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Chemical investigation, toxic potential and acetylcholinesterase inhibitory effect of *Parkia platycephala* leaf and seed extracts

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The present work evaluated the inhibitor potential of acetylcholinesterase, the preliminary toxicity and determination of active components of different leaf and seed extracts of *Parkia platycephala*. All extracts were obtained through hot extraction in a closed system (Soxhlet). To obtain the leaf and seed hexanic, methanolic, and ethanolic extracts (LHE, LME, LEE, and SHE, SME, and SEE), sequential extraction was performed on the same plant sample using hexane, methanol, and hydroethanol solution (70%). Preliminary Phytochemical analysis and the characterization by gas chromatography coupled to the mass spectrometer (GC-MS) was performed. The content of phenols total flavonoids and the antioxidant potential was then quantified.The preliminary toxicity against *Artemia salina* was also evaluated and the potential for acetylcholinesterase inhibition was determined. The presence of tannins, flavonoids, saponins, phytosterols/triterpenoids and alkaloids were detected in phytochemical analysis. The leaf extracts showed antioxidant potential, LEM (IC_{50} = 30.19 ± 0.75 µg/ml) and LEE (IC_{50} = 40.62 ± 0.65 µg/ml). The analysis by GC-MS indicated a diversity of volatile compounds, evidencing urs-12-ene (triterpenoid) and 1,2,3-benzenetriol (phenol) in the leaf extracts, and linoelaidic acid (fatty acid), (Z)-9-octadecenamide, tricycle [20.8.0.0 (7.16)] triacontane,1(22),7(16)-diepoxy-, (Z)-7-hexadecenal (fatty aldehyde) in the seed extracts. The preliminary toxicity analysis demonstrated that the use of *P. platyceplaha* leave and seeds for medicinal purposes is relatively safe. All the extracts inhibited acetylcholinesterase, compared to the physostigmine control, with IC_{50} values in the range of 9.85 to 15.68 mg/ml. Thus, these data support the use of *P. platycephala* as a potential therapy for Alzheimer's disease.

Key words: Phytochemistry, antioxidant activity, toxicity, fava-de-bolota, Alzheimer.

INTRODUCTION

The origin of Alzheimer's disease (AD), despite various indications, is still fueled by the cholinergic hypothesis, which relates the symptoms of dementia to the decrease in the level of acetylcholine, a neurotransmitter and

neuromodulator (Kuppusamy et al., 2017; Sharma, 2019). The availability of acetylcholine is regulated by the enzyme acetylcholinesterase (AChE), that is, the inhibition of this enzyme increases the concentration of acetylcholine in synapses, improving communication between cells (Decker and Duncan, 2020; Mota et al., 2012).

Among the main therapeutic approaches for AD, a compound isolated from the species Galanthus spp. known as galantamine, has the ability to inhibit acetylcholine production (Heinrich, 2010). The interest in plant species (herbs) with medicinal action against Alzheimer's (Soheili et al., 2021) stems from the numerous side effects associated with the synthetic drugs currently available for AChE inhibition (iAChE), including peripheral toxicity, hepatotoxicity, and gastrointestinal tract disorders (Shaikh et al., 2021).

Despite the advancement of scientific research into new actives against AD, data confirming the iAChE effect of Brazilian plants is still insignificant (less than 1%) when compared to national biodiversity (Santos et al., 2018). Aside from the low national rate, regional studies of the Cerrado Biome discovered that only qualitative iAChE tests were conducted, highlighting the need for a more detailed investigation of the species in the region.

Parkia platycephala is popularly known in Brazil as "fava-de-bolota", "faveira", "faveira-preta", "visgueiro", "fava-de-boi" or "sabi" (Chaves et al., 2020). It has an extensive regional representation as the state of Tocantins' symbol tree and also peculiar characteristics resulting from its location on the border between the Cerrado and the Brazilian Caatinga (Lorenzi, 2020; Tocantins, 2012). Despite the fact that its extracts and isolated actives, primarily from pods, are well-known in the fields of nutrition and pharmacology (gastrointestinal infections in animals), little is known about their therapeutic potential (Araújo et al., 2019; Cavada et al., 2020; Santos et al., 2018). There are reports of the presence of phenolic compounds, flavonoids, flavones, phytosteroids, condensed tannins, and saponins in this species. Such compounds are of great interest in the health field (Oliveira et al., 2017). Among its therapeutic uses, the antinociceptive and anti-inflammatory effects were observed in mice, after intravenous application with purified lectin, obtained from the seed of *P. platycephala* (Bari et al., 2016). In addition, gastroprotective and anthelmintic effects have been reported in studies with lectin against multiresistant strains of *Staphylococcus aureus* and *Escherichia coli* following its association with gentamicin (Silva et al., 2019). The ethanolic extract and the hexane, acetatic and methanolic fractions of the leaf of *P. platycephala* inhibited trichostrongylid egg hatching (Figueiredo et al., 2020). Although scarce, there are studies with other therapeutic indications for extracts of the specie *P. platycephala*, such as the preliminary analysis of the iAChE, in which the seed ethanol extracts from plant species of the Brazilian semiarid region were evaluated, indicating anti-cholinesterase activity (71.5%) of this species (Farias et al, 2013). With a view to identifying new iAChEs of natural origin, this research aimed to identify the phytochemical composition, and evaluate the preliminary toxicity of the *P. platycephala* leaf and seed extracts, as well as the potential acetylcholinesterase inhibitory activity of these extracts.

