Phytochemical and physicochemical evaluation, and photoprotection, antioxidant, antifungal, and antibacterial activities of the floral extract of *Schubertia grandiflora* Mart. & Zucc. (Apocynaceae)

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Abstract

Schubertia grandiflora is a species belonging to the floral of the Cerrado domain, presented annually with aromatic flowers. The study aimed to evaluate the hydroethanolic floral extract of *S. grandiflora* for phytochemistry, physicochemical and photoprotection, antioxidant, antifungal and antibacterial activities. Fresh flowers were collected and the floral extract produced by maceration. Qualitative phytochemical tests were carried out for the main phytochemical classes, organoleptic physico-chemical parameters, extract mass, moisture content, pH, relative density, refractive index, phenolic and total flavonoid contents, color density, *Hue* tint, and tests biological factors for protection factor, DPPH free radical reduction activities, antifungal and antibacterial. Phytochemistry has demonstrated the presence of several groups of important metabolites mainly in pharmaceutical use, visual color of the citrus yellow floral extract, aromatic and homogeneous, extract mass = 12.57%, moisture content = 6.21%, pH = 5.85, refractive index = 1.4217 n_D, relative density 0.9044 g mL^{-1} 20 °C, total phenolics = 238.83 mg GAE 100 g^{-1} dried extract, total flavonoids = 17.93 mg QC 100 g^{-1} dried extract, color density = 1.05, *Hue* color = 8.24, protection factor UVA and UVC, antioxidant activity with CI_{50} = 9.44 µg mL^{-1}. Antifungal activity demonstrated inhibition zone only for *Candida tropicalis* between 10-6 mm and for *Candida krusei* between 18-5 mm, and antibacterial for *Escherichia coli* between 13-7 mm, *Pseudomonas aeruginosa* between 21-5 mm and *Enterococcus faecalis* between 32-17 mm. The hydroethanolic floral extract of *Schubertia grandiflora* demonstrated potential biological activities, characterizing this natural product for the development of pharmaceutical, biological and agricultural formulations.

Keywords: Phytomolecules; *Candida* genus; *Staphylococcus aureus*; *Schubertia* genus

Resumo

Schubertia grandiflora é uma espécie pertencente ao domínio floral do Cerrado, apresentada anualmente com flores aromáticas. O estudo teve como objetivo avaliar o extrato floral hidroetanólico de *S. grandiflora* quanto às atividades fitoquímica, físico-química e fotoproteção, antioxidante, antifúngica e antibacteriana. Flores frescas foram colhidas e o extrato floral produzido por maceração. Foram realizados testes fitoquímicos qualitativos para as principais classes fitoquímicas, parâmetros físico-químicos organolépticos, massa do extrato, teor de umidade, pH, densidade relativa, índice de refração, teor de flavonóides fenólicos e totais, densidade de cor, tonalidade e testes de fatores biológicos de proteção fator, atividades de redução do radical livre DPPH, antifúngica e antibacteriana. A fitoquímica tem demonstrado a presença de vários grupos de metabólitos importantes principalmente em uso farmacêutico, cor visual do extrato floral amarelo cítrico, aromático e homogêneo, massa do extrato = 12,57%, teor de umidade = 6,21%, pH = 5,85, índice de refração = 1,4217 nD , densidade relativa 0,9044 g mL^{-1} 20 ° C, fenólicos totais = 238,83 mg GAE 100 g^{-1} extrato seco, flavonóides totais = 17,93 mg QC 100 g^{-1} extrato seco, densidade de cor = 1,05, cor de matiz = 8,24, fator de proteção UVA e UVC, atividade antioxidante com CI50 = 9,44 µg mL^{-1}. A atividade antifúngica demonstrou zona de inibição apenas para *Candida tropicalis* entre 10-6 mm e para Candida krusei entre 18-5 mm, e antibacteriana para *Escherichia coli* entre 13-7 mm, *Pseudomonas aeruginosa* entre 21-5 mm e *Enterococcus faecalis* entre 32-17 mm. O extrato floral hidroetanólico de *Schubertia grandiflora* demonstrou potencial atividade biológica, caracterizando este

produto natural para o desenvolvimento de formulações farmacêuticas, biológicas e agrícolas.

Palavras-chave: Fitomoléculas; Gênero *Candida*; *Staphylococcus aureus*; Gênero *Schubertia*

