ARQUIVOS CIENTÍFICOS (IMMES)

Phytochemical, physicochemical and bioactive activities of hydroethanolic floral extract of *Ceiba pubiflora* A. St. –Hil. (Malvaceae)

Avaliação fitoquímica, físico-quimica e atividades bioativas do extrato hidroetanólico floral de Ceiba pubiflora A. St. –Hil. (Malvaceae)

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Palavras-chave

Alkaloids
Flavonoids
Ceiba Genus
Antioxidant activity
Big-bellied.

Ceiba pubiflora of the Malvaceae family still has few studies on phytochemistry, physicochemistry, and various biological activities. Objective: The study aimed to evaluate the floral extract of C. pubiflora for phytochemical, physicochemical, and bioactive characteristics. Methodology: C. pubiflora flower were collected in the Cerrado area, then the hydroethanolic extract 70% (v/v) was produced by maceration. Phytochemical analyzes were performed using different reagents. Thin-layer chromatography was performed to compare qualitative phytochemical results. The extract yield, pH, refractive index, relative density, reduction of DPPH free radical, and total phenolic compounds by Folin-Ciocalteau were determined. Results: The extract yield was = 6.88%, several phytochemical classes were observed, such as alkaloids, flavonoids, hemolytic saponin, among others, the pH was = 6.41, total solids = 1.12%, refractive index = 1.5369 nD, and relative density of 0.9061 g mL-1 at 20 °C. The best eluent was the combination of acetone and ethyl acetate with 12 revealed compounds, a positive presence was observed for glucose-reducing and fructose sugars and not for sucrose-reducing sugars. The antioxidant activity showed IC50 = 217.4 mg L-1, and total phenolic content = 7.26 mg GAE 100 g-1 in dry mass. Conclusion: In this preliminary study, it was possible to observe that the floral extract of C. pubiflora presents the potential for new biological and chemical studies, due to the large number of phytochemical classes observed, as well as the biological activities evaluated, thus suggesting that future research be carried out so that possibly new phytopharmaceuticals are developed from the floral extract.

Keywords

Alcaloides
Flavonoides
Gênero Ceiba
Actividad antioxidante
Barriguda

Ceiba pubiflora da família Malvaceae, ainda apresenta escassos estudos sobre a fitoquímica, físico-química e diversas atividades biológicas. Objetivo: O estudo teve por objetivo avaliar o extrato floral de C. pubiflora quanto as características fitoquímicas, físico-químicas e bioativas. Métodos: Flores de C. pubiflora foram coletadas em área de Cerrado, em seguida, o extrato hidroetanólico 70% (v/v) foi produzido por maceração. As análises fitoquímicas foram realizadas utilizando diferentes reativos. A cromatografia em camada delgada foi realizada para comparar os resultados fitoquímicos qualitativos. O rendimento de extrato, pH, índice de refração, densidade relativa, redução do radical livre DPPH e compostos fenólicos totais por Folin-Ciocalteau foram determinados. Resultados: O rendimento de extrato foi = 6,88%, várias classes fitoquímicas foram observadas, como alcaloides, flavonoides, saponina hemolítica, entre outras, o pH foi = 6,41, sólidos totais = 1,12%, índice de refração = 1,5369 nD, e densidade relativa de 0,9061 g mL-1 a 20 °C. O melhor eluente foi a combinação entre acetona e acetato de etila com 12 compostos revelados, foi observado a presença positiva para açúcares redutores glicose e frutose, e não redutora para sacarose. A atividade antioxidante apresentou CI50 = 217,4 mg L-1, e conteúdo de fenólico totais = 7,26 mg EAG 100 g-1 em masa seca. Conclusão: Neste estudo preliminar, foi possível observar que o extrato floral de C. pubiflora, apresenta potencial para novos estudos biológicos e químicos, devido ao grande número de classes fitoquímicas observadas, bem como, das atividades biológicas avaliadas, sugerindo assim, que futuras pesquisas sejam realizadas para que possivelmente sejam desenvolvidos novos fitofármacos a partir do extrato floral.

INTRODUCTION

Cerrado domain is a Neotropical savannah, being this strategic environment with rich variability in species of Brazilian and world flora, in addition, it is considered the second-largest in a natural area, occupying an area of about 2 million km^2, or 22% of the National territory (BUENO et al., 2018). In this environment, great diversity is found in plant families such as Malvaceae, subfamily Bombacoideae, and Bombaceae tribe, which includes the *Ceiba* Miller genus, with about 17 species of Neotropical distribution. In Brazil, 11 species of *Ceiba* are registered, *Ceiba pubiflora* being a species native to the Midwest and Northeast and Southeast (GIBBS; SEMIR, 2003; SILVA et al., 2016).

