Phytochemistry and biological activities of latex from *Schubertia grandiflora* Mart. (Apocynaceae)

Márcia Divina Vidal Silva[1], Antonio Carlos Pereira de Menezes Filho[2], Porshia Sharma[3], Rafael Martins da Cruz[2], Amanda de Oliveira Souza[2], Aparecida Sofia Taques[4], Adrielle Pereira da Silva[2], Carlos Frederico de Souza Castro[2] & Matheus Vinicius Abadia Ventura[1,2]

[1] University Center of Southwest Goiano, UniBRAS, Rio Verde, Goiás State, Brazil

[2] Goiano Federal Institute, IF Goiano, Rio Verde, Goiás State, Brazil

[3] Institute for Innovative Learning and Research (IILR) Academy, Indore, India

[4] Mato Grosso Federal Institute, São Vicente, Mato Grosso State, Brazil

Correspondence: Márcia Divina, University Center of Southwest Goiano, UniBRAS, Rio Verde, Goiás State, Brazil. E-mail: silvavidal.md@gmail.com

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Abstract

Schubertia grandiflora is a plant species that produces latex from roots to flowers. This study aimed to evaluate the latex of *S. grandiflora* on qualitative phytochemical analysis and effects of quantitative biological activities. Latex was collected from logs. Phytochemical analysis of precipitation and colorimetry were carried out for several groups of phytogroups. Antifungal activity was performed on *Sclerotinia sclerotiorum*, *Colletotrichum acutatum* and *C. gloeosporioides*, cytotoxic activity on *Artemia salina*, antioxidant activity on DPPH free radical and determination of inhibition on acetylcholinesterase. The latex proved to be rich in phytochemical groups, inhibition capacity of *S. sclerotiorum* with 64% and for *C. gloeosporioides* of 30%, low rate of cytotoxicity with 25% in 1000 µg mL^{-1}, reduction of DPPH free radical of 67% and AChE inhibition of 86%. *Schubertia grandiflora* latex proved to be a potential natural phytochemical agent for diverse biological activities through this study.

Keywords: Apocynaceae family, *Schubertia* genus, phytocompounds, latex, acetylcholinesterase inhibition.

Fitoquímica e atividades biológicas do látex de *Schubertia grandiflora* Mart. (Apocynaceae)

Resumo

Schubertia grandiflora é uma espécie vegetal que produz látex desde as raízes até flores. Este estudo teve por objetivo, avaliar o látex de *S. grandiflora* sobre análises fitoquímicas qualitativas e efeitos de atividades biológicas quantitativa. O láxtex foi coletado de troncos. Análises fitoquímicas de precipitação e colorimetria foram realizadas para Diversos grupos de fitogrupos. A atividade antifúngica foi realizada sobre *Sclerotinia sclerotiorum*, *Colletotrichum acutatum* e *C. gloeosporioides*, atividade citotóxica sobre *Artemia salina*, atividade antioxidante sobre o radical livre DPPH e a determinação de inibição sobre acetilcolinesterase. O látex revelou ser rico em grupos fitoquímicos, capacidade de inibição de *S. sclerotiorum* com 64% e para *C. gloeosporioides* de 30%, baixa taxa de citotoxicidade com 25% em 1000 µg mL^{-1}, redução do radical livre DPPH de 67% e inibição da AChE de 86%. O látex de *Schubertia grandiflora* demonstrou ser um bom agente natural fitoquímico para diversificadas atividades biológicas nesse estudo.

Palavras-chave: família Apocynaceae, gênero *Schubertia*, fitocompostos, látex, inibição da acetilcolinesterase.

1. Introduction

Apocynaceae Juss. family is considered as one of the largest among the Angiosperm botanical families, with more than 5,350 species distributed in 378 genera and five subfamilies found in tropical and subtropical regions

of the planet (Endress et al., 2014, 2018; Campos; Farinaccio, 2021). Among this exuberant flora in Apocynaceae, we can mention *Schubertia grandiflora* Mart. & Zuccarini (1824: 57) found in *Cerrado* domain environments, *Pantanal* and *Caatinga* biomes, and in anthropic areas (side of dirt roads) (Araújo, 2014).

