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Chemical composition and antioxidant activity of crude extracts from *Pachira aquatica* leaves, flowers and seeds

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Abstract

Pachira aquatica, an arboreal species belonging to the family Malvaceae, is widely cultivated as an ornamental plant. Its leaves are used in folk medicine for treating skin rashes and urticaria; its fruit peels find application in the treatment of diarrhea, diabetes, and anemia; and its lipid-rich seeds are used as human and animal food. This study aimed to determine the chemical composition and assess the antioxidant potential of crude extracts from *P. aquatica* leaves, flowers and seeds. The plant material was collected and then dried at room temperature. The crude extracts were prepared by dynamic maceration with solvent depletion. Chemical analysis was performed by ultra-high performance liquid chromatography coupled with high resolution mass spectrometry. Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), ferric reducing antioxidant power (FRAP), β-carotene/linoleic acid co-oxidation, and total phenolic content assays. Eleven flavonoids, four phenolic acids, two organic acids and four coumarins were identified in extracts. Crude extracts from leaves, flowers and seeds had total phenolic contents of 28.74; 75.68; and 14.88 g GAE/mg; half-maximal effective concentrations for DPPH• scavenging activity of 8.02; 0.69; and 4.01 mg/mL; FRAP activities of 0.038; 0.274; and 0.042 µM ferrous sulfate/mg; and the ability to reduce β-carotene/linoleic acid co-oxidation by 42.13%; 64.85%; and 30.37%; respectively. The utilization of antioxidant compounds found in *P. aquatica* leaves, flowers and seeds in industrial applications is a promising strategy to add value to this plant.

Keywords: caffeic acid; catechin; kaempferol; kaempferol-3-*O*-glucorhamnoside; monguba; quercetin.
Abbreviations: ANOVA_analysis of variance; BHA_butyl hydroxyanisole; BHT_butyl hydroxytoluene; CE_crude extract; DPPH•_2,2-diphenyl-1-picrylhydrazyl radical; EC_{50}_ half-maximal inhibitory concentration; FRAP_ferric reducing antioxidant power; GAE_ concentration in gallic acid equivalents; GL_Gay-Lussac; MS/MS_mass spectrometry; *P. aquatica*: *Pachira aquatica*; SisGen_System for the Management of Genetic Heritage and Associated Traditional Knowledge; TBHQ_*tert*-butyl hydroquinone; UHPLC-ESI-QTOF-MS/MS_Ultra-high-performance liquid chromatography coupled to high resolution mass spectrometry.

Introduction

Pachira aquatica is a tree species belonging to the family Bombacaceae, although, according to the Angiosperm Phylogeny Group II classification, it belongs to the family Malvaceae (Apg II, 2003). The species is native to the floodplain regions of the coast of Pará and Maranhão States, Brazil (Lorenzi et al., 2015). *P. aquatica* is commonly known as monguba, wild cocoa, mamorana, embiratanha, Guiana chestnut, chestnut, carolina, munguba, water chestnut, false cocoa, Maranhão chestnut and sapote-grande (Lorenzi, 2016). It has a light brownish to brown trunk, green crown, dark green leaves, a palmate compound and long-petiolate, and buds spathaceous. The flowers are located at the end of branches and characterized by their large size and color. The flower opening occurs at the end of the day, releasing perfume that attracts pollinators. The fruits are woody, brown, and velvety, they grow in the form of capsules containing light-brown seeds (Figure 1) (Peixoto and Escudeiro, 2002; Kinupp and Lorenzi, 2014).

In folk medicine, *P. aquatica* leaves are used to treat skin rashes, urticaria and scratches (Alfaro, 1984). Fruit peels and seeds are used to treat diarrhea, infections, diabetes, skin rashes, wounds and anemia (Coe et al., 2012). Flowers are used for the preservation of diurnal (e.g., bees) and nocturnal (e.g., bats and moths) pollinating animals (Hernández-Montero and Sosa, 2016). There are no studies addressing the biological activity of flowers. *P. aquatica* wood finds application in the production of boxes, matches, frames and cellulose paste (Lorenzi, 2016). The seeds are used as human and animal food, given their high content of lipids (38.39%), of which palmitic acid (44.93%) is the major saturated fatty acid (Jorge and Luzia, 2012). *P. aquatica* (monguba) is the third most predominant species in the city