C. pubiflora occurs mainly in semideciduous forests, and particularly in calcareous soils, and is popularly known as "potbellied or white paineira", with a height between 15-25 m with the canopy; gray stem with conical spines; compound leaves; fruits in capsule, oblong, woody, and brown seeds with ovoid shape. In addition to the Cerrado *C. pubiflora* is inserted in the flora of the Atlantic Forest and Caatinga biomes. In the Midwest region of Brazil, the period of flowering and fruiting is associated with farmers during the rainy and dry seasons. The flowers of *C. pubiflora* are large, pink in color, and have a light aroma which is why it is used in ornamentation and landscape projects in cities (PREVIATTO; DAINEZI; POSSO, 2016; SILVA, 2018; PEZZINI et al., 2021).

Numerous extracts produced from the species of the Malvaceae family present phytocomposites of great phytotherapic interest for the food industries during conservation, in the pharmaceutical industry in the production of topical and internal medicines, and in the agricultural industries such as larvicide, molluscicide and insecticide, and biotechnology mainly in the control of microorganisms (SILVA et al., 2016; SILVA et al., 2017; MEHMOOD et al., 2018; PORTELLES et al., 2019).

Genus *Ceiba* is described for various therapeutic, nutritional, and economic purposes in floristic inventories and ethnobotanical studies based on the gum extracted from the stem bark and roots with aphrodisiac properties (Silva, 2018; MEHMOOD et al., 2018). However, about the phytochemical composition of the floral organ, little is known about the classes of phytocomposites and their possible phytotherapic effects, requiring previous studies on phytochemistry that can be obtained from secondary metabolism.

Considering the scarcity of studies of the *Ceiba* genus, this work aimed to evaluate the hydroethanolic floral extract of *Ceiba pubiflora* on phytochemical, physicochemical, and biological activities.

MATERIALS AND METHODS

Plant material

In natura flowers were collected at the Goiano Federal Institute, Campus Rio Verde, Goias, Brazil (17°48′ 39.4″ S and 50°53′ 57.5″ W), at 8 pm on 25th March 2020. The plant material was identified, and samples were deposited as voucher specimens in the Herbarium at the Goiano Federal Institute, Goias, Brazil (identification number HRV: 12637).

Preparation of hidroethanolic extract

Extraction was carried out with 500 g flowers and 100 mL 70% (*w*/*w*) hydroethanolic solution; it was kept under constant magnetic agitation (Solab, Mod. SL-91/4) for 12 hours. n the dark, contact between solvent and raw material was kept for nine days at room temperature (25 °C). After that, it was manually agitated daily. The mixture that resulted from the extraction was separated by filtration (Unifil, C42), followed by solvent evaporation carried out by a rotary evaporator (Fisaton, Mod. 0641) at reduced pressure. Then, the extract was lyophilized (Liotop, Mod. L101) with negative pressure until constant mass.

Phytochemical assay

The concentrated hydroethanolic floral extract was subjected to a qualitative test for the identification of various phytochemical constituents as per standard procedures for cardiac glycosides, cyanogenic glycosides, alkaloids, organic acids, reducing sugars & non-reducing sugars, coumarins, foamy saponins & hemolytic saponins, polysaccharides, phenols, tannins, flavonoids, purines, resins, catechins, auronas & chalcones, depsides & depsidones, cyanogenic heterosides, benzoquinones, naphthoquinones & phenanthraquinones, anthraquinones, steroids & triterpenoids, sesquiterpenolactones, proteins & amino acids, leucoanthocyanidins, anthocyanins, azulenos, and oxidation time (BARBOSA et al., 2004; BRAGA et al., 2019; HERNÁNDEZ et al., 2019).

These tests were based on the visual observation of color modification or precipitate formation after the addition of specific reagents. The results were expressed by the cross-testing (-) negative, (+) weak positive, (++) moderate positive and (+++) strong positive (Marinho et al., 2021).

Physicochemical analysis

The yield of the dry extract was determined by

calculating the ratio of its weight on a digital analytical scale (Marte, Mod. W220) with respect was to the weight of the plant drug, expressed as a percentage according to equation 1 (ALVES et al., 2011).

$$Yield\% = [(g\ DE * 100)/gVD] \text{ Ec. } [1]$$

Where: *DE* = dry extraction, and *VD* = Vegetable drug.