Resumen

Schubertia grandiflora es una especie perteneciente al dominio floral del Cerrado, presentada anualmente con flores aromáticas. El estudio tuvo como objetivo evaluar el extracto floral hidroetanólico de *S. grandiflora* para actividades fitoquímicas, fisicoquímicas y fotoprotectoras, antioxidantes, antifúngicas y antibacterianas. Se recolectaron flores frescas y el extracto floral se produjo por maceración. Se realizaron pruebas fitoquímicas cualitativas para las principales clases de fitoquímicos, parámetros físico-químicos organolépticos, masa de extracto, contenido de humedad, pH, densidad relativa, índice de refracción, contenido de fenólicos y flavonoides totales, densidad de color, tinte Hue, y pruebas de factores biológicos de protección. factor, DPPH actividades de reducción de radicales libres, antifúngico y antibacteriano. La fitoquímica ha demostrado la presencia de varios grupos de metabolitos importantes principalmente en uso farmacéutico, color visual del extracto floral amarillo cítrico, aromático y homogéneo, masa del extracto = 12,57%, contenido de humedad = 6,21%, pH = 5,85, índice de refracción = 1,4217 nD , densidad relativa 0.9044 g mL-1 20 ° C, fenólicos totales = 238.83 mg GAE 100 g^{-1} extracto seco, flavonoides totales = 17.93 mg QC 100 g^{-1} extracto seco, densidad de color = 1.05, color de tono = 8.24, factor de protección UVA y UVC, actividad antioxidante con CI50 = 9,44 µg mL^{-1}. La actividad antifúngica demostró zona de inhibición solo para *Candida tropicalis* entre 10-6 mm y para *Candida krusei* entre 18-5 mm, y antibacteriana para *Escherichia coli* entre 13-7 mm, *Pseudomonas aeruginosa* entre 21-5 mm y *Enterococcus faecalis* entre 32-17 mm. El extracto floral hidroetanólico de Schubertia grandiflora demostró actividades biológicas potenciales, caracterizando este producto natural para el desarrollo de formulaciones farmacéuticas, biológicas y agrícolas.

Palabras clave: Fitomoléculas; Género *Candida*; *Staphylococcus aureus*; Género *Schubertia*

1. Introduction

The Apocynaceae family, has around 5,100 plant species included in the order *Gentiales* and subclass *Asteridae*. Apocynaceae is one of the largest botanical families with 550 genera, being found with greater diversity in the tropics and subtropics. Consists of tropical trees, shrubs, herbs, stem succulents and vines (Rapini, 2012; Ekalu et al., 2019). In Brazil, more than 400 species distributed in 41 genera are described, where several of these taxa have latex; leaves are simple, opposite or whorled; flowers are large, colourful and slightly fragrant with five contorted lobes; and fruits are in pairs (Pereira et al., 2007; Wong et al., 2013).

The Apocynaceae family is chemically characterized by the frequent occurrence of alkaloids structures. The genus *Schubertia*, is inserted in *Asclepiadeae*, subtribe *Gonolobinae* (Endres et al., 2014), with *Schubertia grandiflora* (Fig. 1) being one of the representative species of this genus.

S. grandiflora popularly known as “maria-da-costa ou cipó-de-leite”, has a herbaceous habit, perennial climbing between 1-3 m height; blooms in the rainy season; with geographic distribution in Brazil, being found in the states of Goiás, Minas Gerais, Distrito Federal and Piauí in areas of the Cerrado domain, in the Paraná Atlantic Forest biome, and in the state of Mato Grosso in the Pantanal biome. The species is not restricted only to Brazil, but is also described in Argentina, Paraguay and Bolivia (Descole & Meyer, 1941; Schessl et al., 1999; Lombardi et al., 2005; Santos-Filho et al., 2015). The fruit, na ovoid follicle with a spiny protuberance, bears fruit at the end of the dry season. The species has ornamental use, and also in folk medicine, the tuber of *S. grandiflora* is used in the form of decoction as na emenagogue and abortifacient (Agra et al., 2007).

Although there are numerous phytochemical studies with Apocynaceae, still, little is known about the genus *Schubertia*, and studies with *S. grandiflora* are scarce as previously noted. The vegetables of this family present a rich chemical constitution from the secondary metabolism in the various organs of the vegetable such as cardenolides, sugars, sterols, lignans, triterpenoids, flavonoids, steroids, glycosides, phenols, lactones and alkaloids (Wen et al., 2016). These phytochemical classes in Apocynaceae are involved in numerous recognized biological activities, with antitumor, antimalarial, anti-inflammatory, antioxidant, larvicidal and cytotoxic action (Joselin & Brintha, 2012; Bhadane et al., 2018; Ekalu et al., 2019).

The study aimed to evaluate the hydroethanolic floral extract of *Schubertia grandiflora* regarding phytochemical, physicochemical parameters and about antioxidant, antifungal and antibacterial activities.

Figure 1. Individual of *Schubertia grandiflora* with in florescence. Source: Authors, 2021.

2. Materials and Methods

Plant material

The flowers of *S. grandiflora* were collected from the city of Rio Verde, Goiás, Brazil, at 6-8 pm on 3st March 2021. Plant identification material was identified by Dra. Isa Lucia de Morais and, samples were deposited as voucher specimens in the Herbarium at the Goiano Federal Institute, Rio Verde Campus, Goiás, Brazil (identification number 18067).

Preparation of plant extract

About 150 g of powdered material was taken in a clean, flat bottomed amber glass container and soaked in 200 mL of 70% hidroethanolic (*v/v*). The container with its contents was kept for a period of 10 days, accompanying ocasional skaking and stirring. The extract is was filtrate through Whatman filter paper no. 1 (Unifil, C42). The hidroethanolic extract was concentrated on a rotary evaporator with reduced pressure. After reduction, the extract was transferred to lyophilizer until constant mass. The extract was kept refrigerated at -12 °C until analysis.