MATERIALS AND METHODS

Plant material

The samples of the leaves and seeds of *P. platycephala* were collected within the Federal University of Tocantins – Campus of Palmas, between the coordinates 10°10'55"S and 48°21'45" W. Anfterwards, were registered and incorporated into Tocantins Herbarium (HTO) collection, located at the Federal University of Tocantins (UFT) in Porto Nacional, under the number HTO 12007. The project is registered with SIGEN under the number A06B860.

Preparation of extracts

The plant materials (leaf and seed) were dried in an oven at 60°C for 48 h, powdered with the aid of the Willye knife mill (Fortinox STAR FT 50) with granulometry below Mesh 20 and stored in a contamination-free glass container, preserving it from moisture. Sequential hot extractions were performed in a closed system, using a Soxhlet apparatus. The extraction with hexane was performed to degrease the sample, for a period of 5 h of reflux, obtaining the leaf hexanic extract (LHE) and the seed hexanic extract (SHE). After completing the extraction time, before starting the extraction with the subsequent solvent, the plant sample was dried at room temperature for 12 h. Then, the extraction process was repeated for each solvent (methanol and 70% ethanol), obtaining the leaf and seed methanol extracts (LME, SME) and the leaf and seed ethanol extracts (LEE, SEE). The solvent from all extracts was removed using a rotary evaporator (FISATOM brand) at -600 mmHg at 45°C. Then the extracts were frozen at -70°C, lyophilized in LIOTOP bench freeze dryer L101 and stored in airtight flasks for further analysis.

Phytochemical prospecting

The qualitative characterization of the main classes of secondary metabolites was performed through analysis based on precipitation and/or staining reactions, with specific reagents for flavonoids, tannins, phytosterols, terpenoids, quinones, saponins and alkaloids (Matos, 2009; Saraiva et al., 2018; Simões et al., 2017).

Characterization by gas chromatography mass spectrometry (GC-MS)

The extracts were analyzed by GC-MS using a Shimadzu®

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chromatograph model QP2020 Ultra equipped with a ZB-5HT column (30 m long ×0.25 mm internal diameter x 0.25 µm film thickness). It was performed under the following conditions: heating at 50°C for 1 min, until reaching 320°C in 35 min. Injection temperature: 320°C; Interface temperature: 320°C; carrier gas (Helium): 1 ml/min; The electron energy was 70 eV and the temperature of the ion source was 320°C; scan mode. 1 µl of each extract was injected, in which the constituents were identified by comparison with the mass spectra of the NIST 14 library.

Determination of the content of total phenolic compounds

The quantification of the total phenolic content was performed using the Folin–Ciocalteu method (Amorim et al., 2012), with modifications, using gallic acid as standard. Samples (0.2 ml) of methanol solutions (1 mg/ml, p/v) from extracts (LHE, LME, LEE, SHE, SME, SEE) or from the standard (4 to 100 µg/ml, p/v) were mixed with an aqueous solution of the Folin-Ciocalteu reagent (0.5 mL to 10%, v/v), sodium carbonate (1 ml to 7.5%, p/v) and 8.3 ml of distilled water, carefully stirred and kept for 30 min protected from light. The absorbance was measured at 760 nm using a UV-VIS HACH DR 5000 spectrophotometer. The total phenol content was determined by interpolating the absorbances of the samples against a calibration curve constructed with the different concentrations of the gallic acid standard (y = 0.006x + 0.0449, adjusted R^2 = 0.997), expressed as milligrams equivalent of gallic acid per gram of lyophilized extract (mg EAG/g). All experiments were performed in triplicate.

Determination of total flavonoid content

The quantification of total flavonoids was performed according to Peixoto-Sobrinho (2008), with modifications (Soares et al., 2017). By mixing 0.5 ml of methanol solution (1mg/ ml, p/v) of the extracts (LHE, LME, LEE, SHE, SME, SEE) or the standard (10 to 400 µg/ml, p/v) with an aqueous solution of acetic acid (0.5 ml to 60%, v/v), methanolic solution of pyridine (2 ml to 20%, v/v), aluminum chloride (1 ml to 5%, p/v) and distilled water (6 ml). The blank was constructed by joining all the components of the reaction and the extract or standard, replacing the aluminum chloride with water. The reaction complex and the blank were carefully shaken and kept for 30 minutes protected from light, and the absorbances were measured at 420 nm in a spectrophotometer. Total flavonoid contents were determined by interpolating the absorbances of the samples (discounting the blank absorbance) against a calibration curve constructed with the different concentrations of the standard rutin (y = 0.0022x – 0.0124, adjusted R^2 = 0.999), expressed in equivalent milligrams of rutin per gram of lyophilized extract (mg ER/g). All experiments were performed in triplicate.

Determination of antioxidant activity

The antioxidant capacity was measured by the elimination of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Peixoto-Sobrinho et al. (2011), using the rutin pattern as a positive control. In triplicate, 0.5 ml of different concentrations of extracts or standards (10 – 200 µg/ml, p/v) were added to a methanolic solution of DPPH (3 ml to 40 µg/ml, p/v). The blank was constructed by replacing DPPH with methanol in the reaction medium. The reaction complex and the blank were shaken and kept for 30 min protected from light, and the absorbances were measured at 517 nm in a spectrophotometer calibrated with methanol. The absorbance of the DPPH solution at 40 µg/ml was also measured and used as a negative control. The free radical scavenging activity or antioxidant activity (AA) was expressed as the percentage of inhibition determined by the equation:

$$AA(\%) = \frac{(Ac - (Aa - Ab))}{Ac} \times 100$$

where AA (%) is the percentage of antioxidant activity; Ac, the absorbance of the negative control; Aa, the absorbance of the sample; Ab, the absorbance of the blank. The IC_{50} (µg/ml) was obtained using the calibration curves obtained by plotting the different concentrations in relation to AA%.