The pH was performed in a digital pHmeter (Lucadena, Mod. 210-P), the total solids content in an oven at 105 °C (Nova Ética, Mod. 400-3ND), and the refractive index in a refractometer digital (Hanna Instruments, Mod. HI96800) with a refraction range between 1.3330 to 1.5080 n_D. The relative density was determined in a 10 mL graduated cylinder (Pyrex) and expressed in g mL^{-1} at 20 °C (VALDÉS et al., 2015; DOMÍNGUEZ et al., 2019).

Chromatography determination was performed by thin-layer chromatography (TLC) for the extract using chromatoplates (DC-Fertigfolien Alugram® Xtra SIL G/UV_{254}) (Macherey-Nagel). Approximately 10 µL of the extract was deposited on the chromatoplates cut into strips of (2 x 10 cm^2 in length). The eluents used were (ethyl alcohol, chloroform, ethyl acetate, and acetone), and the following developers used (sulfuric vanillin, ultraviolet light, long and short wavelengths, and ferric chloride) were used. The retention factor (R*fs*) was determined according to the points observed for each developer (ALVES et al., 2011). The hydroethanolic floral extract was scanned between 250 nm and 900 nm in a UV-*Vis* spectrophotometer (Belphotonics, Mod. M-51), the data were generated by UV-*Vis* professional two software processed in Origin (version 2018).

Antioxidant activity and total phenolic compounds

The antioxidant capacity was determined using the DPPH (2,2-diphenyl-1-picrilhydrazyl) reduction method. The DPPH sequestration method was performed in microdilution in 96-well microplates (Global Plast) modified (MEZZA et al., 2018). For each well, 100 µL of an ethanolic solution with DPPH at the concentration of 0.06 mMol L^{-1} and 100 µL of a hydroethanolic extract solution at different concentrations. The microplate was kept in a place protected from light and heat for 1 hour. Then, the microplate spectrophotometer (Scientific Hexis, SpectraMax Plus, Mod. 384) was read at a wavelength of 517 nm. The percentage of antioxidant capacity was determined according to equation 2. The inhibition concentration (IC_{50}) was determined in (mg L^{-1}) in the sequestration of 50% of the initial concentration of the standard solution of DPPH 0.06 mMol L^{-1}.

$$\%Red=\left(\frac{(CSabs - ESabs)}{CSabs}\right)*100 \text{ Eq. } [2]$$

Where: *CSabs* = absorption of the control solution, and *ESabs* = absorption of the extract solution containing the radical DPPH.

The total phenolic content was assessed with the Folin-Ciocalteau assay described by Prakash and Vedanayaki (2019). Briefly, 1 mL of floral extract was mixed with 5 mL of distilled water, and then 1 mL of 1 Mol Folin-Ciolateaus reagent was added. The solution was homogenized for 5 min and was allowed to React for another 5 min period. Then, 2.5 mL of conc. 7.5% Sodium Carbonate (Na_2CO_3) solution was added. After incubation at room temperature for 60 min, the absorbance of each solution was measured at 760 nm in a UV-*Vis* spectrometer (Belphotonics, Mod. M-51). The same procedure was also used for the standard solution curve was obtained 1000-5 ppm), and a standard curve was obtained. The total phenolic contents were expressed in mg of Gallic Acid equivalent 100 g^{-1} dry mass floral extract (mg GAE 100 g^{-1}).

RESULTS

C. pubiflora floral period in the collection area in the municipality of Rio Verde, Goias, Brazil, in 2020 was between March to May, with a high intensity of flowering in May. Figure 1 shows a flower of *C. pubiflora* used in the production of hydroethanolic floral extract of this study.

Figure 1. *Ceiba pubiflora* flower used in the hydroethanolic floral extract, collected in Rio Verde, Goias, Brazil in 2020. Bar: 10 cm.

Source: Authors, 2021.

The hydroethanolic floral extract of *C. pubiflora* was presented transparency, crystallinity, pink color, and slight aroma. The percentage of dry extract yield equal to 6.88% (*v*/*v*). Phytochemical prospection of *C. pubiflora* hydroethanolic floral extract indicated the presence of different secondary metabolites classes, except for foamy

saponins, polysaccharides, resins, benzoquinones, anthraquinones, proteins, and amino acids. Table 1 shows the results of the phytochemical screening carried out on the hydroethanolic floral extract.

Table 1. Phytochemical prospecting of the hydroethanolic floral extract of *Ceiba pubiflora*.