Phytochemical analysis

The phytochemical assays to detect the presence of foamy saponins, hemolytic saponins, reducing sugars, no-reducing sugars, tannins, cyanogenic heterosides, polysaccharides, proteins and amino acids, benzoquinones derivatives and naphthoquinones and phenanthraquinones, sesquiterpenolactones, flavonoids, steroids and triterpenoids, cumarines, organic acids, alkaloids, phenols, resins, azulenos, depsides and depsidones, anthraquinones, purines, and cardiac glycosides were performed following the method described by Barbosa et al. (2004). These tests were based on the visual observation of color modification or precipitate formation after the addition of specific reagents. The presence of these compounds, was evaluated qualitatively through the non-parametric system of crosses described by Camacho-Campos et al. (2020). Presence (+++) abundant. (++) moderate. (+) low. (-) absent.

Physicochemical parameters

The organoleptic characteristics were determined for smell, visual and homogeneity of the solution extract. The mass of the extract after lyophilization was determined on digital analytical balance and the result expressed as a percentage according to equation [1].

$$\text{Yield\%} = (\text{g dry extract/g vegetable drug})*100 \quad \text{Eq. [1]}$$

The moisture content (%) was determined gravimetrically as described by Menezes Filho et al. (2020). Through the difference in mass in na oven at 105 °C until constant mass. pH of the floral extract was determined using a pH meter at room temperature. The relative density of the floral extract was determined in a 10 mL volumetric flask. The result was expressed in g mL^{-1} at 20 °C. The refractive index was obtained using a digital refractometer, with a working range between 1.3330 to 1.5080 n_D (Menezes Filho et al., 2020).

The total phenolic content in flower extract was determined, by the Folin-Ciocalteu method by Nagadesi and Kannamba (2019), modified. The extract 50 mg was mixed with Folin-Ciocalteu reagent 0.5 mL and distilled water 7.5 mL. The solution was hept at room temperature for 10 minutes and then 10 mL of 7,5% sodium carbonate aqueous solution was added to the mixture and then incubated for 60 minutes at room temperature. The absorbance against the reagent blank was determined at Abs = 760 nm. The total phenolic compounds was calculated from calibration curve of gallic acid (R^2 = 0.9998) and expressed as mg of gallic acid (mg GAE 100 g^{-1}) dry extract. The sample was analyzed in triplicate.

The total flavonoids content in flower extract was determined aluminum chloride colorimetric method described by Chang et al. (2002) modified. 1 mL (150 µg mL^{-1}) of the floral extract, was diluted with distilled water 4 mL in a 10 mL volumetric flask. Initially, 5% sodium nitrite aqueous solution 300 µL was added to each volumetric flask. After, 5 minutes, 10% aluminum chloride aqueous solution 300 µL was added and 2.5 mL distilled water then 3 added to the reaction flask and solution well. The absorbance was read at Abs = 415 nm for Quercetin. The total flavonoids content was calculated from calibration curve Quercetin (QC) (R^2 = 0.9999). The amount of 5% aluminum chloride solution was substitute by the same amount of distilled water in blank. The total flavonoids content of the floral extract was expressed as (mg QC 100 g^{-1}) dry extract. The sample was analyzed in triplicate.

Color density and hue tint were measured by diluting 1 mL of the sample in 3 mL of a buffer solution at pH 3. The samples (200 µL) were placed in a UV-*Vis* spectrophotometer and absorbance at 420, 520 and 700 nm was measured. The measurements were done in triplicate for each sample. Color density and hue tint were calculated as described by Jung et al. (2013), equation [2, 3]. The analysis was performed in triplicate.

$$\text{Color density} = [(Abs_{420}\ nm - Abs_{700}\ nm) + (Abs_{520}\ nm - Abs_{700}\ nm)] \quad \text{Eq. [2]}$$

$$\text{Hue tint} = (Abs_{420}\ nm - Abs_{700}\ nm) / (Abs_{520}\ nm - Abs_{700}\ nm) \quad \text{Eq. [3]}$$

Photoprotection assay

The photoprotection effect was determined using the UV-*Vis* spectrophotometric method (Menezes Filho; Santos; Castro, 2020). The crude extract was added in a single-field quartz cuvette (1 cm) with na optical path, and the photoprotection determination carried out by scanning at critical wavelengths between 200-400 nm.

Antioxidant activity

The DPPH colorimetric method described by Ávila-Reyes et al. (2018) was used to evaluate the free radical scavenging activity. A standard curve of DPPH* (Abs_{523}nm and correlation coefficient R^2 = 0.9999) was used to estimate the 2,2-diphenyl-1-picrylhydrazyl (DPPH*) concentration (µg mL^{-1}) in the reaction medium. Antiradical activities were expressed in terms of inhibition concentration (IC_{50}) expressed in µg mL^{-1}. Ascorbic acid was used as reference samples and assayed in the same manner. The sample was analyzed in triplicate.