Determination of preliminary toxic activity

The analysis of the preliminary toxicity was performed using the *Artemia salina* method according to the methodology of Lacerda et al. (2011) with adaptations. Initially, 1 L of 3% saline solution was prepared, with synthetic sea salt, pH adjusted between 8 to 9 (with 1 M sodium carbonate), for incubation of *A. salina* eggs (0.1 g), which were exposed to artificial light (60W incandescent light bulb) for 24 h to hatch.

To perform the bioassay, ten nauplii were placed in triplicate in test tubes containing, separately, 5 mL of solution of the extracts (LHE, LME, LEE, SHE, SME, SEE) diluted in saline solution of DMSO (1%) in concentrations of 4, 200, 1000 and 4000 µg/ml. A control group was prepared containing only DMSO solution (1%) diluted in saline solution (3%). After a period of 24 h, the number of static nauplii was counted and the percentage of mortality was determined. The mortality percentages of each concentration were used for the linear regression calculations, from which it was possible to determine the IC_{50} of each extract. The classification of the extract followed the criteria established by NGUTA et al. (2011). Both organic extracts and aqueous extracts with IC_{50} values < 100 µg/ml have high toxicity, 100 ≤ IC_{50} ≤ 500 µg/ml have moderate toxicity and IC_{50} > 1000 µg/ml are considered non-toxic.

Determination of anticholinesterase activity

The inhibiton of the acetylcholinesterase enzyme (AChE) was measured in 96-well flat-bottom plates using an Elisa BIOTEK reader, model ELX 800, software “Gen5 V2.04.11”, based on the methodology described by Ellman et al. (1961). The following solutions were used per well: 25 µl of acetylthiocholine iodide (15 mM), 125 µl of 5.5'-dithiobis-[2-nitrobenzoic] in the Tris/HCl solution (50 nM, pH = 8, with 0.1 M of NaCl and 0.02 M of $MgCl_2.6H_2O$. (3 mM, DTNB or Ellman's reagent)), 50 µl of the Tris/HCl solution (50 nM, pH = 8, with 0.1% bovine serum albumin (BSA)), 25 µl of the extract sample dissolved in DMSO (1%) and diluted 10 times in Tris/HCl solution (50 mM, pH = 8) to obtain a final concentration of 0.2 mg/mL (Rhee et al. 2001; Trevisan and Macedo, 2003).

The absorbance was measured at 405 nm for 30 seconds. Then, 25 µl of the enzyme acetylcholinesterase (0.25 U/ml) was added and the absorbance was measured once per minute for a total of 25 min of incubation of the enzyme. As a negative standard, all solutions were used except for the sample. The dilutions of the samples and the positive standards used in the quantitative evaluations in microplate, starting from a stock solution with a concentration of 2 mg/ml were: 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/ml. All samples were analyzed in triplicate. The values referring to the natural colorings of the extracts were extinguished from the analysis. The percentage of inhibition of acetylcholinesterase was calculated by comparing the reaction rates (substrate hydrolysis) of the samples in relation to the blank (considered total AChE activity, 100%). The standard used as a positive control was physostigmine (eserine).

Statistical analysis

The content of total phenols, flavonoids, antioxidant activity and

Table 1. Preliminary phytochemical screening of *P. platycephala* leaf and seed extracts.

CLASS	LHE	LME	LEE	SHE	SME	SEE
Flavonoids	+	+++	+++	-	+++	+++
Tannins	-	+++	+++	-	+++	+++
Phytosterols/Triterpenes	++	+	+	++	+	+
Quinones	-	-	-	-	-	-
Saponins	-	+	+	-	+	-
Alkaloids	+	+	-	+	+	-

(+) weak presence; (++) average presence; (+++) strong presence; (-) absence (LHE - leaf hexane extract; LME – leaf methanol extract; LEE - leaf ethanol extract; SHE - seed hexane extract; SME - seed methanol extract; SEE - seed ethanol extract).

anticholinesterase activity are presented in mean ± standard deviation (SD) of three determinations. The data were analyzed using the SISVAR version 5.6 program. The IC_{50} was determined using the GRAPHPAD PRISM program (v.8.0). Analyses of variance (ANOVA) was used to compare the mean values obtained in the analysis and the $p < 0.05$ values were considered statistically significant by the Tukey test.

RESULTS AND DISCUSSION

Phytochemical screening

The results obtained for the phytochemical screening of extracts of *P. platycephala* are shown in Table 1. The qualitative phytochemical screening of leaf and seed extracts of *P. platycephala* revealed the presence of flavonoids, tannins, phytosterols/triterpenoids, saponins and alkaloids. There are records of the presence of flavonoids, alkaloids, phenols, tannins, saponins, glycosides, terpenoids, anthraquinones and sterols in the leaf, bark, seed and pod of the genus *Parkia* (*P.*) (Chanu et al., 2018; Chhikara et al., 2018; Sá-Santos et al., 2018).

It is noticed that extraction with nonpolar solvent (LHE, SHE) was proved to be more efficient in the phytochemical screening of the phytosterol/triterpene metabolites, due to the fact that these compounds are fat soluble (Cheok et al., 2014). The flavonoid and tannin metabolites had a more pronounced presence in the extracts obtained after degreasing (LME, LEE, SME, SEE), that is, with polar solvents. Thus, sequential extraction optimizes the obtainment of secondary metabolites. Extraction with nonpolar solvent eliminates the competition between some metabolites, which have no selectivity when only polar extraction occurs (Soares et al., 2017).