of Umuarama-Paraná (Brazil), representing 10.80% of the afforestation of the central city area with 796 catalogued trees (Pozzobom et al., 2020). This species is known as a non-conventional food plant (NCFP); because its young leaves, after blanching, can be consumed stewed and crushed. The young flowers consumed sautéed, in salads decorating dishes, and, the roasted ground seeds, are transformed into flours used for the production of paçocas, cakes, breads, puddings, farofas, dumplings, breading fish (Kinupp and Lorenzi, 2014). Consumption in human and animal food is justified by the nutritional value of its seeds, which contain lipids (38.39%), proteins (11.86%), carbohydrates and fibers (41.60%), and the oil extracted from the seeds represents more than 30% of the total mass (Jorge and Luzia, 2012). Considering that each *P. aquatica* tree produces 10 fruits (woody capsules) and has an average of 39 seeds inside, an adult tree can produce about 390 seeds (Espitia et al., 2018), thus showing the importance of this species as a food source also bauru almonds (*Dipteryx alata* Voge) has nutricional importance as a viable food, furthermore, both are native from Brazilian savanna. The bauru almonds flour show potential to be used for enrichment to other kind of food being an alternative and gluten free option (Silva et al., 2022).

Regarding extraction, most studies report the extraction of oil and its chemical composition (Lawal et al., 2015; Gamal et al., 2018; Zoghbi et al., 2003; Rodrigues et al., 2019). Lawal et al. (2015) extracted essential oil from *P. aquatica* leaves via hydrodistillation and identified the following major compounds: 9-octadecenamide (35.1%), phytol (31.2%) and methyl palmitate (19.1%). Gamal et al. (2018) found that palmitic acid methyl ester (21.08%) and 9,12-octadecadienoic acid ethyl ester (26.20%) were the major compounds of *P. aquatica* leaf essential oil. Zoghbi et al. (2003) obtained essential oil from *P. aquatica* flowers and identified the following major compounds: *trans*-*trans*-α-farnesene (19.2%), β-caryophyllene (11.5%) and *trans*-linalool oxide (7.2%). Rodrigues et al. (2019) obtained fixed oil from *P. aquatica* seeds by Soxhlet extraction and found a lipid content of 43.42%, with palmitic acid (60.92%) and oleic acid (7.67%) as the major compounds.

Few studies have addressed the extraction and biological activity of *P. aquatica* leaves, flower and seed extracts. Souza et al. (2012) reported that seed extract showed insecticidal and repellent activity against *Hypothenemus hampei*, inhibiting 80% of insect attraction to coffee fruits. Halili et al. (2019) obtained *P. aquatica* leaves extract and identified alkaloids, glycosides and tannins as secondary metabolite constituents. The current study is the first to investigate *P. aquatica* flower extract. However, there is no information on the biological activity, including antioxidant activity, of flower extracts. The imbalance between pro-oxidant and oxidant homeostasis in the body can lead to the generation of toxic radical species, which damage molecules such as membrane lipids and DNA; leading to the development of diseases such as type 2 diabetes, aging, cardiovascular diseases and cancer (Uttara et al., 2009). On the other hand, oxidation in foods can generate undesirable odors and flavors, as well as changes in color and texture, interfering in the sensory and nutritional quality of foods (Gutiérrez-del-Rio et al., 2021). Thus, the search for biomolecules with natural antioxidant capacity has been the goal of the food, cosmetic and pharmaceutical industries, intending to replace synthetic antioxidants such as butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and *tert*-butyl hydroquinone (TBHQ) (Jardini and Mancini Filho, 2007), which are harmful to health (Oyetayo, 2009). The Brazilian Ministry of Health determined the following upper limits for synthetic antioxidants in food products: 200 mg kg^{-1} for BHA and TBHQ and 100 mg kg^{-1} for BHT (Brasil, 2001). Given the above, this study aimed to analyze the chemical composition and antioxidant activity of crude extracts from *P. aquatica* leaves, flowers and seeds.

Results

Yield of crude extracts from leaves, flowers and seeds of P. aquatica

The yield (%) of crude extracts of leaves, flowers and seeds of P. aquatica was 15.13%; 36.88%; and 51.93%; respectively.

Chemical composition of crude extracts of leaves, flowers and seeds of P. aquatica by UHPLC–ESI-MS/MS

Eleven flavonoids, four phenolics acids, two organic acids and four coumarins were identified in the crude extracts (Table 1). In the leaves extracts, five flavonoids (rutin, kaempferol-3-O-glucorhamnoside, quercetin-3-glucopyranoside, quercitrin and kaempferol) and a phenolic acid (p-coumaric acid) were found. In the flower extracts, six flavonoids (rutin, kaempferol-3-O-glucorhamnoside, quercetin-3-glucopyranoside, quercitrin, kaempferol and quercetin), four phenolic acids (caffeic acid, quinic acid, chlorogenic acid, and p-coumaric acid), two organic acids (malic acid and benzoic acid) and four coumarins (dicaffeoylquinic acid, 5 hydroxyethoxy ayapin, esculetin and 5 methoxyscopoletin) were identified. In the seed extracts, four flavonoids (catechin, rutin, quercetin and quercetin-3-glucopyranoside), two phenolic acids (chlorogenic acid and caffeic acid) and one organic acid (benzoic acid) (Figure 2) were identified.