Antifungal assay

Antifungal test of flower extract from *S. grandiflora* was tested against *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 4563), *Candida guilliermondii* (ATCC 90877), and *Candida krusei* (ATCC 34135) by using the agar diffusion method (disc-diffusion). Antifungal assay was performed as described by Cavalcanti et al. (2011) modified. The strains were resuspended in 25 mL of sterile Sabouraud Dextrose Broth (SDB). The suspension was shaken on a shaking table with incubation for 24 h at 36 °C. Starting from this culture inoculum containing approximately (1 x 10^6 CFU mL^{-1}) were prepared according to the turbidity in a tube of 0.5 on the McFarland scale, in a UV-*Vis* spectrophotometer. The antifungal test, was carried out in a solid medium (SDB) using sterile filter paper discs with a diameter of 7 mm.

Four paper disks containing 50 µL in different concentrations of floral extract (500; 250; 100; 50 and 25 mg mL^{-1}), diluted in 70% hydroethanol solution, were added to each *Petri* dish. As a negative control 70%

hydroethanolic solution was used, absence of interferents and, as a positive control, Ketoconazole (50 µg mL^{-1} disc). Zones of inhibition were examined after 24-46 h, measured and recorded as the diameter (mm) of complete growth-inhibition, obtained with the aid of a digital caliper. The minimum inhibition zone considered was 5 mm.

Antibacterial assay

Antimicrobial assay of flower extract from *S. grandiflora* was tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella sorovar* Thyphymurium (ATCC 14028), *Salmonella sorovar* Enteritidis (ATCC 13076), and *Enterococcus faecalis* (ATCC LB 29212) by using the agar diffusion method by Carvalho et al. (2020) modified. The bacterial lines were cultivated in Brain Heart Infusion Broth (BHI) and incubated at 36 °C for 36 h. After this period, they were replicated on *Petri* dishes containing 60 mL the Muller Hilton Agar (MHA) with turbidity cells equivalent to the 0.5 of the McFarland scale (1 x 10^6 CFU mL^{-1}) in a UV-*Vis* spectrophotometer. Then, the plates were incubated at 36 °C for 24 h for subsequent observation of the inhibition halos. The plates containing the bacterial inoculum were then perforated and the cavities (7 mm) were filled with 100 µL of the floral extract solutions at (500, 250, 100, 80 and 50%) concentrations.

As a negative control, the solvent hydroethanolic solution; and as a positive control, the antibacterial azithromycin (15 µg disc), Cephalexin (30 µg disc), Tigecycline (15 µg disc) and Amikacin (30 µg disc) was used. Analyzes were performed in quadruplicate. The halo of antibiosis was determined in (mm), using a digital caliper. The minimum inhibition zone considered was 5 mm.

Statistics analysis

All values are expressed as mean (±) S.E. Statistical significance of the difference was assessed by Tukey's test. *p* values lower than 0.05 were considered significant.

3. Results

Results of preliminary phytochemical analysis of the flower extract of *S. grandiflora* showed the presence of hemolytic saponins, reducing sugars, no-reducing sugars, tannins, flavonoids, coumarins, organic acids, alkaloids, phenols, depsides and depsidones, and purines (Tab. 1).

The floral extract of *S. grandiflora* showed a visual citrine yellow color, sweet aroma and homogeneous liquid without turbidity in the organoleptic analysis, as well as a considerable yield rate = 12%, low moisture content = 6%, slightly acidic pH corroborating the qualitative analysis for organic acids pH = 5 and refraction index = 1.4217 n_D. The relative density similar to countless other extracts of equal polarity = 0.90 g mL^{-1} 20 °C, expressive phenolic content = 238 mg GAE 100 g^{-1} and total flavonoids = 17 mg QC 100 g^{-1} dried base (Tab. 2). Both the color density = 1, and the Hue tint = 8, are tests considered new for the study of extracts, presenting brief tests in the literature, but it can be evaluated in this study, that the extract presents a strong tendency in yellow color. The color density value is considered high for the hydroethanolic extract, which is associated with a darker color (Tab. 2).

Table 1. Phytochemical prospecting of the hydroethanolic floral extract of *Schubertia grandiflora*.

Classes	Results
Foamy saponins	-
Hemolytic saponins	+
Reducing sugars	+
No-reducing sugars	++
Tannins	Gr
Cyanogenic heterosides	-
Polysaccharides	-
Proteins and amino acids	-
Benzoquinones derivatives and naphthoquinones and phenanthraquinones	-
Sesquiterpenolactones	-
Flavonoids	+++
Steroids and triterpenoids	-
Coumarins	+++
Organic acids	++
Alkaloids	+++
Phenols	+++
Resins	-
Azulenes	-
Depsides and depsidones	++
Anthraquinones	-
Purines	++
Cardiac glycosides	-

Presence (+++) abundant. (++) moderate. (+) low. (-) absent. (Gr) Green condensed or catechetical tannins. Source: Authors, 2021.

Table 2. Physicochemical properties of hydroethanolic floral extract of *Schubertia grandiflora*.