Analysis by GC-MS

Figures 1 and 2 show the chromatograms obtained by GC-MS from the extracts of the leaves (LHE, LME, LEE, Figure 1) and seeds (SHE, SME, SEE, Figure 2) of *P. platycephala*. Figures 1 and 2 show the results obtained by GC-MS. It is verified in each extract a variability of compounds, influenced by the polarity of the solvents. Table 2 lists the dominant compounds of LHE, LME, LEE, SHE, SME and SEE.

The compounds (methylsulfinyl)(methylthio)-methane, n-hexadecanoic acid (palmitic acid), (Z)-9-octadecenamide (oleamide) and stigmasterol were detected both in the leaf and seed of *P. platycephala*. It is worth noting that of these, 9-octadecenamide-(Z) is present only in ethanol extracts, mostly in the SEE (24%). The 1,2,3-benzenetriol compound (pyrogallol) was the majority in the LME and LEE, showing greater affinity for extraction with polar solvents (above 40%). Other compounds exclusive to polar leaf extraction are 4-O-methylmannose and beta-sitosterol.

In the hexane extracts, there were some compounds with highly non-polar characteristics, urs-12-ene (23.93%), 4, 4, 6a, 6b, 8a, 11, 11,14b-octamethyl-1,4,4a, 5, 6.6a, 6b (12.04%), lup-20(29) -en-3-one (20.15%) and lupeol (17.13%) in LHE and 2-cyclopenten-1-one, 3-methyl- (9.60%) and linoelaidic acid (23.51%) in SHE. It should be noted that the compounds 3-methyl-2-cyclopenten-1-one, linoelaidic acid, tricyclo[20.8.0.0(7.16)]triacontane,1(22),7(16)-diepoxy and (Z)-7-hexadecenal were detected only in the extracts of the seed of *P. platycephala*. There are reports of the presence of the compounds 1,2,3-benzenetriol and 4-O-methyl-mannose in extracts of the bark and root of *P. biglobosa* sequentially extracted with hexane, ethyl acetate, ethanol and water (Ibrahim et al., 2013). Gnansounou et al. (2019) detected stigmasterol and sitosterol derivatives in *P. biglobosa* leaf and bark, while Shahidah et al. (2019) confirmed (Z)-9-octadecenamide-in seeds. Lupeol was detected in various parts of the species *P. biglobosa*, *P. bicolor* and *P. speciosa* (Saleh et al., 2021). Palmitic acid (hexadecanoic acid), which is also found in the genus *Parkia*, was identified in this study (Olowokere et al., 2018; Sangodare et al., 2017).

As well as urs-12-ene detected in LHE, there are reports of the presence of ursolic acid in the leaves of the

