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Environment

Biotic and abiotic factors influence the chemical composition, toxicity, and biological potential of *Serjania marginata* Casar

Fatores bióticos e abióticos influenciam a composição química, toxicidade e potenciais biológicos da *Serjania marginata* Casar

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ABSTRACT

Serjania marginata Casar. is a medicinal species found in various Brazilian biomes, exhibiting diverse potential uses, safe to use and cultivable. This study aims to investigate the impact of seasonality and different harvesting sites on the chemical composition, biological properties, and toxicity of aqueous extracts derived from the leaves and stems of *S. marginata*. Plant materials were harvested in the spring and autumn of 2020 from two distinct locations: Lagoa Grande Settlement (LG) and Santa Madalena Farm (SM) in Dourados, MS, Brazil. In the aqueous extracts, antioxidant antibacterial, and photoprotective (SPF) potentials and toxicity were analyzed, and phenolic compounds, flavonoids, and tannins were quantified. The harvesting site, plant organ, and season choice depends on the intended application. Significantly higher levels of phenolic compounds were observed in SM stems throughout the year, and in LG leaves harvested in the autumn. Leaves from SM exhibited higher flavonoid content, regardless of the season, while LG leaves harvested in the autumn displayed elevated flavonoid levels. Tannin levels were highest in leaves harvested in the spring, regardless of the location. Leaves of *S. marginata*, regardless of harvesting site or season, demonstrated superior SPF. Stems harvested in the spring in LG exhibited greater antioxidant potential. All aqueous extracts exhibited antibacterial properties, and none displayed toxicity at the tested concentrations.

Keywords: Cipó-timbó; Sapindaceae; Seasonality

RESUMO

Serjania marginata Casar. é uma espécie medicinal encontrada em diversos biomas brasileiros, com diferentes potenciais de uso, de uso seguro e cultivável. O objetivo do estudo foi verificar a influência da sazonalidade e diferentes locais de coleta no perfil químico, potenciais biológicos e toxicidade dos extratos aquosos de folhas e caules de S. marginata. Os materiais vegetais foram coletados na primavera e no outono de 2020, em dois locais (Assentamento Lagoa Grande–LG e Fazenda Santa Madalena-SM), em Dourados-MS, Brasil. Os extratos aquosos foram analisados quanto aos potenciais antioxidante, antibacteriano e fotoprotetor (FPS), toxicidade e quantificados os compostos fenólicos, flavonoides e taninos. A escolha do local de coleta, órgão da planta e época do ano dependem da finalidade do uso. Maior teor de compostos fenólicos foi observado para os caules de SM de qualquer época do ano e as folhas de LG colhidas no outono. De flavonoides, houve maior teor nas folhas da SM de qualquer época e de LG colhidas no outono. De taninos, as folhas colhidas na primavera, em qualquer local, apresentaram maiores teores. Folhas da S. marginata colhidas em qualquer local e época apresentaram FPS superiores. Os caules colhidos na primavera, no LG, apresentaram melhor atividade antioxidante. Todos os extratos aquosos apresentaram propriedades antibacterianas e nenhum apresentou toxicidade nas concentrações testadas.

Palavras-chave: Cipó-timbó; Sapindaceae; Sazonalidade

INTRODUCTION

Scientific research has expanded the possibilities to treat disease conditions, but adverse effects and inadequate drug absorption continue to be some of the main limitations of conventional treatments. Considering these limitations, formulations employing medicinal plants have been devised, demonstrating unequivocal efficacy in treating ailments, notably skin diseases. This efficacy can be attributed to the inherent bioactive compounds present within these plants and their medicinal properties, encompassing antioxidative, antibacterial, and anti-inflammatory attributes (Majtan et al., 2021).