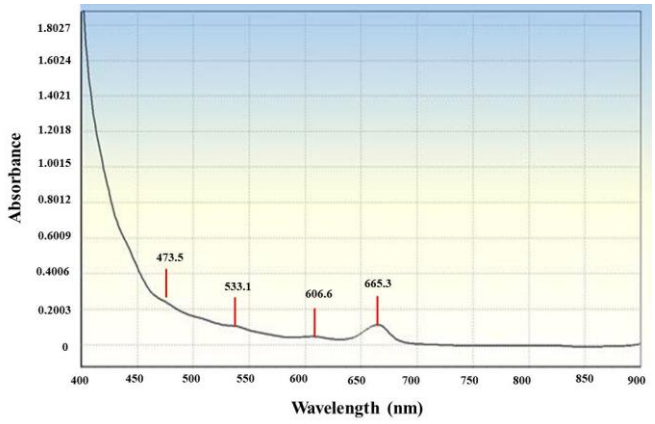
Physicochemical properties	Results*
Yield (%)	12.57 ± 0.16
Moisture content (%)	6.21 ± 0.08
pH	5.85 ± 0.07
Refraction index (n_D)	1.4217
Relative density (g mL^{-1} 20 °C)	0.9044 ± 0.00
Total phenolic content (mg GAE 100 g^{-1})	238.83 ± 0.22
Total flavonoids content (mg QC 100 g^{-1})	17.93 ± 0.11
Color density	1.05 ± 0.00
Hue tint	8.24 ± 0.01

*Values are mean (±) SD. Source: Author, 2021.

Bands between 400-450 nm and between 650-670 nm suggest the presence of chlorophyll (*a*). It suggests that in the spectrum between 473-560 nm there is the presence of flavonoids. These bands can be seen in Figures (2 and 3) separately. Corroborating thus with the result of the phytochemical prospecting (Tab. 1). The qualitative

spectrophotometric result corroborates with the quantitative result using a standard dosage curve of a pure substance belonging to the phytochemical class evaluated.

Figure 2. Scanning in UV-*Vis* spectrophotometer of the hydroethanolic floral extract of *Schubertia grandiflora*. Source: Authors, 2021.



The floral extract showed high absorption efficiency between the UVC (100 to 280 nm) and UVA (315 to 400 nm) wavelengths (Fig. 3) characterizing the floral extract of *S. grandiflora* as a possible new natural agent for the production of sunscreens in the natural product line.

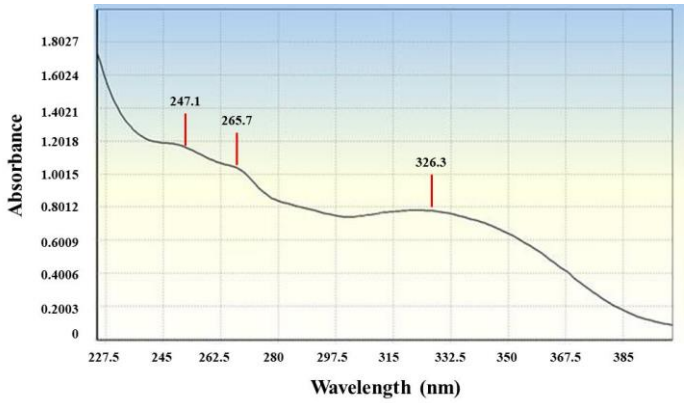


Figure 3. Critical wavelength scanning in a UV-*Vis* spectrophotometer of the hydroethanolic floral extract of *Schubertia grandiflora*. Source: Authors, 2021.

The antioxidant activity of the floral extract of *S. grandiflora* showed was inhibition concentration IC_{50} = 9.44 ± 0.13 µg mL^{-1}, and acid ascorbic IC_{50} = 7.17 ± 0.06 µg mL^{-1}.

In Table 3, it is possible as observed the floral hydroethanolic extract of *S. grandiflora* has good inhibition potential for *C. krusei* with a maximum inhibition of 18 mm, and with 10 mm for *C. tropicalis* both in the highest concentration of 500 mg mL^{-1}, presenting statistical difference by Tukey's test ($p < 0.05$). Even with antifungal activity, the extract showed less activity than the reference antifungal Ketoconazole 50 µg mL^{-1}. Strains of the *C. albicans* and *C. guilliermondii* proved to be resistant to the extract, corroborating what was previously discussed the resistance of this strain to reference antifungal agents.

Table 3. Antifungal activity of hydroethanolic floral extract of *Schubertia grandiflora*.

Microorganisms	Concentrations Inhibition zone (nm)					Reference antifungal (mm)
	500	**250**	**100**	**50**	**25**	**Ke**
C. tropicalis	10b	6b	NI	NI	NI	28a
C. albicans	NI	NI	NI	NI	NI	27
C. guilliermondii	NI	NI	NI	IN	NI	30
C. krusei	18b	15b	11c	5d	NI	33a

*Inhibition zone, diameter measured in (mm), disc diameter 5 mm, average of triplicate assay. Ke: Ketoconazole (50 µg mL^{-1} disc). NI: No inhibition. The disc papers were impregnated with different concentrations of the hydroethanolic floral extract.