Phytochemical prospecting	Results
Cardiac glycosides	+++
Cyanogenic glycosides	++
Alkaloids	+++
Organic acids	+++
Reducing sugars	+++
Non-reducing sugars	++
Coumarins	+
Foamy saponins	-
Hemolytic saponins	+++
Polysaccharides	-
Phenols	+++
Tannins	Gr
Flavonoids	+++
Purines	+
Resins	-
Catechins	++
Auronas & Chalcones	++
Depsides y depsidones	+++
Cyanogenic heterosides	+++
Benzoquinones, naphthoquinones & phenanthraquinones	-
Anthraquinones	-
Steroids & triterpenoids	++
Sesquiterpenolactones	+
Proteins & amino acids	-
Leucoanthocyanidins	+++
Anthocyanins	+++
Azulenos	++
Oxidation time	5 sec.

(-) negative. (+) weak positive. (++) moderate positive. (+++) strong positive. Tannins: Green (Gr) condensed or catechic. Blue (Bl) hydrolyzable or gallic. Source: Authors, 2021.

Qualitative hemolytic assay on human erythrocytes demonstrated potential cytotoxicity with a minimum of 5 min. and complete hemolysis with 10 minutes. After five minutes, deformation of the human erythrocytes can be observed, followed by rupture and worsening of hemoglobin (Fig. 2). Figure 2 shows the hemolysis assay on human erythrocytes from the hydroethanolic floral extract of *C. pubiflora*.

The pH presented a result of 6.41 ± 0.02, total solids of 1.12 ± 0.09, refractive index of n_D 1.5369 ± 0.13, and relative density of 0.9061 ± 0.01 g mL^{-1} to 20 °C. Acetone and ethyl acetate were the eluents with the highest number of R*fs* (12), respectively. The presence of sugars reducing, glucose and fructose, and no-reducing sucrose was observed by thin-layer chromatography analysis in the hydroethanolic floral extract of *C. pubiflora*. Table 2 showed summarized the results of the thin-layer chromatography of the hydroethanolic floral extract of *C. pubiflora* in different eluents.

The UV-*Vis* spectrophotometric analysis, between 850-250 nm short and intense bands were observed, suggesting the presence of phenolic and flavonoid compounds that absorb in UV energy sources between 200-300 nm and between 400-500 nm, respectively. Figure 3 shows the UV-*Vis* spectrophotometry scan of the hydroethanolic floral of *C. pubiflora* between 850-250 nm.

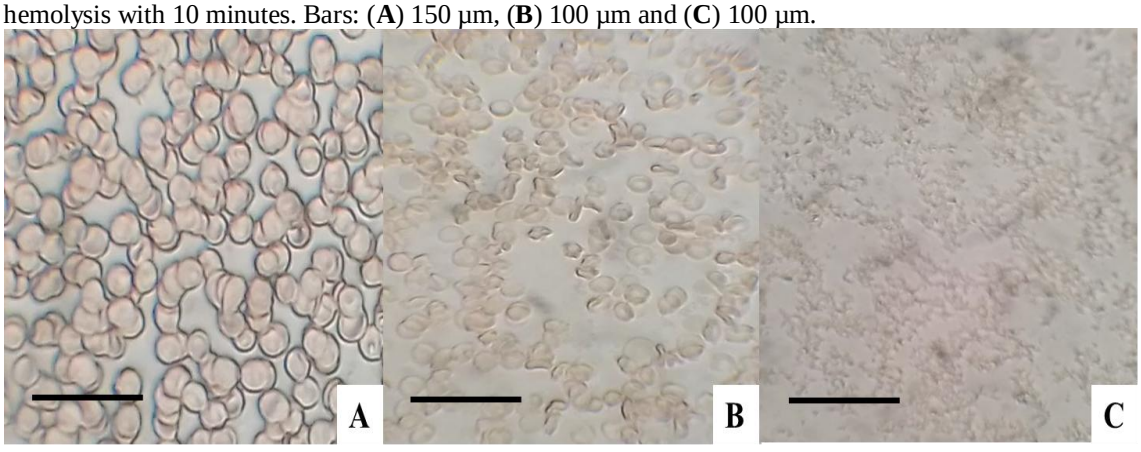
The antioxidant activity of the hydroethanolic floral extract of *C. pubiflora* presented inhibition concentration (IC_{50}) = 217.4 ± 0.19 mg L^{-1} and total phenols content of 7.26 ± 0.16 mg AGE 100 g^{-1} in dry extract.