Antioxidant activity of crude extracts of leaves, flowers and seeds of P. aquatica by UHPLC–ESI-MS/MS

The antioxidant activities of crude extracts from *P. aquatica* leaves, flowers and seeds are described in Tables 2 and 3. The concentration of leaf, flower and seed extracts required to inhibit 50% of $DPPH^{\bullet}$ (IC_{50}) was 8.03; 0.69; and 4.01 mg/mL, respectively. Flowers extract had the highest activity, but was 69 times lower than quercetin control. The capacity of leaf, flower and seed extracts to reduce ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron, as assessed by the FRAP assay, was 0.04; 0.27; and 0.04 μM $FeSO_4$/mg sample, respectively, and not showed significant difference. The total phenolic content was 28.74; 75.68; and 14.88 μg GAE/mg sample, respectively.

Flower extract had a 2.63 and 5.08-times higher total phenolic content than leaf and seed extracts, respectively, demonstrating the antioxidant potential of *P. aquatica* flowers.Table 3 shows the oxidation inhibition potential of leaves, flowers and seeds crude extracts, as assessed by the β-carotene/linoleic acid co-oxidation assay. The increase in the concentration of extracts contributed to increase the antioxidant activity. Flowers crude extracts (1.00 mg/mL) showed the highest antioxidant activity (64.95%). In a complementary way, a bibliographic survey of the biological activities of the compounds found in the leaves, flowers and seeds of *Pachira aquatica* was carried out (Table 4).

Discussion

P. aquatica seeds were extracted by using 96° GL alcohol, as per the method described by Souza et al. (2012). However, for the liquid extraction conditions to obtain crude extracts of leaves and flowers of *P. aquatica,* those proposed by previous studies on extracts of the Malvaceae family, to which *P. aquatica* belongs, were used. Thus, leaf extracts were obtained by using 80° GL alcohol (Francis et al., 2018) and flower extracts by using 77° GL alcohol (Artanti et al., 2018). It is important to highlight that the alcoholic concentration of the solvent used directly influences the extraction of compounds present in the plant (Souza et al., 2012; Francis et al., 2018; Artanti et al., 2018), but there is still no standardization for *P. aquatica* leaves, flowers and seeds.

Chemical characterization revealed the presence of flavonoids, phenolic acids, organic acids and coumarins in extracts of leaves, flowers and seeds of *P. aquatica.* There are few reports of the chemical composition of *P. aquatica* in the literature (Rodrigues et al., 2019; Rezende et al., 2021). The compounds identified in crude seed extracts are in agreement with those reported by Rodrigues et al. (2019), who identified UHPLC–ESI-MS/MS caffeic acid, ferulic acid, 4-hydroxybenzoic acid and γ-tocopherol in defatted *P. aquatica* seeds extracted in methanol-acetone-water (7:7:6, v/v/v). Rezende et al. (2021) analyzed the ethanolic extract of *P. aquatica* seeds by UHPLC-QDa-MS and identified the phenolic acids caffeic, chlorogenic, ferulic and quinic acids, as well as the flavonoids catechin, epicatechin and kaempferol; in ethanolic leaf extracts, the authors identified chlorogenic acid, *p*-coumaric acid, rutin and kaempferol.

The flower extract showed the highest content of total phenolics and antioxidant activity by DPPH$^{\bullet}$ method. Such findings can be explained by the fact that the flower extract contains a greater number of flavonoids and phenolic acids. The flavonoids and phenolic acids identified in *P. aquatica* (Rodrigues et al., 2019; Rezende et al., 2021) and in the present study showed antioxidant activity. The antioxidant potential can be attributed to the presence of the identified phenolic compounds, since the relationship of these compounds with antioxidant activity is reported in the literature (Lima and Bezerra, 2012). Kaempferol, a compound present in flowers crude extract, is a tetrahydroxyflavone that has hydroxy groups (Hua et al., 2015). This compound has been isolated by Hyun et al. (2006) from the ethyl acetate fraction of *Ginkgo biloba* leaves and found IC_{50} of 2.86 μM for scavenging peroxynitrite, which is an oxidizing molecule involved in lipid peroxidation. Tian et al. (2021) reported that kaempferol exhibited stronger antioxidant activity with IC_{50} DPPH$^{\bullet}$ of 4.506 μg/mL. Sun et al. (2019) reported that kaempferol-3-O-glucorhamnoside from *Thesium chinense* Turcz. reduced reactive oxygen species and oxidative stress, generated by exposure to *Klebsiella pneumoniae,* in mice lungs and cultured cells.