This species is characterized by vine-like appearance, white laxex (Figure 1, A and B), pubescent branches, leaves with oval blade, umbelliform summits, subaxillary, calyx with oval lobes, acuminate, abaxially pubescent, corolla rotaceous, alvescent, abaxially glabrous, adaxially pilose with elongated trichomes, corona with target segments that are longer than the anthers and oval follicles with longitudinally distributed spinous protuberances.

The plant bears flowers between January and March, with fruiting in September (Fontella-Pereira, 2005). It is distributed in more than 16 Brazilian states, including Paraguay, Bolivia and Argentina. In Brazil, there are no scientific records that *S. grandiflora* has individuals in the states of Acre, Alagoas, Amapá, Espírito Santo, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina and Sergipe (Flora do Brasil, 2020) .

Species of the Apocynaceae family have a formidable phytochemical constitution with numerous groups of phytocompounds in special metabolism such as sugars, steroids, lignins, triterpenoids, flavonoids, glycosides, phenols, lactones and alkaloids, which have various biological activities such as antitumor, antimalarial, anti-inflammatory, antioxidant, larvicidal and cytotoxic (Joselin et al., 2012; Bhadane et al., 2018; Ekalu et al., 2019; Menezes Filho et al., 2022).

Schubertia grandiflora produces throughout its length (roots, branches, leaves and flowers) a white latex without aroma that becomes rigid with time. This latex is still unknown at a scientific level regarding its phytochemical composition and possible and attractive biological activities, thus leading, in this study, to determine which are the phytochemical groups and some biological activities of great medical interest.

This study aimed to qualitatively evaluate which groups of phytocompounds and quantitatively some biological activities such as antifungal, antibacterial, cytotoxic, antioxidant and acetylcholinesterase inhibition evaluating the latex extracted from the branches of *Schubertia grandiflora*.

Figure 1. Trunk of *Schubertia grandiflora* in (**A**) with exudate, and in (**B**) exudate collected in glass vials. Source: Authors, 2023.

2. Material and Methods

2.1 Identification and deposit of the specimen

The plant species was identified by the second author of this study, and a specimen was deposited in the Herbarium in the Laboratory of Vegetal Systematic belonging, to the Department of Biological Sciences of the Goiano Federal Institute, Rio Verde, Goiás, Brazil, with the following Voucher: HRV 13509.

2.2 Latex collection

Latex collection was performed manually in the morning between 7 and 8 h (am) by cutting branches of *S. grandiflora* with a sterile stainless steel knife. The latex was collected using a sterile disposable syringe and sterile glass vials. The sample was added 1 mL of sterile water to 5 mL of latex (1:5, *v/v*). The vials containing

exudate were kept refrigerated at -2 °C in a thermal box and transported to the laboratory for analysis. 95 g of latex *in natura* were obtained from 45 samples of *S. grandiflora*.

2.3 Phytochemical screening

Phytochemical analysis was performed for the main groups of special metabolites present through staining, and qualitative precipitation techniques. With the latex of *S. grandiflora*, the following tests were performed for: organic acids, reducing and non-reducing sugars, alkaloids, anthraquinones, catechins, depsides and depsidones, coumarins, steroids, phenols and tannins, flavonoids, polysaccharides, proteins, amino acids, purines, foaming and hemolytic saponins, sesquiterpenolactones, organic acids, and azulenes proposed by (David et al., 2019).

2.4 Antifungal assay

The agar diffusion method was used to determine the antifungal activity on *Sclerotinia sclerotiorum*, *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* as described by Toigo et al. (2022) adapted. The strains used are: SS12-21, CA15-67 and CG 16-21 respectively, from the second author's mycological bank. The fungal strains were cultured at 20 °C for 10 days for *S. sclerotiorum* and 28 °C for 3-5 days for the other strains.