Serjania marginata Casar. (Sapindaceae), known in Brazil as “cipó-timbó,” “cipó-uva,” or “barbasco” is a species with substantial potential for medicinal and phytocosmetic applications. Indigenous to Brazil, it thrives in various Brazilian biomes including the Amazon, Caatinga, Cerrado, Atlantic Forest, and Pantanal (Reflora, 2023). Bourdy, Michel, and Roca-Coultthard (2004) documented its traditional use in alleviating gastric issues. Subsequent studies conducted since 2015 have corroborated not only the antiulcerogenic potential (Perico et al., 2015) of hydroalcoholic leaf extracts of *S. marginata* but also their antioxidant (Heredia-Vieira et al., 2014), antimicrobial (Perico et al., 2015), anticancer (Serpeloni et al., 2021), and anti-inflammatory properties (Leitão et al., 2022). Moreover, these extracts exhibited no discernible toxicity or mutagenicity (Perico et al., 2015), underscoring their suitability for therapeutic use.

Given the prevalent mode of employing medicinal plants – typically as aqueous extracts or teas – Moreira et al. (2019) evaluated the aqueous leaf extract of *S. marginata*, confirming its nontoxic and nonmutagenic nature. Furthermore, recent research has unveiled this extract's antinociceptive and anti-inflammatory potential (Leitão et al., 2023). For other species within the *Serjania* genus, the ethanolic stem extract of *S. lethalis* demonstrated antibacterial potential against *Staphylococcus aureus* (Lima et al., 2006) and anti-inflammatory properties (Napolitano et al., 2005). Similarly, the ethyl acetate stem extract of *S. schiedeana* exhibited anti-inflammatory potential (Salinas-Sánchez et al., 2017), signifying the therapeutic promise not only of leaves but also of stems within the *Serjania* genus. Regrettably, no scientific literature to date has explored the stems of *S. marginata*.

In the development of herbal medicines and phytocosmetics, cultivating medicinal species in their natural habitats, even when abundant, is crucial for obtaining standardized materials and preserving the environment by Brazil's Interministerial Ordinance no. 2,960/2008 (Portaria Interministerial nº 2.960, 2008). Tabaldi et al. (2012) and Alves (2015) have established *S. marginata* as a cultivable *ex situ* species in this context. Emphasizing the importance of optimizing the therapeutic potential of medicinal plants, Heredia-Vieira et al. (2017) investigated *S. marginata* leaves haversted in Dourados, MS, at three stages of the plant's growth cycle – before, during, and after flowering. Their findings indicated higher yields of hydroalcoholic extracts and greater antioxidant potential in specimens haversted before and after flowering, with higher phenolic compound levels observed post-flowering and elevated flavonoid levels pre-flowering. Conversely, Zannata et al. (2021) reported that different seasons influenced the concentration of saponins synthesized by *S. marginata*, with higher concentrations observed in spring and autumn. Nevertheless, there is a dearth of literature comprehensively examining the impact of geographic origin and seasonality on the chemical profile and biological properties of *S. marginata*. Given the widespread utilization of this species for medicinal purposes and its occurrence across various Brazilian biomes, highlighting its considerable potential, standardizing extracts for public consumption is justifiable. Furthermore, the choice of harvesting location, season, and plant organ (leaves or stems) is contingent upon the intended application.

Within this context, this research aims to assess the influence of seasonality and harvesting locations on the chemical composition, toxicity, antioxidant, photoprotective, and antibacterial potential of aqueous extracts derived from the leaves and stems of *S. marginata*.

2 MATERIALS AND METHODS

2.1 Location and climate

Leaves and stems of *S. marginata* (registered in the National System of Management of Genetic Heritage and Associated Traditional Knowledge under no. AF5A35E) were haversted in two locations within the municipality of Dourados, MS, Brazil: Santa Madalena Farm and Lagoa Grande Settlement (Table 1). A voucher specimen was identified by the specialist Professor Dr. Maria do Carmo Vieira, and deposited in the Herbarium Collection of Universidade Federal da Grande Dourados (DDMS 4677).

Table 1 – Identification of *Serjania marginata* Casar. samples based on haversting locations, seasons, and plant organs

Harvesting location in Dourados – MS	Organ/Season	TAG
Lagoa Grande Settlement (LG) 22º18'23.24" S and 55º08'16.84" W	Leaf – Autumn Stem – Autumn	LGLF LGStF
	Leaf – Spring Stem – Spring	LGLS LFStS
Santa Madalena Farm (SM) 21°59'41.8" S and 55°19'24.9" W	Leaf – Autumn Stem – Autumn	SMLF SMStF
	Leaf – Spring Stem – Spring	SMLS SMStS

Source: Prepared by the Authors (2023)

The Santa Madalena Farm and Lagoa Grande Settlement present typical vegetation of Cerrado stricto sensu, characterized by low, inclined, and tortuous trees. The soil in the study areas has the chemical characteristics at a 0-0.20 m depth in Table 2.