Quercetin, also identified in the present study is a 3,3',4',5,7-pentahydroxyflavone, a compound present in flowers crude extract is a natural polyphenolic flavonoid compound (Rauf et al., 2018) and used as a control in several antioxidant protocols, such as in DPPH$^{\bullet}$ scavenging assays (Hyun et al., 2006). This compound has strong antioxidant activity and the capacity to eliminate oxygen-reactive species such as $O_2^{\bullet-}$ (Kukongviriyapan et al., 2012) $ONOO^-$ (Kim et al., 2013) and NO^- (Luangaram et al., 2007). Its antioxidant property is related to the hydroxyl group on the third carbon, the double bond between the second and third carbons, the carbonyl group on the fourth carbon, and the aromatic rings (Cook et al., 1996). In a study reported by Zhang et al. (2011), quercetin exhibited antioxidant activity by reducing the generation of reactive oxygen species by lipopolysaccharides. Lesjak et al. (2018) reported that quercetin showed IC_{50} of <1.33 μg/mL by DPPH$^{\bullet}$ method. Quercetin has showed biological activities as anti-inflammatory effects (Kleemann et al., 2011), anti-carcinogenic (Kumari et al., 2010), antiviral (Ganesan et al., 2012) and antibacterial (Rattanachaikunsopon and Phumkhachorn, 2010).

Quercitrin is the glycosylated form of quercetin. Chen et al. (2022) and Yin et al. (2013) showed that this compound minimized the generation of reactive oxygen species induced by UVB radiation. Miliauskas et al. (2004) identified the flavonoid glycoside quercetin-3-glucopyranoside in *Geranium macrorrhizum* crude extract, which inhibited 93% of DPPH$^{\bullet}$. Catechin is a flavonoid with two pharmacophore groups, a resorcinol on the A ring and a catechol on the B ring, as well as a hydroxyl in the 3 '- position of the C ring (Janeiro and Brett, 2004). Grzesik et al. (2018) evaluated the free radical reducing capacity estimated by the reduction of ABTS, since catechin (3,965 mol Trolox equivalents/mol) was 4 times more efficient than trolox (1 mol Trolox equivalents/mol).

The flavonoid kaempferide, present in flower crude extract in the current study, was also identified in the extract of *Alpinia oxyphylla* fruits and the concentration required to inhibit 50% of DPPH$^{\bullet}$ was 97.58 μg/mL (Bian et al., 2013). Marques et al. (2020) performed antioxidant activity using 1% malic acid and obtained as a result 22.41% of DPPH inhibition (47.50 uM Trolox eq_sL-1). As reported by Pero et al. (2009), quinic acid is an antioxidant prometabolite of nicotinamide and tryptophan. Sato et al. (2011) reported that caffeic acid and chlorogenic acid exert protective effects against ischemia/reperfusion injury in the rat small intestine, with caffeic acid (10.1 ± 9.32 μM) having a stronger antioxidant activity than chlorogenic acid (41.0 ± 12.1 μM) because of its faster absorption. Kiliç and Yesiloglu (2013) demonstrated that 45 μg/mL *p*-coumaric acid was capable of inhibiting 71.2% of lipid peroxidation.

Morelloflavone, a compound identified in flowers crude extract is a bioflavonoid found in the acetonic extract of *Garcinia dulcis* fruits. It has been shown to have metal-chelating antioxidant activity, with a Fe^{2+} binding ratio of 5.3 (moles of bound Fe^{2+}/mole of compound) (Towatana et al., 2007).

Among the coumarins present flowers extract in this study, dicaffeoylquinic acid was identified in fresh leaves of *Inula viscose* and used at 12 and 40 μM to inhibit 50% of ABTS and DPPH$^{\bullet}$, respectively (Danino et al., 2009). Esculetin from cortex extract of *Fraxinus chinensis* inhibited 50% of DPPH$^{\bullet}$ at 2.1 μg/mL (Lee et al., 2007).