The bacterial assay using different concentrations of the floral extract of *S. grandiflora*, potential inhibitory activity was observed only for *E. coli* with inhibition halos between 13-7 mm, *P. aeruginosa* between 21-5 mm, and especially for *E. faecalis* between 32-17 mm. When compared to the reference antibacterials, a statistical difference was observed by the Tukey's test ($p < 0.05$) for *E. coli*, *P. aeruginosa* and *E. faecalis*. It is also observed in (Tab. 4) at concentrations 500, 250 and 100 mg mL^{-1}, they presented was inhibition halo higher than the reference antibiotics Azithromycin (15 µg) and Tigecycline (15 µg).

Table 4. Antibacterial activity of hydroethanolic floral extract of *Schubertia grandiflora*.

Microorganisms	Concentrations Inhibition zone (mm)					Reference antibiotics (mm)			
	500	**250**	**100**	**80**	**50**	**Az**	**Ce**	**Ti**	**Am**
S. aureus	NI	NI	NI	NI	NI	24	27	-	-
E. coli	13b	10b	7b	NI	IN	19a	20a	-	-
P. aeruginosa	21a	18a	14b	9c	5d	20a	-	-	22a
S. Thyphymurium	IN	NI	NI	IN	IN	29	28	-	-
S. Enteritidis	IN	IN	IN	IN	IN	28	25	-	-
E. faecalis	32a	29a	25b	21c	17d	20c	-	22c	-

*Inhibition zone, diameter measured in (mm), disc diameter 5 mm, average of triplicate assay. Az: azithromycin (15 µg disc)™. Ce: Cephalexin (30 µg disc)™. Ti: Tigecycline (15 µg disc)™. Am: Amikacin (30 µg disc)™. NI: No inhibition. (-) No determined. The cavities were impregnated using a concentration in (%) of floral hydroethanolic extract. Source: Authors, 2021.

4. Discussion

Different phyto-constituents such as alkaloids, phenolic compounds, flavonoids, saponins, glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, proteins and carbohydrates were identified in the floral extracts of *Allamanda cathartica*, *Allamanda violacea*, *Wrightia tinctoria*, and *Nerium oleander* in the Apocynaceae family evaluated in the study by Joselin et al. (2012). Alkaloids, tannins, phenol, flavonoids, sterols, anthraquinones, proteins and quinones were present in the ethanolic flower extract of *Calotropis gigantea* (Apocynaceae) observed in the study of Dhivya and Manimegalai (2013). The phytochemical analysis of the floral extracts of *Alstonia scholaris* (Apocynaceae), indicated presence of a variety of compounds such as alkaloids, amino acids, carbohydrates, phenols, tannins, cardiac glycosides, saponins, terpenoids and steroids, flavonoids, fixed oils and fats (Thankamani et al., 2011).

In the review study proposed by Bhadane et al. (2018) the researchers discuss the use of floral extracts with pharmacological principles. Among the studies, the research carried out by Arambewela and Ranatunge (1991) evaluated 11 indole alkaloids including voacangine, voacristine, vobasine, and tabernaemontanine from different extracts of *Tabernaemontana divaricata* (Apocynaceae) leaves, stem bark, and flowers. The presence or absence of the phyto-constituents depended upon the solvent medium used for extraction, it can be single or conjugated

in different polarities (Joselin et al., 2012; Yalavarthi; Thiruvengadarajan, 2013; Pandey; Tripathi, 2014).

The according to Yadav et al. (2017), the phytochemicals like reducing sugars, no-reducing sugars, alkaloids, flavonoids, tannins, saponins, carbohydrates, purines, cardiac glycosides, phytosterols, phenols, protein and amino acid, diterpens etc. are known to show medicinal activity as well as exhibit physiological actions. The alkaloids have a wide range of pharmacological properties including anti-asthma, antimalarial, antitumor, anticancer properties as reported by Ajuru et al. (2017), and Kittakoop et al. (2014); flavonoids has properties antioxidant, estrogenic, anti-allergic, vascular, anti-inflammatory, anti-carcinogenic, anti-microbial, and anti-viral activity (Harborne 2000; O'Neil et al., 2000; Sonam et al., 2017).

Coumarins has properties edema modification, faster reabsorption of edematous fluids and treatment of lymphedema (Casley-Smith, 1993); phenols has properties as protecting agents against pathogens, certains type of cancers, neurodegenerative disease, anti-inflammatory, hormone modulators, and diabetes (Ajuru et al., 2017); saponin have been considered as bioactive antibacterial and antifungal agent (Sonam et al., 2017); tannins possess the potential properties as cytotoxic, anti-diarrhoeal and antihemorrhagic agents (Dhivya; Manimegalai, 2013; Sonam et al., 2017); terpenoids are credited for analgesic and anti-inflammatory actions (Sonam et al., 2017); the anthraquinones has properties responsible for the antibacterial and antifungal activity (Oladeji et al., 2020); and purines have anticonvulsants, antileukemic and anti-carcinogenic activities (Řezníčková et al., 2019).