Tabela 2. Chemical attributes of soils of Santa Madalena farm and Lagoa Grande Settlement

Locations	O.M.[1]	pH	P[2]	K[2]	Al[3]	Ca[3]	Mg[3]	
	g kg	$CaCl_2$	mg dm[3]		cmol$_c$ dm[3]			
Santa Madalena Farm	1,42	3,74	1,03	0,03	1,50	0,18	0,07	
Lagoa Grande Settlement	16,13	4,00	3,91	0,07	1,12	0,27	0,10	
	H+Al	SB[4]	CEC[5]	V	Cu[2]	Mn[2]	Fe[2]	Zn[2]
		cmol$_c$ dm[3]		%		mg kg^{-1}		
Santa Madalena Farm	3,43	6,21	9,65	64,38	3,64	4,28	42,49	0,86
Lagoa Grande Settlement	3,25	0,44	8,20	15,37	0,59	4,68	79,66	0,19

[1] O.M.: Organic matter, [2]Mehlich (P, K, Fe, Cu, Zn, Mn); [3]KCl 1N (Ca, Mg e Al), [4]SB: Sum of bases; [5]CEC: Cation exchange capacity; V: Base saturation. Source: Prepared by the Authors (2023)

The haversting of *S. marginata* occurred in two distinct seasons in 2020, namely, in the autumn (June, average temperature of 22.0 °C) and spring (November, average temperature of 28.3 °C). According to the Köppen and Geiger climate classification, the Dourados, MS, region boasts a tropical climate characterized by humidity during summers and aridity in winters, classified as Aw (EMBRAPA, 2020). This climate pattern delineates two discernible seasons: a rainy season spanning from October to April and a dry season from May to September. The average annual temperature in Dourados is 23.4 °C, with the region receiving an average annual precipitation of 1,419 mm (Souza et al., 2013).

2.2 Preparation of aqueous extracts

To prepare the aqueous extracts, 20 g of dried and pulverized stems or leaves were macerated in 200 mL of distilled water for 24 hours period at room temperature (approximately 25 °C) and then lyophilized.

2.3 Antioxidant potential

The assessment of antioxidant potential employed the methodology elucidated by Kumaran and Karunakaran (2006), utilizing the DPPH radical (1,1-diphenyl-2-picrylhydrazyl; 0.004% in methanol). A total of 3,000 μL of DPPH solution was introduced to 100 μL of the sample. The samples were assessed at different concentrations ranging from 50 to 1,000 μg mL^{-1}. After a 30-minute incubation period under light and at room temperature, readings were recorded using a spectrophotometer (wavelength: 517 nm). The sample inhibition percentages were determined and results were expressed in terms of the minimum inhibitory concentration (CI_{50}), with due consideration to a dilution factor of 31 times.

2.4 Phenolic compounds, flavonoids, and tannins

For the quantification of phenolic compounds, the Folin-Ciocalteau colorimetric method was adopted (Djeridane et al., 2006). Specifically, 500 μL of Folin-Ciocalteau reagent (1:10 v/v) was combined with 1,000 μL of distilled water and 100 μL of the sample. After 1 minute, 1,500 µL of 20% aqueous sodium carbonate solution (w:v) were added, and the solution rested for 30 minutes. The same procedure was applied to all samples, with spectrophotometric readings taken at 760 nm. Quantification relied on an analytical gallic acid standard curve (R^2 = 0.9992) at concentrations ranging from 10 to 1,000 µg mL^{-1}, and results were expressed as mg of gallic acid equivalent per g of extract (mg GAE g^{-1}).

For flavonoid quantification, 1,000 µL of 2% aluminum chloride in methanol (w:v) and 1,000 µL of each sample were combined. After a 15-minute incubation, spectrophotometric readings were obtained at 430 nm. Flavonoid concentrations were determined using an analytical rutin standard curve (R^2 = 0.9990) within the range of 1 to 50 µg mL^{-1}, and results were expressed as mg of rutin equivalent per g of extract (mg RE g^{-1}).