The β-carotene/linoleic acid co-oxidation assay showed that extracts differed significantly in antioxidant potential. As reported by Hassimoto et al. (2005), extracts can be classified as having high, intermediate, or low inhibition potential according to the following percentages: ≥70%, 40–70% and <40% inhibition, respectively. Flower and leaf extracts exhibited intermediate oxidation inhibition potential (64.85% and 42.13%, respectively), whereas seed extracts provided low oxidation inhibition (30.37%) at 1.0 mg/mL (Table 3). In the current study, the intermediate

Table 1. Chemical composition of crude extracts of *Pachira aquatica* leaves, flowers and seeds assessed by UHPLC-ESI-QTOF-MS/MS.

Compounds	Molecular formula	Theoretical *m/z* $[M-H]^-$	Experimental *m/z* $[M-H]^-$	Error (ppm)	RT (min)	Sample (CE)
Flavonoids						
Catechin	$C_{15}H_{14}O_6$	289.0712	289.0697	5.18	4.05	Seeds
Rutin	$C_{27}H_{30}O_{16}$	609.1456	609.1419	6.07	4.27	Flowers
			609.1426	4.92	4.29	Seeds
			609.1430	4.26	4.40	Leaves
Kaempferol-3-O-glucorhamnoside	$C_{27}H_{30}O_{15}$	593.1507	593.1466	6.91	4.40	Flowers
			593.1474	5,56	4.44	Leaves
Quercetin-3-glucopyranoside	$C_{21}H_{20}O_{12}$	463.0877	463.0849	6.04	4.42	Flowers
			463.0867	2.15	4.47	Seeds
			463.0859	3.88	4.48	Leaves
Quercitrin	$C_{21}H_{20}O_{11}$	447.0928	447.0903	5.59	5.59	Flowers
			447.0910	4.02	4.61	Leaves
Kaempferol	$C_{15}H_{10}O_6$	285.0399	285.0379	7.01	5.11	Flowers
			285.0395	1.40	5.16	Leaves
Quercetin	$C_{15}H_{10}O_7$	301.0349	301.0333	5.31	5.23	Flowers
			301.0341	2.65	5.18	Seeds
Morelloflavone	$C_{30}H_{20}O_{11}$	555.0921	555.0891	5.40	5.32	Flowers
Apigenin	$C_{15}H_{10}O_5$	269.0459	269.0436	8.55	5.43	Flowers
			269.0449	3.72	5.41	Seeds
Kaempferide	$C_{16}H_{12}O_6$	299.0555	299.0541	4.68	5.46	Flowers
Amentoflavone	$C_{30}H_{18}O_{10}$	537.0816	537.0787	5.39	5.55	Leaves
Phenolic acids						
Caffeic acid	$C_9H_8O_4$	179.0345	179.0337	4.47	4.20	Flowers
			179.0339	3.35	4.16	Seeds
Quinic acid	$C_7H_{12}O_6$	191.0556	191.0550	3.14	0.88	Flowers
Chlorogenic acid	$C_{16}H_{18}O_9$	353.0873	353.0852	5.94	3.96	Flowers
			353.0855	5.09	4.24	Seeds
P-coumaric acid	$C_9H_8O_3$	163.0395	163.0388	3.07	4.63	Flowers
			163.0390	3.07	4.64	Leaves
Organic acids						
Malic acid	$C_4H_6O_5$	133.0131	133.0137	-4.51	0.92	Flowers
Benzoic acid	$C_7H_6O_2$	121.0280	121.0286	-4.96	5.11	Flowers
			121.0290	-8.26	5.11	Seeds
Coumarins						
Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	515.1184	515.1205	-4.08	5.86	Flowers
5 hydroxyethoxy ayapin	$C_{12}H_{10}O_6$	249.0398	249.0386	4.82	5,78	Flowers
Esculetin	$C_9H_6O_4$	177.0187	177.0184	1.69	4,94	Flowers
5 methoxyscopoletin	$C_{11}H_{10}O_5$	221.0449	221.0442	3.17	4.82	Flowers

RT: retention time; CE: crude extract.

Fig 1. Representative photographs of *Pachira aquatica* (a) leaves, (b) flower, and (c) fruit with seeds. Source: The authors.

Table 2. Antioxidant activity, as assessed by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging, ferric reducing antioxidant power (FRAP) assays, and total phenolic content of crude extracts from *Pachira aquatica* leaves, flowers, and seeds.