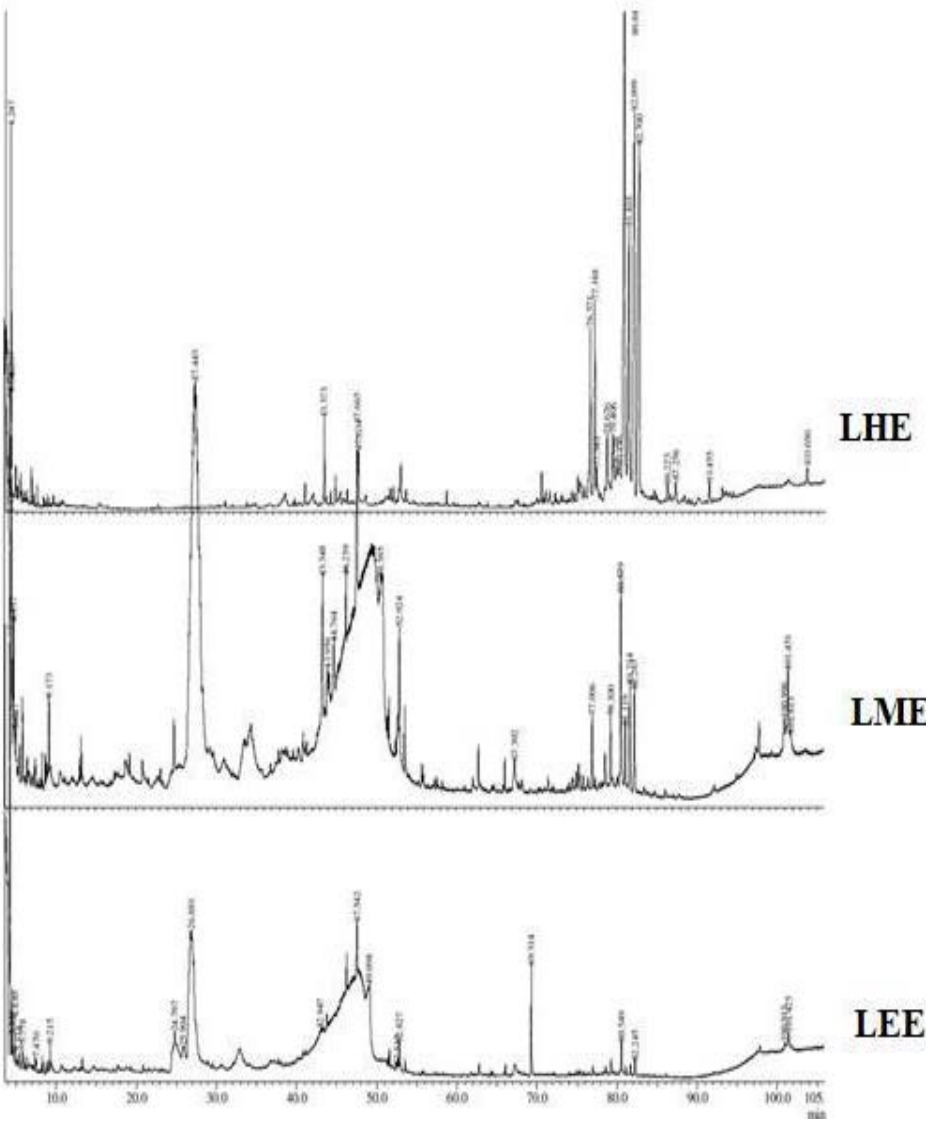

Figure 1. Comparison between chromatograms obtained by GC-MS analysis for *Parkia platycephala* leaf extracts obtained by different extraction methods. (LHE - leaf hexanic extract; LME - leaf methanolic extract; LEE - leaf ethanolic extract).

species *P. javanica* (Dinda et al., 2009), both derived from ursane. Derivatives of pentacyclic triterpenoid compounds are highly attractive due to the promising expectations given the variability of biological properties of this class (Luchnikova et al., 2020). The main compounds in Table 2 are biologically active. According to Beulah et al. (2018) pyrogallol has an antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide, carcinogenic and cytotoxic effect. Palmitic acid, in addition to antioxidant and hypocholesterolmeic activity, has nematicide, pesticide, lubricant, antiandrogenic, hemolytic and 5-alfareductase inhibitor effects (Beulah et al., 2018). Not least, (Z)-7-hexadecenal has antiviral activity (Devakumar et al., 2017), and (Z)-9-octadecenamide has high anti-inflammatory power (Ano et al., 2015).

Pentacyclic triterpenoids (urs-12-ene, lup-20(29)-en-3-one, lupeol) have anti-cancer activity (SHAN et al., 2016), chemopreventive (Prasad et al., 2007), anti-inflammatory (Feng et al., 2018), anti-HIV (Smith et al., 2007) and antimicrobials (Duric et al., 2013). The compound tricyclo[20.8.0.0(7.16)]triacontane,1(22),7(16)-diepoxy, despite being mentioned in some studies of chemical characterization of plant extracts (Mohiuddin et al., 2018), is not related to specific studies of bioactive compounds. The compounds methane(methylsulfinyl)(methylthio), 3-methyl-2-cyclopenten-1-one, 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b, lup-20(29)-en-3-one,