DISCUSSION

There is a minimal number of phytochemical studies with the *Ceiba* genus. There are no studies with *C. pubiflora* on the floral organ, which in this study exhibited a high qualitative content of phytochemical groups, this species promising for several biological studies. Despite the scarce phytochemical reports and biological activities, this study tried to reconcile *Ceiba* species, thus raising a discussion about the lack of preliminary studies.

The genus *Ceiba* presents in the literature its primary focus some phytochemical studies focused on the stem and leaf organ (MUÑOZ-CÁZARES et al., 2018; SANTOS et al.,

Figure 2. Hemolysis assay in 5% blood cells concentrate. In (**A**) normocytic blood cells, in (**B**) hemolysis with 5 minutes, and (**C**) hemolysis with 10 minutes. Bars: (**A**) 150 µm, (**B**) 100 µm and (**C**) 100 µm.



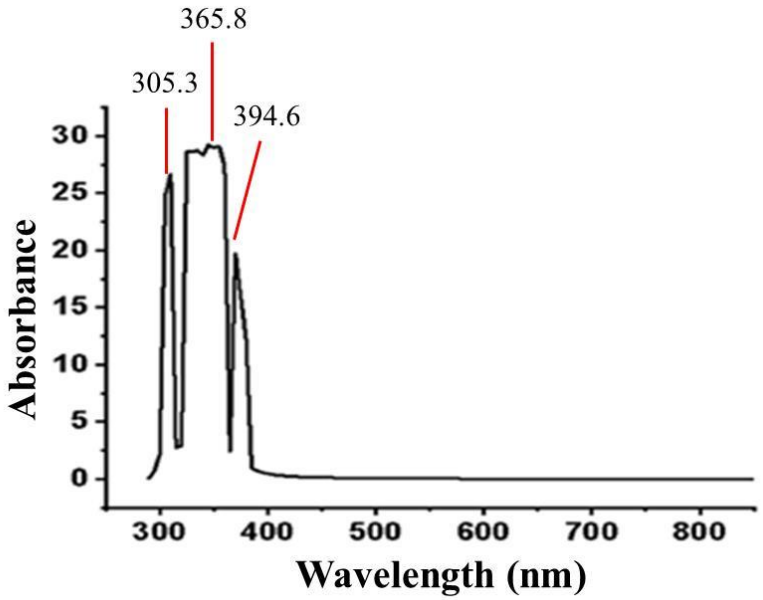
Source: authors, 2021.

Table 2. Results of thin-layer chromatography of the hydroethanolic floral extract of *Ceiba pubiflora*.

Eluents	UV 254mm	UV 365mm	Sulfuric vanillin	$FeCl_3$	I_2	$KMnO_4$	$C_{21}H_{14}Br_4O_5S$	H_2CrO_4
C_3H_6O	0.69	0.45 0.30	0.75 0.26	0.33 0.08	0.35	0.11	0.51 0.42	0.23
$CHCl_3$	-	0.63	0.65	0.57 0.45 0.15	0.45	0.54 0.25	0.75	0.75
$C_4H_8O_2$	0.09	0.19 0.15	0.70 0.62	0.65	0.71	0.65	0.72	0.75 0.69 0.05
C_2H_5OH	-	0.65	0.75	0.12	0.75 0.15	0.55 0.35 0.26	0.35	0.05

Results in R*fs* (Retention factor), determined in millimeters (mm). Source: Authors, 2021.

Figure 3. UV-*Vis* spectrophotometry of the hydroethanolic floral extract of *Ceiba pubiflora* between 850-250 nm.



Source: Authors, 2021.

2019). According to a study by Muñoz-Cázares et al. (2018), the research evaluated the stem bark of *Ceiba pentandra* and *C. aesculifolia* in different solvents (hexane, dichloromethane, and methanol). It exhibited in this study the exclusive presence of terpene-type secondary metabolites, steroids and flavonoids, and the absence of tannins and phenols.

Several phytochemical studies have demonstrated the presence of phytomolecules isolated from the anthocyanin classes expressed in cyanidin-3-glucoside for *Ceiba acuminata*, *Ceiba speciosa*, from Cyanidin-3,5-diglucoside for *C. speciosa*, flavonoids apigenin-7-*O*-β- d-rutinoside in *Ceiba insignis*, catechin in *Ceiba pentandra*, 5-hydroxy-7,4',5'-trimethoxy isoflavone-3'-*O*-α-l-arabinofuranosyl(1-6)-β-d-glucopyranoside, kaempferol in *C. pentandra* (DAS et al., 2021).