Samples (CE)	DPPH$^{\bullet}$ IC_{50} (mg/mL)	FRAP (μM ferrous sulfate/mg)	Total phenols (μg GAE/mg)
Leaves	8.027 ± 1.522^{d}	0.038 ± 0.080^{b}	28.741 ± 0.240^{b}
Flowers	0.690 ± 0.048^{b}	0.274 ± 0.193^{b}	75.680 ± 0.481^{a}
Seeds	4.010 ± 1.584^{c}	0.042 ± 0.010^{b}	14.881 ± 0.841^{c}
Quercetin	0.010 ± 0.001^{a}	-	-
Trolox	-	9.175 ± 0.001^{a}	-

Values are the mean ± standard deviation of triplicate assays. Data were subjected to analysis of variance (ANOVA), and differences between means were assessed by Tukey's test ($p \leq 0.05$). Values in the same column followed by different letters are significantly different ($p \leq 0.05$). Quercetin (0.0103 mg/mL) and Trolox (9.175 mg/mL) were used as positive controls in DPPH$^{\bullet}$ and FRAP assays, respectively. Abbreviations: IC_{50}: half-maximal inhibitory concentration; DPPH$^{\bullet}$: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; CE: crude extract.

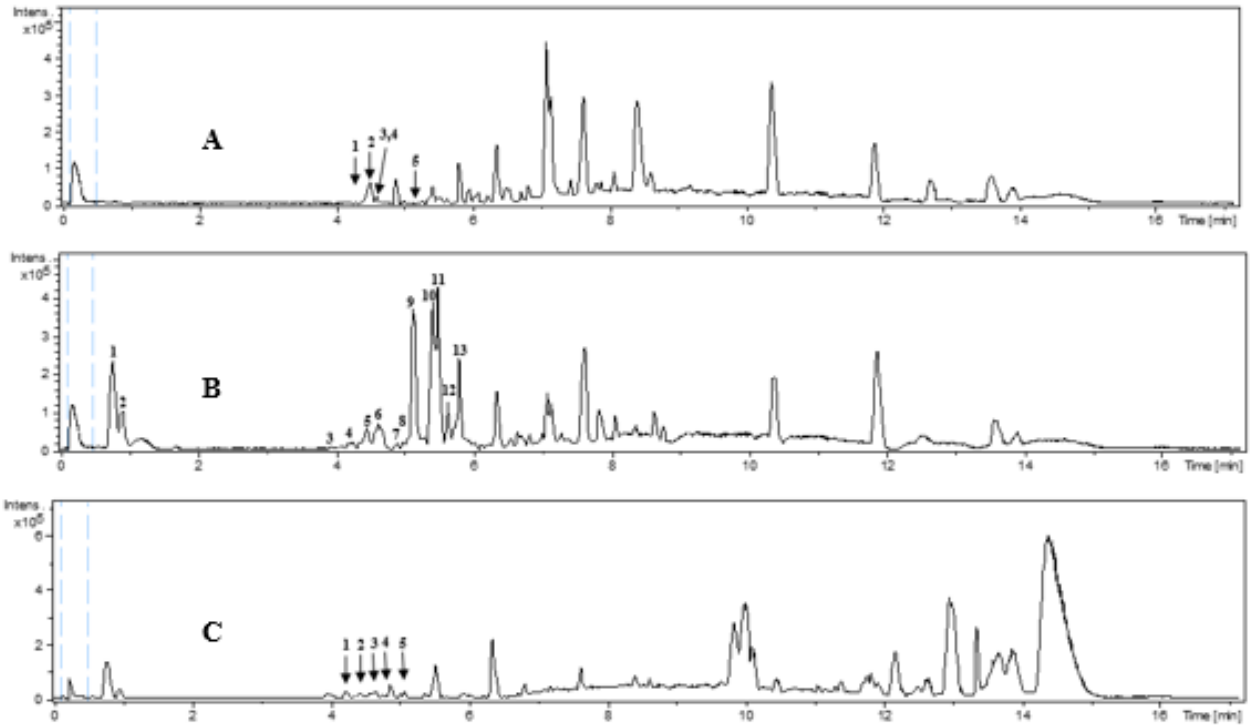

Fig 2. Chromatogram of crude extracts from *Pachira aquatica*. **Leaves (A):** 1. Kaempferol-3-O-glucorhamnoside; 2. Quercetin-3-glucopyranoside; 3. P-coumaric acid; 4. Quercetrin; 5. Kaempferol. **Flowers (B):** 1. Quinic acid; 2. Malic acid; 3. Chlorogenic acid; 4. Cafeic acid; 5. Quercetin-3-glucopyranoside; 6. Kaempferol-3-O-glucorhamnoside; 7. Quercetrin; 8. P-coumaric acid; 9. Kaempferol; 10. Quercetin; 11. Morelloflavone; 12. Kaempferide; 13. Dicaffeoylquinic acid. **Seeds (C):** 1. Cathechin; 2. Cafeic acid; 3. Chlorogenic acid; 4. Quercetin-3-glucopyranoside; 5. Quercetin; obtained by ultra-high performance liquid chromatography/high-resolution mass spectrometry.