Regarding tannin quantification, the methodology of Pansera *et al.* (2003) was adopted, with volume-based adaptations while preserving proportions. Specifically, 500 µL of Folin-Denis 1:9 (v:v) were combined with 500 µL of each sample. After a 3-minute incubation, 500 µL of 8% sodium carbonate (w:v) were added. Spectrophotometric readings were taken at 430 nm after two hours of reaction. Tannin concentrations were determined utilizing an analytical tannic acid standard curve (R = 0.9983) across concentrations ranging from 0.5 to 80 µg mL^{-1}, with results expressed as mg of tannic acid equivalent per g of extract (mg TAE g^{-1}).

2.5 Toxicity

Toxicity analysis was conducted by exposing nauplii of *Artemia salina* to varying concentrations, ranging from 15 to 1,000 µg mL^{-1}, following adaptations to the methodology of Meyer et al. (1982). The hatching of *A. salina* eggs necessitated preparing a solution containing sea salt (30 g L^{-1}) and pH adjustment between 8.0 and 9.0 using a 0.1 mol L^{-1} NaOH solution. Eggs were then subjected to hatch in this solution over a 48 hours, with continuous aeration at room temperature. Subsequently, ten

A. salina larvae were transferred to test tubes containing different concentrations of aqueous extracts (ten larvae per test tube). A negative control was included, prepared using ten *A. salina* larvae and the saline solution employed for hatching. After 24 hours, both deceased and surviving organisms were tallied.

2.6 Antibacterial activity

Assessment of antibacterial activity followed the methodology outlined by Bernardi et al. (2017) and encompassed extract concentrations spanning from 15 to 1,000 μg mL^{-1}. Bacterial strains utilized in this study encompassed *Burkholderia cepacia* (NEWP 0059), *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC 38731), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus epidermidis* (ATCC12228), *S. aureus* (ATCC 25232), and *S. saprophyticus* (ATCC15305). Tetracycline was employed as a positive control at a concentration of 15 μg mL^{-1}, with distilled water as the negative control. Results were expressed in terms of minimum inhibitory concentration (μg mL^{-1}).

2.7 Photoprotective activity

To determine the sun protection factor (SPF), we adhered to the method described by Dutra et al. (2004). To this end, solutions of each sample were prepared at 1 mg mL^{-1} in distilled water and diluted to achieve a concentration of 0.2 mg mL^{-1}. SPF was calculated by spectrophotometric measurements conducted at wavelengths from 290 to 320 nm, with 5-nm intervals, using distilled water as a blank for each sample.

2.8 Statistical analyses

Data on the levels of phenolic compounds, flavonoids, tannins, SPF, and antioxidant potential were subjected to variance analysis. In instances where significance was ascertained via the F-test, the subsequent analysis involved a t-test (LSD) at a 5% probability level (SAEG, 2007). The application of the Pearson correlation test facilitated the assessment of the degree and statistical significance of interrelationships among variables. Antibacterial activity data were presented as means.

RESULTS AND DISCUSSION

3.1 Quantification of phenolic compounds, flavonoids, and tannins

All attributes assessed in the aqueous extracts of *S. marginata* leaves and stems were influenced by the interaction between location, harvest time, and plant organ, except for tannin content, which was influenced by the interaction between harvesting time and plant organ (Table 3).

Table 3 – Analysis of variance for the contents of phenolic compounds (Phenol. Comp.), flavonoids, tannins, sun protection factor (SPF), and antioxidant potential (Antiox.) of aqueous extracts from leaves and stems (LFSt) of *Serjania marginata* Casar. haversted at two different locations and seasons