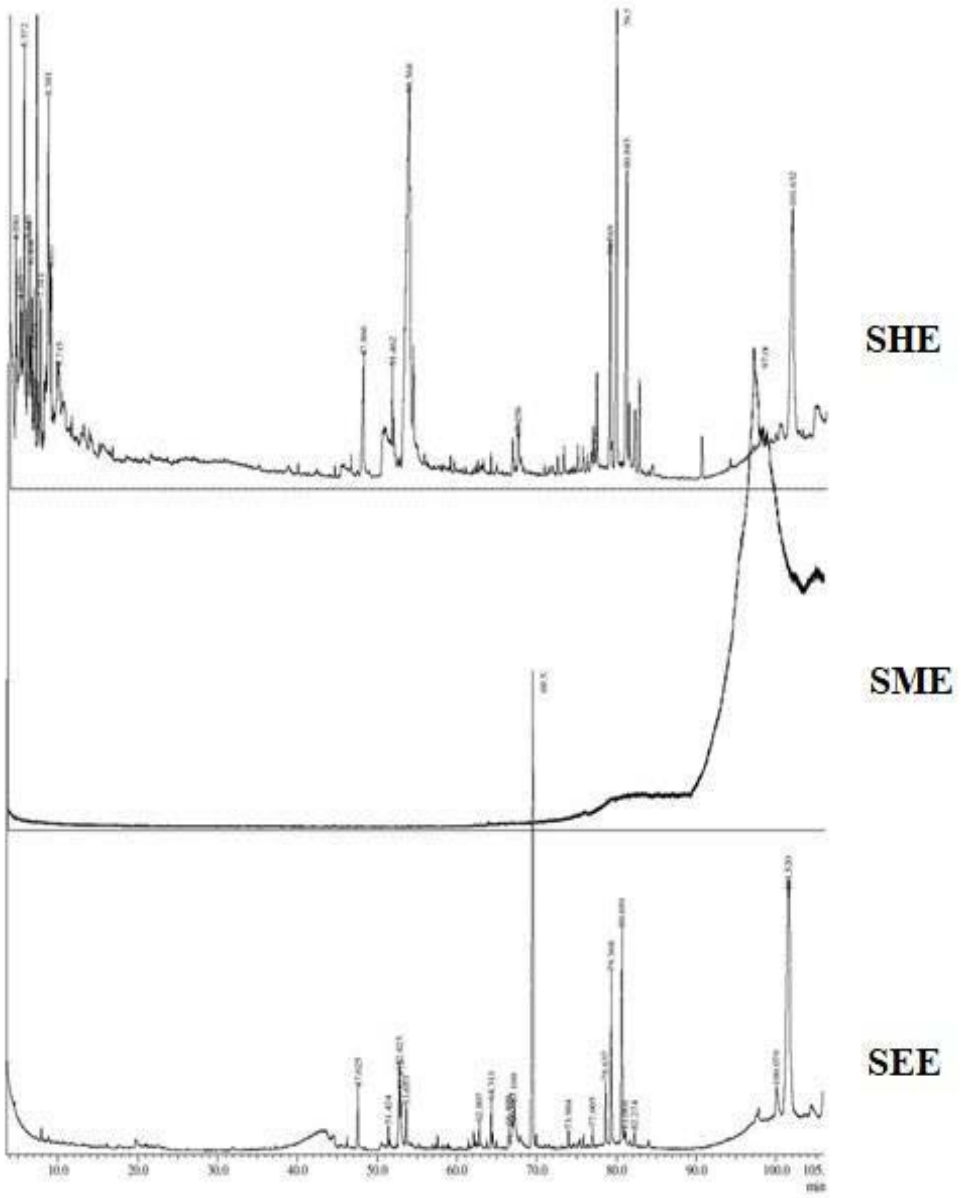

Figure 2. Comparison between chromatograms obtained by GC-MS analysis for *Parkia platycephala* seed extracts obtained by different extraction methods. (SHE - seed hexanic extract; SME - seed methanolic extract; SEE- seed ethanolic extract).

tricyclo[20.8.0.0 (7,16)]triacontane,1(22),7(16)-diepoxy- and (Z)-7-hexadecenal appear for the first time in the profile of volatile compounds of a species of the genus *Parkia*.

Content of phenolic, flavonoids and evaluation of antioxidant activity

The analysis of the contents of total phenols, total flavonoids and the antioxidant activity of *P. platycephala* leaf and seed extracts are shown in Table 3. In the leaf extracts, it was detected contents of total phenols superior to the seed extracts. Regarding phenols, it was also noticed that there was no significant difference between LME (85.11 ± 0.25 mg GAE/g) and LEE (84.17 ± 0.33 mg GAE/g) and that these values 84.57 ± 1.06 mg GAE/g are approximate to those reported in the literature for the ethanol extract of *P. biglobosa* leaf, obtained after sequential extraction (hexane, ethyl acetate, and ethanol) (Ibrahim et al., 2013). However, for the flavonoid content, this equivalence is not maintained, with the LEE (25.64 ± 0.91 mg RE/g) being 100% higher than the LME (11.85 ± 0.69 mg RE/g).

Table 2. Main chemical constituents identified by GC-MS in *Parkia platycephala* extracts.

RT (min)	Compound	Peak área (%)					
		LHE	LME	LEE	SHE	SME	SEE
4.168 - 4.616	(methylsulfinyl)(methylthio)-methane	4.31	-	14.07	2.80	-	-
8.381	3-methyl-2-cyclopenten-1-one	-	-	-	9.60	-	-
26.881- 27.443	1,2,3-benzenetriol	-	55.62	41.16	-	-	-
47.513 - 47.665	n-hexadecanoic acid	0.72	3.48	7.30	2.53	-	2.39
49.098 - 50.585	4-O-methylmannose	-	4.86	5.47	-	-	-
53.568	linoelaidic acid	-	-	-	23.51	-	-
69.314 - 69.531	(Z)-9-octadecenamide	-	-	4.10	-	-	24.52
78.913 - 79.592	stigmasterol	2.58	0.98	-	9.20	-	5.42
80.549 - 80.579	beta-sitosterol	-	3.67	1.81	-	-	-
80.691 - 80.843	gamma-sitosterol	-	-	-	6.43	-	8.84
80.840	urs-12-ene	23.93	-	-	-	-	-
81.119 - 81.418	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a, 5,6,6a,6b	12.04	1.08	-	-	-	-
81.714 - 82.099	lup-20(29)-en-3-one	20.15	1.70	-	-	-	-
82.267 - 82.700	lupeol	17.13	1.93	0.81	-	-	0.59
97.068	tricyclo[20.8.0.0(7,16)]triacontane1(22),7(16)-diepoxy	-	-	-	-	100.00	-
101.520	(Z)-7-hexadecenal	-	-	-	10.70	-	36.26

LHE - leaf hexane extract; LME – leaf methanol extract; LEE - leaf ethanol extract; SHE – seed hexan extract; SME – seed methanol extract; SEE - seed ethanol extract

Table 3. Quantification of the contents of total phenolics, total flavonoids and antioxidant activity of *Parkia platycephala* leaf and seed extracts.

Extract	Total phenols (mg GAE/g)	Total flavonoids (mg RE/g)	DPPH IC_{50} (µg/ml)
LHE	*	**	ND
LME	85.11 ± 0.25^{a}	11.85 ± 0.69^{c}	30.19 ± 0.75^{b}
LEE	84.17 ± 0.33^{a}	25.64 ± 0.91^{a}	40.62 ± 0.65^{c}
SHE	*	**	ND
SME	*	**	ND
SEE	*	20.86 ± 2.25^{b}	ND
RUT	-	-	15.77 ± 0.08^{a}

*phenol content < 4 mg AGE/g; ** flavonoids content < 10 mg RE/g; ND- not detected;
Values followed by the same letter indicate significant similarities in the same column ($p < 0.05$, ANOVA followed by Tukey's test) (LHE - leaf hexanic extract; LME - leaf methanolic extract; LEE - leaf ethanolic extract; SHE - seed hexanic extract; SME - seed methanolic extract; SEE - seed ethanolic extract) and of rutin (RUT) (positive control).