A mycelium disc with a diameter of 7 mm was transferred to the center of *Petri* dishes with a diameter of 10 cm containing sterile potato, dextrose and agar (PDA) medium. Different latex concentrations were used, dissolved in 0.1% Triton X-100 to render doses between 100-500 µL mL^{-1}, in each plate 500 µL of the concentration was pipetted. The plates were transferred to an incubator at 20 °C (10 days) and 28°C (3-5 days), respectively. The diameter of the zone of inhibition was measured and recorded as an indicator of antifungal activity and expressed in percentage (%) using a digital caliper. The commercial reference fungicide Frowncide 500 SC was used as a positive control (dose of 10 µL mL^{-1}).

Triton X-100 emulsifier was also evaluated at the lowest dose under investigation (100 µL mL^{-1}). The agar diffusion assays applied against the three fungi were performed in quadruplicate. Mycelial growth was obtained daily until complete fungal growth separately on control plates. The experimental design was completely randomized. The data were submitted to analysis of variance (ANOVA) and the averages of the treatments were evaluated by the *Scott-Knott* test with 5% of significance using the statistical program ASSISTAT. The percentage of mycelial growth inhibition was calculated by the following equation 1.

$$MGI = (Control - Treatments)/Control*100 \qquad Eq.\ 1$$

WHERE: Control, (control growth); Treatments, (treatment growth).

2.6 Citotoxicity assay

Cytotoxicity assay on *Artemia salina* was based on the technique described by Araújo et al. (2010). *Artemia saline* water solution was prepared (500 mL) concentration 35.5 g L^{-1} for preliminary incubation of 50 mg of *A. salina* eggs. The solution containing eggs of *A. salina* was maintained under homogenization on a magnetic table at 50 rpm exposed to white artificial light for 24 h for hatching of the larvae (metanauplii). Soon after, the metanauplii were collected and kept for another 24 h for the nauplii stage.

The exudate solution of *S. grandiflora* was prepared containing 62.5 mg of exudate *in natura*, added to 28 mL of saline solution and 2 mL of dimethylsulfoxide (DMSO). Ten nauplii were selected in triplicate, divided into six concentrations (1000; 750; 500; 250; 100 and 50 µg mL^{-1}). In each tube, an aliquot of the exudate solution ranging from 3000 to 100 µL was added and the volume was completed to 5 mL with saline solution, where concentrations ranging from 1000 to 1 µg mL^{-1} were obtained. The data were submitted to analysis of variance (ANOVA) and the averages of the treatments were evaluated by the *Scott-Knott* test with 5% of significance using the statistical program ASSISTAT.

2.7 DPPH reducing activity

The antioxidant assay was performed using the methodology as described by David et al. (2019) adapted, in the reduction of the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH). DPPH stock methanolic solution, was prepared with a concentration of 40 µg mL^{-1} and kept refrigerated at -8 °C in a place free of light. The *in natura* exudate was diluted in methanol in a 1:5 ratio (*v/v*). Then, an aliquot of 0.8 mL of the solution was added to 0.2 mL of the DPPH stock solution. The mixture was then kept at rest protected from light for 30 min in a

refrigerator at -4 °C. After this time, the sample was read in a UV-*Vis* spectrophotometer at a wavelength of 555 nm. Distilled water was used as a negative control, and a standard ascorbic acid solution was used as a positive control. The ability to reduce DPPH was expressed in percentage (%) of sequestration according to equation 2.

$$\%AA = 100 - \{[(Abs\ sample - Abs\ blank/Abs\ control)100]/Abs\ control\} \quad \text{Eq. 2}$$

WHERE: %AA = Abs absorbance of the sample; Abs absorbance of the blank, and Abs absorbance of the control.