The physicochemical characteristics of the floral extract are fundamentally important first in the knowledge of the new phytocomposites obtained, and also as a starting point for the development of new products especially, for the pharmaceutical industry of topical or internal drugs. The behavior of the extract says a lot when preparing the formulation and its interaction with other substances, such as moisture, pH and relative density. The extraction yield should also be observed, mainly for solvents with low toxicity such as ethanol and its conjugates (ethanol/water).

Thankamani et al. (2011) evaluated different solvents in the production of floral extracts from *Alstonia scholaris* (Apocynaceae), where they obtained a yield of 8.6 g% (hexane), 3.8 g% (benzene), 3.41 g% (propanol), 3.41 g% (ethyl acetate), 0.57 g% (methanol), 0.66 g% (acetonitrile) and 23.04 g% (water). Sethi (2012) evaluating different fractions of the floral hydroethanolic extract of *Allamanda violacea* obtained the following yields: petroleum ether 0.052%, ether 0.115%, chloroform 0.092%, chloroform:methanol 0.083%, and chloroform:methanol 0.068%.

The floral extract in the qualitative and quantitative analyzes demonstrated the potential for further studies on the isolation of these phytomolecules, possibly a new source of this class of phytocomposites can be extracted from the floral material of *S. grandiflora*. Numerous investigations have demonstrated the benefits of phenolic compounds for human health. The phenolic groups have important biological activity antioxidant, anticancer, anti-inflammatory effect and the way these compounds exert their therapeutic action have also been analyzed in numerous studies with plant extracts (María et al., 2018).

Other species of Apocynaceae exhibit potential as observed in Ribeiro study et al. (2020) on the seasonal influence on the content of total phenolic compounds in *Secondatia floribunda* (Apocynaceae), evaluating the ethanol extract of peel bark and heartwood observed a variation between 33.93-68.96 and 9.20-17.62 µg GAE 100 g^{-1}, respectively. Yadang et al. (2019) evaluated different extracting solvents for the leaves of *Carissa edulis* (Apocynaceae), where they obtained high quantitative levels of phenolics for the hydroethanolic 139.27 mg GAE 100 g^{-1}, methanolic 146.82 mg GAE 100 g^{-1}, and aqueous 147.05 mg GAE extracts 100 g^{-1}. The total flavonoids content for the peel bark and heartwood ethanolic extracts in *S. floribunda* was between 4.08-18.05 and between 3.95-10.46 µg QE 100 g^{-1} respectively, in the study by Ribeiro et al. (2020). Yadang et al., (2019) found expressive levels of total flavonoids in different extracts on the leaves of *C. edulis*, 14.84 mg RU 100 g^{-1} hydroethanolic, 12.02 mg RU 100 g^{-1} methanolic and 5.88 mg RU 100 g^{-1} to aqueous.

Color chromas influence the content of some important groups of phytomolecules, where from the colorimetric results performed in UV-*Vis* spectrophotometry it is possible to predict their intensity. Hue tint is a measurement of color degradation in anthocyanin, flavones, flavonoids and xanthones containing products. A higher hue tint value was associated with na increase in Abs at 420 nm (yellow tones) in relation to that at Abs 520 nm (red tones); according Jung et al. (2013), this is undesirable because it is na indication of anthocyanin and derivatives flavonoids degradation. The degradation of phytomolecules can occurrence in many ways, the main ones being heat (evaporation of the solvent used in the production of the extract) or light (where some colored compounds are reduced due to photosensitivity) (Isabel et al., 2007).

Flavonoids represent na important class of phytomolecules in numerous plant groups, being found from the roots to the seeds. It is also na important class mainly for the pharmaceutical industry, since, when used in extracts,

fractions, subfractions or isolated in emulsions or for internal use, they have potential activities guaranteeing the maintenance of human and animal health. Groups of the numerous flavonoids when dispersed in ethanol, can present bands of different intensities between 240-280 nm and between 300-550 nm (Bobin et al., 1994; Borges; Amorim, 2020; Martucci et al., 2021). In order to guarantee and maintained health, pharmaceutical groups continuous investments in the development of photoprotective formulations in the natural line, where they use plant extracts with photoprotective action against different types of radiant energizes. Sunscreens have photoprotective activity, protecting the dermis from the harmful effects caused by different ultraviolet (UV) radiation. Synthetic photoprotective solutions have maximum absorption in different regions of the ultraviolet spectrum.

Absorption between 100 to 290 nm corresponds to sunscreens that absorb UVC ultraviolet radiation, between 290 to 320 nm corresponds to UVB sunscreens, and between 320 to 400 nm absorb UVA radiation (Violante et al., 2009). The same ratio is equivalent to photoprotective solutions with incorporated plant extracts that act with the same function to the synthetic, protecting the skin against burns caused by exposure to sunlight. As described by Souza et al. (2005), Di Mambro and Fonseca (2006), and Violante et al. (2009), plant extracts with photoprotection activity present in their complex phytochemical composition groups such as flavonoids, tannins, anthraquinones, alkaloids and polyphenols with proven action against the sun's rays. It is worth mentioning that despite being na *in vitro* assay, the evaluation of the critical wavelength presents results similar to those observed by the *in vivo* assays (Santos et al., 1999; Violante et al., 2009), in addition, between 280-320 nm appear bands that suggest the presence of alkaloids (Martínez et al., 2016).