Table 3. β-Carotene/linoleic acid co-oxidation inhibition potential of crude extracts from *Pachira aquatica* leaves, flowers, and seeds.

Samples (CE)	Concentrations (mg/mL) 1.00	0.75	0.50	0.25
Leaves	42.13 ± .1,65^{bA}	31.83 ± 0.88^{bB}	21.53 ± 2.93^{bC}	11.10 ± 0.72^{cD}
Flowers	64.85 ± 1.08^{aA}	54.37 ± 0.48^{aB}	43.21 ± 1.32^{aC}	38.94 ± 1.64^{aD}
Seeds	30.37 ± 1.02^{cA}	24.11 ± 0.55^{cB}	22.68 ± 0.25^{bB}	20.42 ± 0.44^{bC}

Values are the mean ± standard deviation of triplicate assays. Data were subjected to analysis of variance (ANOVA), and differences between means were assessed by Tukey's test ($p \leq 0.05$). Values in the same column followed by different lowercase letters and values in the same row followed by different uppercase letters differ significantly ($p \leq 0.05$). Trolox (0.2 mg/mL) was used as positive control. CE: crude extract.

potential of the extracts can be explained by the fact that this assay is indicated for lipophilic compounds (Rufino et al., 2010).

We highlight the added value of *P. aquatica* flowers, which are under-utilized, since the tree species is mostly used for shading sidewalks, parks and gardens. Given the high total phenolic content of *P. aquatica* flowers, it is possible to say that this plant material is a promising natural source of antioxidant compounds, with potential application in the pharmaceutical, food and cosmetics industries. In a complementary way, a bibliographic survey of the biological activities of the compounds found in the leaves, flowers and seeds of *Pachira aquatica* was carried out (Table S1).

Materials and methods

Plant material

The leaves, flowers and seeds of *P. aquatica* were collected in 2017 in the city of Umuarama, northwestern region of the state of Parana, Brazil (latitude 23º46'16''S; longitude 53º19'38''W; altitude 442 m). The botanical identification was carried out and a specimen was deposited in the collection of the Physical Garden under number 330. The species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A69024F.

Preparation of crude extracts from P. aquatica leaves, flowers and seeds

P. aquatica leaves, flowers and seeds were dried at room temperature, ground and subjected to dynamic maceration extraction with solvent depletion using 80° GL ethanol for leaves (Francis et al., 2018), 77° GL ethanol for flowers (Artanti et al., 2018) and 96° GL for seeds (Souza et al., 2012). Then, the resulting solutions were concentrated in a rotary evaporator (TE-210) at 40 °C to obtain crude extracts. Extract yield (%) was calculated by dividing the dry weight (g) of leaves (220.5 g), flowers (226 g) and seeds (347 g) by the weight of crude extracts obtained from leaves (33.37 g), flowers (83.37 g) and seeds (180.23 g) multiplying them by 100.

Ultra-high-performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-ESI-QTOF-MS/MS)

Crude extracts from *P. aquatica* leaves, flowers and seeds were analyzed on an ultra-high-performance liquid chromatography (Nexera X2, Shimadzu) coupled to a high-resolution mass spectrometer (QTOF Impact II, Bruker Daltonics Corporation, USA) equipped with an electrospray ionization source. The capillary voltage was operated in negative ionization mode at 4500 V with an end-plate potential of −500 V. Dry gas parameters were set at 8 L/min and 200 °C with a nebulization gas pressure of 4 bar. Collision-induced dissociation was performed using argon gas and collision energy of 15 to 30 eV. Data were acquired in the 50–1300 *m*/*z* range using an acquisition rate of 5 spectra per second. Ions of interest were selected by automatic fragmentation of tandem mass spectrometry (MS/MS) data. Chromatographic separation was achieved using a C18 column (75 × 2.0 mm i.d., 1.6 µm Shim-pack XR-ODS III) and a gradient mixture of solvents A (H_2O) and B (acetonitrile), as follows: 5% B from 0 to 1 min, 30% B from 1 to 4 min, 95% B from 4 to 8 min, and maintained at 95% B from 8 to 17 min at 40 °C. Compound identification was performed as proposed in review studies on the genus *Pachira* (Matlawska and Sikorska, 2004; Valiei et al., 2011; Dinu et al., 2012; Morais et al., 2017), by calculating the mass error and comparing the results with data from MassBank (http://www.massbank.jp/) and Human Metabolome Database (http://www.hmdb.ca/).