2.8 acetylcholinesterase inhibition

The colorimetric method for determining AChE inhibition followed as proposed by Miloševića et al. (2020). The enzyme conc. (0.09 U mL^{-1}), conc. (0.014 M) and DTNB (0.01 M) were dissolved in conc. (0.1 M, pH = 8), and the diluted extracts conc. 1 mg mL^{-1} in phosphate buffer solution + 10% DMSO (*v/v*). The latex was serially diluted (40 µL) prepared directly in a 96-well microplate, so that the concentration range in the final volume was between 0.4-400 µM.

The solutions were adjusted to 160 µL with phosphate buffer (0.1 M, pH = 8) and the enzyme was added (20 µL). After 15 min of incubation in D.B.O. without photoperiod at 25 ºC, aliquots of DTNB (10 µL) and AChE (10 µL) were added to the wells. Then, the plates were homogenized and the incubation continued for another 40 min. Absorbance (Abs) was read at 405 nm, in a UV-*Vis* microplate reader. As a blank, phosphate buffer (180 µL) and DTNB (10 µL) and AChE (10 µL) solutions were used. Maximum enzymatic activity was obtained by replacing the latex sample with 10% DMSO phosphate buffer solution and the latex Abs by replacing the enzymatic solution with phosphate buffer. A solution of conc. (10 µM) was used as a positive control (standard inhibitor). The percentage (%) of enzyme reaction inhibition was calculated according to equation 3.

$$AChE\ (\%) = [(A-B) - (C-D)]/(A-B)*100 \quad \text{Eq. 3}$$

WHERE: A, B, C and D are the absorbances of the maximum enzymatic activity, reaction blank, enzymatic activity in the presence of the latex sample and the color of the sample solutions, respectively. The AChE assay was performed in triplicate.

3. Results and Discussion

Phytochemical analysis conducted on the *S. grandiflora* plant látex, revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant latex revealed the presence of phytochemicals namely phenols, organic acids, alkaloids, no-reducing sugars, steroids, tannins, carboxilic acids, foaming saponins and hemolytic saponins.

The phenolic compounds possess biological properties such as anti-microbial agents (Hussain et al., 2011), anti-apoptosis, bactericides, antiaging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, inhibition of angiogenesis and cell proliferation activities (Han et al., 2007; Yadav; Agarwala, 2011). Alkaloids have been closely associated with medicinal uses since centuries and one of their common biological properties is their cytotoxicity (Nobori et al., 1994), analgesic, antispasmodic and bactericidal activities (Hussain et al., 2011).

Several researches have reported the analgesic (Antherden, 1969; Harborne, 1973), antispasmodic and antibacterial (Stray, 1998; Okwu; Okwu, 2004) properties of alkaloids. Tannins bind to proline rich protein and interfere with protein synthesis, and anti-microbial agents (Naveen Prasad et al., 2008). According to Raquel (2007), Yadav & Agarwala (2011); steroids have been reported to possess antibacterial and antifungal properties, and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001).

It was also observed that the latex contains saponins which are known to contribute inhibitory effect against inflammation (Just et al., 1998). Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties (Sodipo et al., 2000; Okwu, 2004; Hussain et al., 2011).

Table 1. Phytochemical prospection of the *Schubertia grandiflora* latex.

Phytochemical groups	**Reaction**
Phenols	+

Flavonoids	-
Organic acids	+
Azulenes	-
Alkaloids	+
Reducing sugars	-
No-reducing sugars	+
Anthraquinones	-
Cathequins	-
Coumarins	-
Depsides and depsidones	-
Steroids	+
Tannins	Green
Polysaccharides	-
Proteins	-
Amino acids	-
Carboxylic acids	+
Purines	-
Foaming saponins	+
Hemolytic saponins	+
Sesquiterpenolactones	-

Note: (-) Absent. (+) Positive. Source: Authors, 2023.