The floral extract yield was high, although it is not possible to compare it with other floral extracts of the *Ceiba* genus due to lack of study. Nevertheless, through phytochemical prospecting of the hydroethanolic floral extract of *C. pubiflora*, it was possible to determine the presence of diverse classes of secondary metabolites that show a wide variety of biological activities such as antimicrobial, antioxidant, antitumor, antiophidic, photoprotection, and photosensitizing (OKUDA; YOSHIBA; HATANO, 1989; ESQUENAZI et al., 2002; BARREIROS; DAVID, 2006; MATIAS et al., 2010).

Among some of these secondary metabolites, we can mention. In the tannins, the antimicrobial properties appear to be associated with the hydrolysis of an ester bond with

gallic acid, thereby serving as a natural defense mechanism against microbial infections. Flavonoids are synthesized by plants in response to microbial infection and are effective against a broad range of microorganisms. Flavonoids are the largest groups of phenolic compounds, including antioxidant, antiparasitic, antibacterial, and free radical elimination properties (MATIAS et al., 2011; SILVA et al., 2019; BATIHA et al., 2020).

Saponins are a group of compounds associated with toxicity capable of causing damage to the cell membranes of erythrocytes. This effect results from the ability to interact between the components of the cell membrane and the sterol molecules such as cholesterol, inducing deformation in the membrane and consequently the leakage of the hemoglobin content (CRUZ et al., 2007; NUNES, 2015). Depsides and depsidones are an interesting group of secondary metabolites. The basis of their structure is two or more molecules of hydroxybenzoic acids linked by an ester linkage (STUDZIŃSKA-SROKA; DUBINO, 2018). The depsides have anti-inflammatory properties, in the study of Bugni and others (2009), the researchers described the effect of a cyclooxygenase inhibitor. In addition, tannins were studied and reported to draw tissues together, thus restricting blood flow, which helps to heal wounds, and anti-inflammatory activity (KUMARI et al., 2017).

Although some reagents may reveal more than one phytoclass, this is only a preliminary result, requiring further tests using quantitative results that can be obtained by high-performance liquid chromatography and compared with classical standards. The expressive content of phenols in the floral extract of *C. pubiflora* has important reported biological actions in several other studies. For example, according to Oliveira and others (2016), Haslam (1996), and Dixon and others (1983), phenolic compounds have an action to inhibits lipid peroxidation and lipoxygenase *in vitro*.

The oxidation time was slightly fast, suggesting that it is an excellent antioxidant extract, where it also showed good efficiency in reducing free radicals from DPPH. A large amount of R*fs* is observed in the chromatographic assay for the four eluents evaluated and their respective developers. Only polar eluents were used, observing that the compounds of the flower extract showed to be less polar and were more easily eluted. The elotropic sequence of the eluents was: $CHCl_3$ 10 R*fs*> $C_4H_8O_2$ 12 R*fs*> C_3H_6O 12 R*fs*> C_2H_5OH 10 R*fs*, he R*fs* showed high values in mm, which confirms that they have a greater amount of apolar compounds in the extract. Especially acetone and ethyl acetate proved to be the best eluents.

The developers UV_{254} nm light shows phytochemicals that absorb light, often they are conjugated substances and aromatic systems, and for UV_{365} nm light, the compounds naturally fluoresce; sulfuric vanillin is especially sensitive to the presence of alcohols and terpenoids; the ferric chloride complex with phenols and enolizable compounds; complex I2 with structures of amino acids, indoles, alkaloids, steroids, purines, and lipids; potassium permanganate easily stains oxidizable compounds, olefins, alkynes and aromatics; bromocresol green shows organic acid compounds; and chromic acid difficult substances (SHERMA; FRIED, 2005; RODRIGUES et al., 2009).

The results obtained for both qualitative tests and TLC showed that both are suitable for a preliminary analysis of phytochemicals, presenting speed, economy, safety, and easy handling (RODRIGUES et al., 2009). The presence of reducing sugars was more intense both in the qualitative test (Tab. 1) and in the TLC, with two reducing sugars (glucose and fructose) and one non-reducing sugar (sucrose).

CONCLUSIONS

The results allow us to conclude that the data obtained in the present study are pioneering and promising and may encourage future studies with *C pubiflora* on the aspects of phytochemical, toxicological, and chemical screening of the floral extract. In addition, further studies should be carried out to investigate possible biological activities such as antifungal, antibacterial, and anti-inflammatory from the floral extract of *C. pubiflora*.

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