The importance of this new methodology makes the photoprotection characterization test, a quick solution to which you want to verify that among the groups of phytomolecules of na extract independent of the extraction solvent there are or not classes of secondary metabolites capable of interacting with the environment blocking sources highly energetic. In the literature it presents considerable number of the studies such as that by Violante et al. (2009) where the researchers evaluated hydroethanolic extracts from the aerial parts of *Macrosiphonia velame*, *Lafoensia pacari* and *Oxalis hirsutissima*, where through the analysis in critical wavelength, they were able to determine the type of UV absorption, with results at 318 nm (UVB), 356 and 324 nm (UVA), respectively.

The floral extract showed potential antioxidant activity when compared to the reference ascorbic acid. With this, new studies should be carried out to better evaluate the antioxidant activity of the floral extract of *S. grandiflora*. According by Mezza et al. (2019) free-radical scavenging activity is mediated by na electron donor molecule, called of antioxidant. Several phytochemical classes such, as flavonoids, tannins and phenolics, have a great reaction preventing chain reaction from oxidation radicals and delaying reactions in the process of degradation (Matthäus, 2002; Orak et al., 2019).

The production of reactive oxygen species (e.g. singlete oxygen, and other), occurs physiologically in living organisms, but can also be purchased in the environment. According to Skenderidis et al. (2018), the reactive oxygen species, are useful molecules at low concentrations, since they regulate growth, differentiation, proliferation, and apoptosis cell. Several pathologies are linked to several reactive species such as cardiovascular diseases, neurodegenerative diseases and several types of cancers.

Several studies are reported evaluating the antioxidant activity in the Apocynaceae family, although there are still few studies for the genus *Schubertia*. Yadang et al. (2019) obtained excellent free radical reduction potential in different solvent polarities using *C. edulis* leaves, with IC_{50} = 0.32; 0.31 and 0.30 mg mL^{-1} for hydroethanolic, methanolic and aqueous extracts.

The numerous actions can be obtained from a single extract, using one or solvent conjugates in the extraction of certain phytochemical classes. In addition to the activities already mentioned, the hydroethanolic floral extract of *S. grandiflora* demonstrated fungistatic and bacteriostatic action for several groups of microorganisms. Several studies discuss the resistance of numerous strains of *Candida* that cause the disease popularly known as "candidiasis". Several reference antifungals no longer have antifungal and fungistatic action for this yeast genus, especially for *C albicans*, such as amphotericin B, fluconazole and itraconazole (Almeida et al., 2017; Kordalewska; Perlin, 2019). The fungi as demonstrate high capacity to proliferate especially in immunosuppressed patients, with the acquired human immunodeficiency virus (HIV). There are several reports of dermal and internal infections that can reach organs of great importance for the maintenance of life (Arendrup; Patterson, 2017; Baptista et a., 2020).

In the study of Pattnaik et al. (2016) the ethanol extracts of *Calotropis procera* and *Calotropis gigantea* (Apocynaceae), leaves showed a high inhibition 10 and 15 mm against *Aspergillus niger* respectively, and no

activity against *C. albicans*. It is suggested that the phytochemical composition with the presence of organic acids, tannins and flavonoids with a proven influence on antibacterial and bacteriostatic activity (Duarte; Mota; Almeida, 2014; Sonam et al., 2017). Although the *S. aureus*, *S. serovar* Thyphimurium and *S. serovar* Enteritidis strains showed resistance in the highest concentration, further studies should be carried out isolation the chemical constituents of the extract by high performance liquid chromatography and testing higher dosages for other discussions regarding the antibacterial action. In the study by Thankamani et al. (2011) the researchers evaluated the antibacterial activity of different floral extracts of *A. scholaris* (Apocynaceae), with high antibacterial potential, showing inhibition between 16-25 mm for *Bacillus cereus*, between 11-25 mm for *S. aureus*, between 11.5- 22 mm for *Lactobacillus* sp., between 13-17 mm for *P. aeruginosa*, between 12.25-21 mm for *E. coli*, and between 12-22 mm for *Salmonella typhi*. The floral ethanolic extract of *Catharanthus roseus* (Apocynaceae) has potential antibacterial activity for *P. aeruginosa* and *S. aureus* (Wong et al., 2013).

In conclusion, the phytochemical analysis of the hydroethanolic floral extract of *S. grandiflora* showed the presence of a wide variety of chemical compounds with importance in the pharmaceutical, food, and agricultural fields. The extract showed photoprotection action and antioxidant activity. Furthermore, the floral extract showed antifungal and antibacterial activities. The results contribute to a better understanding of the chemical composition of *Schubertia grandiflora* floral extract and further strengthen its application in traditional and modern medicine practices. Thus opening new possibilities for the use of native plant material from the cerrado in the development of products based on natural molecules, thus ensuring the preservation of natural areas and genetic heritage.

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