The total phenol content of the seed extracts was less than 4.00 mg GAE/g. Ghasemzadeh et al. (2018) quantified the content of total phenols and flavonoids for the seed extracts of the species *P. speciosa* and despite the superiority of the total phenol content (14.9 ± 2.03 mg GAE/g) in relation to the seed extracts of the species *P. platycephala*, the levels of flavonoids (12.4 ± 3.51 mg RE/g) of *P. speciosa* were lower than the SEE (20.86 ± 2.25 mg RE/g) of *P. platycephala*.

P. platycephala is described as a species with a high percentage of phenolic compounds, which are normally directly associated with the antioxidant potential (Chun et al., 2005; Figueiredo et al., 2020). The analysis of the antioxidant activity of the extracts, according to the classification adopted by Melo et al. (2010), indicates that the LME and LEE of *P. platycephala* have high potential, since they do not exceed the limit of three times the coefficient of the positive control, in this case, the rutin (IC_{50} = 15.77 ± 0.08 µg/ml). In addition, the antioxidant potential of LME (IC_{50} = 30.19 ± 0.75 µg/ml) is statistically superior to that of LEE (IC_{50} = 40.62 ± 0.6 µg/ml). Dluya et al. (2017) detected an IC_{50} = 45.72 µg/ml for the radical scavenging activity (DPPH) of methanol extracts of the species *P. biglobosa*, which when compared to the

Table 4. Lethal concentrations of 50% (IC_{50}) of extracts of *Parkia platycephala* by the *Artemia salina* test. (LHE - leaf hexanic extract; LME - leaf methanolic extract; LEE - leaf ethanolic extract; SHE - seed hexanic extract; SME - seed methanolic extract; SEE - seed ethanolic extract).

Extract	IC_{50} (µg/ml)	Toxicity *Artemia salina*
LHE	*	Non-toxic
LME	825.60 ± 0.01	Low
LEE	2987.00 ± 0.01	Non-toxic
SHE	*	Non-toxic
SME	598.30 ± 0.01	Low
SEE	326.80 ± 0.01	Moderate

*IC_{50} not established up to a concentration of 4000 µg/mL. (LHE - leaf hexanic extract; LME - leaf methanolic extract; LEE - leaf ethanolic extract; SHE - seed hexanic extract; SME - seed methanolic extract; SEE - seed ethanolic extract).

antioxidant potential of the LME (IC_{50} = 30.19 ± 0.75 µg/mL) indicates superiority of the methanol extract of *P. platycephala.* SHE, SME, SEE and LHE did not reach a 50% dose-response in this test, indicating that their antioxidant activity was low. Farias et al. (2013) confirm the difficulty of quantifying the antioxidant potential of the seed extract of this species. For hexane extracts (LHE, SHE), the low antioxidant activity is justified due to the low affinity of phenolic compounds for nonpolar solvents.

Toxicity

Table 4 defines the IC_{50} values and toxicity ratios for each *P. platycephala* extract. LHE and SHE were found to be non-toxic, with IC_{50} > 4000 µg/ml and LEE with IC_{50} = 2987.00 ± 0.01 µg/ml. It is noteworthy that the sequential extraction in the seed enabled the isolation of some compounds, which caused the SEE to have moderate toxicity, that is, 100 < IC_{50} < 500 µg/ml. Figueiredo (2014) reported the non-toxicity of *P. platycephala* leaf extracts (IC_{50} > 1000 µg/ml).

In *in vivo* tests on mice, Fernandes et al. (2010) stated that the leaf ethanolic extracts of *P. platycephala* did not present acute toxicity (1000 mg/kg) and neither cytotoxicity in erythrocytes of rats (100 µg/ml).

Saleh et al. (2021) confirm these results, stating that the acute toxicity in fish is within the range of 500-5000 mg/kg of body weight, and in rats the IC_{50} > 5000 mg/kg (Builders, 2012), suggesting that the seed extracts of the genus *Parkia* are not potentially dangerous as they present moderate or low toxicity.

Anticholinesterase activity

With the extracts chemically characterized and their toxicities established, we can strengthen the potential of the biological activities of *P. platycephala* and emphasize the inhibitory evaluation of acetylcholinesterase as shown in Figure 3. It is reported that only extracts with iAChE values equal to or greater than 50% have a potential for successful fractionation and isolation of the active ingredients capable of inhibiting the enzyme acetylcholinesterase (Trevisan and Macedo, 2003). In this case, as shown in Figure 3, all extracts analyzed in this study showed this characteristic of percentage inhibition, with emphasis on the SHE that obtained the highest percentage of inhibition (77.40%). Previous studies with the seed ethanolic extract of *P. platycephala* and aqueous from *P. biglobosa*, the latter defatted, confirm iAChE > 50% (Ademiluyi, 2018; Farias et al., 2013). Table 5 shows all the lethal concentrations of 50% of the extracts of *P. platycephala*.

According to Table 5, using physostigmine (IC_{50} = 1.15 ± 0.05 µg/ml) as a positive control, the seed hexanic extract of *P. platycephala* showed the best iAChE activity, (IC_{50} = 9.85 ± 0.76 µg/ml). It is noteworthy that the LME, LEE, SEE, SME had significantly similar iAChE activity. These data are the first quantitative reports of the species *P. platycephala* of the potential for inhibition of this enzyme. In this study, the inhibition of acetylcholinesterase from extracts of *P. platycephala* can be related to the pyrogallol compound, which, according to Sarikaya (2015), exhibits potent iAChE activity (IC_{50} = 10.2 µM), and to palmitic acid with moderate inhibitory power of AChE (IC_{50} = 33.9 µM or 8.69 µg/mL) (Fang et al., 2010).

