratap, R. (2010). The biological potential of flavones. *Natural Product Report*, 27(11), 1571-1593.

Visintini Jaime, M. F., Redko, F., Muschietti, L. V., Campos, R. H., Martino, V. S., & Cavallaro, L. V. (2013). In vitro antiviral activity of plant extracts from Asteraceae medicinal plants. *Virology journal*, 10, 245-245. https://10.1186/1743-422X-10-245.

Zida, A., Bamba, S., Yacouba, A., Ouedraogo-Traore, R., & Guiguemdé, R. T. (2016). Anti-*Candida albicans* natural products, sources of new antifungal drugs: A review. *Journal de Mycologie Médicale*, 27(1), 1-19. https://doi.org/10.1016/j.mycmed.2016.10.002.

Wang, X., Ding, G., Liu, B., & Wang, Q. (2020). Flavonoids and antioxidant activity of rare and endangered fern: *Isoetes sinensis*. *Plos One*, 15(5), e0232185. https://doi.org/10.1371/journal.pone.0232185.

Wang, Y., Yang, L., He, Y. Q., Wang, C. H., Welbeck, E. W., Bligh, S. W. A., & Wang, Z. T. (2008). Characterization of fifty-one flavonoids in a Chinese herbal prescription Longdan Xiegan Decoction by high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection. *Rapid Communication Mass Spectrometric*, 22, 1767–1778. https://doi.org/10.1002/rcm.3536.

Waksmundzka-Hajnos, M., Sherma, J., & Kowalska, T. (2008). Thin layer chromatography in phytochemistry. Boca Raton/London/New York: CRC Press.

Woisky, R.G., & Salatino, A. (1998). Analysis of propolis: some parameters andprocedures for chemical quality control. *Journal of Apicultural Research*, 37(2), 99–105. https://doi.org/10.1080/00218839.1998.11100961.