Antioxidant activity of crude extracts from P. aquatica leaves, flowers and seeds

Determination of antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging assay

DPPH$^{\bullet}$ assays were performed according to Rufino et al. (2007). A 10 µL aliquot of crude extract from *P. aquatica* leaves, flowers and seeds at different concentrations (1.0, 0.7, 0.5, and 0.2 mg/mL) was added to 290 µL of methanolic DPPH$^{\bullet}$ solution (60 µM). The negative control was 10 µL of methanolic DPPH$^{\bullet}$ solution (60 µM). Mixtures were kept in the dark at room temperature for 30 min. The reduction in absorbance was measured at 515 nm by using a Spectra Max Plus 384 microplate reader. The total antioxidant capacity of extracts was calculated by using a standard solution of quercetin (60 µM) as a 100% reference. The concentration required to scavenge 50% of free radicals (IC_{50}) was determined from absorbance versus sample concentration curves.

Determination of antioxidant activity by the ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the procedures described by Benzie and Strain (1996) and modified by Rufino et al. (2006, b). Briefly, the FRAP reagent was prepared by mixing 25 mL of acetate buffer (0.3 M), 2.5 mL of an aqueous solution of 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ, 10 mM) and 2.5 mL of an aqueous solution of ferric chloride (20 mM). Then, 10 µL of crude extract from *P. aquatica* leaves, flowers and seeds at different concentrations (1.00, 0.75, 0.50, and 0.25 mg/mL) and 290 µL of FRAP reagent were added to the wells of a 96-well microplate. The microplate was placed on a Spectra Max Plus 384 reader, homogenized by vigorous shaking, and kept at 37 °C for 30 min. The change in absorbance was read at 595 nm. Antioxidant activity was calculated against a standard curve of ferrous sulfate (1000 µM).

Determination of total phenolic content

Total phenolics in crude extracts from *P. aquatica* leaves, flowers and seeds were determined by visible spectroscopy. The Folin–Ciocalteu method was performed as described by Swain and Hillis (1959) with the modifications proposed by Sousa de Sá et al. (2012). Extracts were diluted to 1.0 mg/mL in methanol. The reaction medium was prepared by adding 155 µL of Folin–Ciocalteu solution, 125 µL of sodium carbonate, and 20 µL of diluted sample to the wells of a microplate. The mixture was left to rest in the absence of light for 60 min, and absorbance was read in triplicate on a Spectra Max Plus 384 microplate reader at 760 nm.A calibration curve (Eq. 1, R^2 = 0.9997) was constructed using seven concentrations of gallic acid (0–100 µg/mL) and subjecting the data to linear regression:

$$A = 0.0196C - 0.031 \quad (1)$$

Where, *A* is the sample absorbance and *C* the concentration in gallic acid equivalents (GAE). Results are expressed as µg GAE/mg sample.

Determination of antioxidant activity by the β-carotene/linoleic acid co-oxidation assay

The ability of crude extracts from *P. aquatica* leaves, flowers and seeds to inhibit β-carotene/linoleic acid co-oxidation was assessed according to Rufino et al. (2006, a). To a beaker were added 20 µL of linoleic acid, 265 µL of Tween 40, 25 µL of β-carotene solution (20 mg/mL) and 0.5 mL of chloroform. The solvent was removed using a dryer. Then, the emulsion was dissolved in 20 mL of hydrogen peroxide. The antioxidant activity was determined by adding 280 µL of emulsion and 20 µL of extract at different concentrations (1.00, 0.75, 0.50, and 0.25 mg/mL), incubating the samples for 120 min, and measuring the absorbance at 470 nm. A Trolox solution was used as control. The results are expressed as percentage of oxidation inhibition, as given by Eqs. (2), (3) and (4).

$A_{red} = A_i - A_f$ (2)

$O = [(A_{redsample} \times 100]/(A_{redsystem})$ (3)

$I = 100 - (O)$ (4)

where A_{red} is the reduction in absorbance, A_i the initial absorbance, A_f the final absorbance, O the oxidation percentage and I the inhibition percentage.

Statistical analysis

All experiments were performed in triplicate. Data were subjected to analysis of variance (ANOVA) and differences between means were determined by Tukey's test at the 5% significance level using Minitab version 17 software.

Conclusions

Chemical analysis revealed the presence of flavonoids and phenolic acids in crude extracts from *P. aquatica* leaves, flowers and seeds. The crude flower extract has presented a high content of phenolic compounds (75.68 µg GAE/mg sample), showing potential as a natural source of antioxidants for pharmaceutical, food and cosmetic applications.

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