Ayaz et al. (2017) detected in *in vitro* and *in vivo* studies that the beta-sitosterol compound has a strong iAChE (IC_{50} = 55 µg/ml) with double efficiency in the tests, inhibiting the enzyme acetylcholinesterase and eliminating free radicals. The presence of beta-sitosterol prevents oxidative stress induced by glucose oxidase and lipid peroxidation, presenting a high potential against neurodegenerative diseases such as AD (Shi et al., 2013). Inhibition of acetylcholinesterase has also been observed in studies using stigmasterol (Gade et al., 2017).

Recent studies have verified the iAChE effect of a linoleic acid isomer, demonstrating a threefold reduction in acetylcholine enzyme activity (Cigliano et al., 2019). In

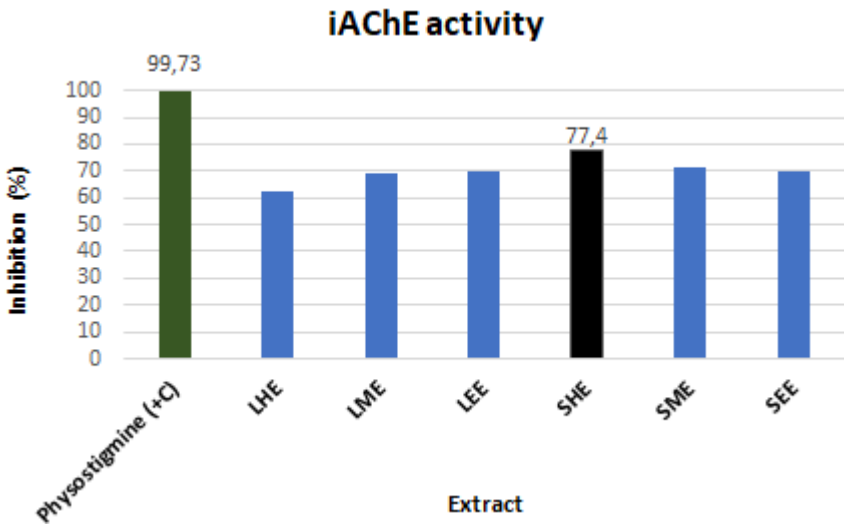

Figure 3. Acetylcholinesterase inhibition percentage of *Parkia platycephala* extracts (LHE - leaf hexanic extract; LME - leaf methanolic extract; LEE - leaf ethanolic extract; SHE - seed hexanic extract; SME - seed methanolic extract; SEE - seed ethanolic extract).

Table 5. Lethal concentrations of 50% (IC_{50}) of the extracts of *P. platycephala* for inhibition of acetylcholinesterase.

Extract	IC_{50} (µg/ml)
Physostigmine	1.15 ± 0.05^{a}
LHE	15.68 ± 0.4^{d}
LME	13.02 ± 0.29^{c}
LEE	12.87 ± 0.1^{c}
SHE	9.85 ± 0.76^{b}
SME	12.14 ± 0.12^{c}
SEE	12.90 ± 0.14^{c}

Lower case letters indicate significant similarities between the lines ($p < 0.05$, ANOVA followed by Tukey's test). LHE - leaf hexanic extract; LME - leaf methanolic extract; LEE - leaf ethanolic extract; SHE - seed hexanic extract; SME - seed methanolic extract, SEE - seed ethanolic extract) and Fisostigmine (positive control).

this context, tests with a nanoplatform with this same isomer suggested a delivery system actively directed at the central nervous system, with a sophisticated potential to reduce the symptoms of AD (Agwa et al., 2020). The compound oleamide is a chemopreventive agent against AD, has protective activity against scopolamine-induced memory loss, and also regulates microglial activity (Ano et al., 2015; HEO et al., 2003).

In SME, the iAChE effect may be related to the presence of the triacontane derivative. Dalai et al. (2014) reported inhibition of AChE, IC_{50} = 45.88 ± 1.94 µg/ml, after tests with cinnamon oil, which contained the triacontane compound in its composition. It is also evident that flavonoids, present in the LME, LEE, SME and SEE, have neuro and nephroprotective activities (Athira et al., 2016; Xicota et al., 2017). The iAChE potential of the flavonoids is justified due to the presence and position of the hydroxyl group (OH) in ring A and ring B, and also due to the unsaturation of ring C (Khan et al., 2018). As seen in this study, Morais et al. (2013) stated that there is no direct relationship between antioxidant activity and acetylcholinesterase inhibition, but there is a dependence on these activities due to their structural patterns (Karmakar et al., 2019).

According to Santos et al. (2018), studies relating data from plant species and iAChE, have shown relevant limitations in not associating the antioxidant and toxic effects of the extracts. This research brings concern

about minimizing this limitation and confirms that extracts with high iAChE power, IC_{50} < 20 µg/ml (Santos et al., 2018) do not need to present high toxicity (Huq et al., 2014; Nwidu et al., 2017). Thus, it is thought that it is important to continue research with the extracts evaluated, since they have a remarkable potential for inhibiting acetylcholinesterase and there are few studies on this species.

Conclusion

This study provides the first evidence of the inhibition of acetylcholinesterase from the leaf and seed of *P. platycephala*. Sequential extraction with increasing polarity helped in optimizing the extraction of volatile chemicals and it was efficient in terms of toxicity and acetylcholinesterase inhibition. These data indicate the potential therapeutic use of this plant in medical conditions related to Alzheimer's disease.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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