The latex demonstrated effective antifungal activity against *S. sclerotiorum* and *C. gloeosporioides* with maximum growth inhibition of 64 and 30% at the maximum concentration of 500 µL mL^{-1}, respectively. For *C. acutatum*, no inhibition activity was observed for this strain at different latex concentrations, demonstrating that this variety is resistant to *S. grandiflora* phytocompounds (Table 2). Oliveira-Tavares et al. (2019) obtained promising results for *Jatropha multifida* latex on *Candida grablata* and *Candida tropicalis* with MIC of 100 µg mL^{-1}. Corroborating our phytochemical findings, the antifungal action observed in Table 2 suggests that phenolic compounds, organic acids, alkaloids and tannins have antifungal action (Duarte et al., 2014; Menezes Filho et al., 2021; De Morais et al., 2021).

Table 2. Antifungal activity of *Schubertia grandiflora* latex on agricultural fungal strains.

Strains	**Latex – Concentrations in µL mL^{-1} (%)**				
	100	**200**	**300**	**400**	**500**
S. sclerotiorum	15f	26e	41d	50c	64b
C. acutatum	0b	0b	0b	0b	0b
C. gloeosporioides	0f	11e	23d	28c	30b

Note: Frowncide Fungicide 500 SC (100%) inhibition positive Control. Negative control Triton X-100 (0%) inhibition. Source: Authors, 2023.

The lethality bioassay on *A. salina* is used with reliable evidence about the toxicity of a given cell sample, especially in humans and animals. Our results demonstrates that *S. grandiflora* latex has a low rate of cytotoxicity even at the highest concentration of 1000 µg mL^{-1} with only 25% mortality. After 24 h of exposure (Table 3). In the negative control DMSO there was no mortality in any of the evaluated larvae, and in the

positive control using potassium dichromate there was 100% mortality. In the study, by Menezes Filho et al. (2022) evaluating the latex of *Manilkara zapota*, the researchers obtained mortality between 65 and 100% on *A. salina*, with lethal concentration (LC_{50}) of 17.9 µg mL^{-1}.

Table 3. Cytotoxicity index of latex of *Schubertia grandiflora* against *Artemia salina*.

Concentration (µg mL^{-1})	**(%) Mortality rate**
1000	25.1 ± 0.04 b
750	21.7 ± 0.05 c
500	13.9 ± 0.11 d
250	4.5 ± 0.05 e
100	0.0 ± 0.00 f
50	0.0 ± 0.00 f

Source: Authors, 2023.

The percentage of DPPH free radical inhibition was 67.36% and AChE with 86.13% for *S. grandiflora* latex. The most widely used DPPH and AChE activity assay is the 96-well microplate assay. This is due to the economy of reagents which are mostly expensive but generates quick results in a short time.

4. Conclusions

Schubertia grandiflora latex presents a formidable number of groups of phytocompounds, in addition to important antifungal activity against *Sclerotinia sclerotiorum* and *Colletotrichum gloeosporioides*, low cytotoxic action against *Artemia salina*, an important antioxidant agent in the reduction of free radical DPPH and strong AChE inhibition activity. New studies should be carried out evaluating other biological activities using latex from *S. grandiflora*, which proved to be attractive at a scientific level with important results in fungal and cytotoxic inhibition, in combating the action of free radicals and in inhibiting acetylcholinesterase.

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6. Author contributions

Márcia Divina Vidal Silva: project writing, latex collection, laboratory analysis, and article writing. *Antonio Carlos Pereira de Menezes Filho*: identification and collection of the plant specimen, laboratory analysis, corrections, translation and submission. *Porshia Sharma*: correction of English writing and grammar. *Rafael Martins da Cruz*: specimen preparation and laboratory analysis. *Amanda de Oliveira Souza*: study review. *Aparecida Sofia Taques*: study review. *Adrielle Pereira da Silva*: study review. *Carlos Frederico de Souza Castro*: search for funds for the purchase of equipment, glassware and reagents and verification of chemical calculations. *Matheus Vinícius Abadia Ventura*: advisor, study analysis, writing verification, presentation of results.

7. Conflicts of interest

There are no conflicts of interest.

8. Ethical approval

Not applicable.

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