Source of variation	DF	Average squares				
		Phenol. Comp.	Flavonoids	Tannins	SPF	Antiox.
Location	1	96.02667^{ns}	764.5423^{ns}	396.5459^{ns}	2.070938^{ns}	23.72352^{ns}
LFSt	1	510.2963^{ns}	4576.224^{ns}	119.5086^{ns}	110.0388^{ns}	34.21801^{ns}
LFSt x Location	1	6,800.442**	503.4116**	91.6073^{ns}	1.06260**	10.1879**
Error a	2	193.0056	0.623454	143.2984	0.05233	0.25627
Season (S)	1	6,890.741**	311.3190**	224.0735^{ns}	3.5037**	33.5585**
Seasons x Location	1	10,086.27**	171.6328**	35.8521^{ns}	14.3067**	11.9104**
Seasons x LFSt	1	626.8267**	45.09005**	1,226.306**	0.9640**	3.2582**
S x LFSt x Location	1	1,370.376**	45.91180**	145.5881^{ns}	9.3375**	23.5631**
Residue	14	74.11667	0.6612	107.8392	0.1065	0.28722
C.V. (%)		3.75	3.75	3.98	6.77	3.59
General average		229.80	21.68	260.86	4.82	14.92

Values followed by ns is not significant and by **p<0.01 by t test (LSD). DF: Degree of freedom. Source: Prepared by the Authors (2023)

The levels of phenolic compounds were notably higher in the aqueous extracts of stems from SM, irrespective of the harvesting time, and in the aqueous extracts of leaves harvested in the autumn in LG. The aqueous extract of leaves harvested in the autumn in LG exhibited a higher phenolic compound content than those harvested in SM in the same period. As for flavonoid levels, the highest concentrations were found in the aqueous extracts of SM leaves, regardless of the harvest time. In the aqueous extracts of LG leaves, the levels were higher in specimens harvested in the autumn. On the other hand, tannin levels were higher in the aqueous extracts of leaves harvestedd in the spring, regardless of the location, and the levels were comparable in both the aqueous extracts of leaves and stems harvest in the autumn in SM. In the LG harvest, tannin levels were higher in the aqueous extract of leaves harvested in the spring and in the aqueous extract of stems in the autumn (Table 4).

Table 4 – Contents of phenolic compounds, flavonoids, tannins, pH, SPF, and antioxidant potential of aqueous extracts from leaves and stems of *Serjania marginata* Casar. harvested in the spring and autumn at Santa Madalena Farm and Lagoa Grande Settlement in Dourados, MS, Brazil

(continue)

Santa Madalena Farm				
Season	**Spring**	**Autumn**	**Spring**	**Autumn**
Leaves/Stems	**Phenolic compounds (mg AGE g^{-1})**		**Flavonoids (mg RE g^{-1})**	
Leaves	209.47 ± 9.34 a B	200.80 ± 6.01 a B	39.45 ± 0.92 a A	41.78 ± 1.37 a A
Stems	250.13 ± 1.68 a A	257.47 ± 16.74 a A	10.90 ± 0.86 a B	12.84 ± 1.14 a B
	CV (%): 3.75		CV (%): 3.75	
	Tannins (mg TAE g^{-1})			
Leaves	265.85 ± 5.53 a A		255.07 ± 1.41 b A	
Stems	248.73 ± 2.45 b B		255.07 ± 0.68 a A	
	CV (%): 3.98			
	SPF		**Antioxidant potential (μg/mL^{-1})**	
Leaves	8.20 ± 0.25 a A	7.01 ± 0.74 a A	14.82 ± 0.83 a A	12.36 ± 0.16 b B
Stems	2.72 ± 0.02 a B	2.85 ± 0.13 a B	14.06 ± 0.70 a A	14.61 ± 0.44 a A
	CV (%): 6.77		CV (%): 3.59	
Lagoa Grande Settlement				
Season	**Spring**	**Autumn**	**Spring**	**Autumn**
Leaves/Stems	**Phenolic compounds (mg AGE g^{-1})**		**Flavonoids (mg RE g^{-1})**	
Leaves	198.13 ± 9.71 b A	300.80 ± 11.55 a A	17.41 ± 0.48 b A	31.39 ± 0.20 a A
Stems	195.47 ± 8.35 b A	243.47 ± 2.77 a B	7.21 ± 0.36 b B	12.94 ± 0.28 a B
	CV (%): 3.75		CV (%): 3.75	
	Tannins (mg TAE g^{-1})			
Leaves	262.29 ± 23.44 a A		235.18 ± 3.06 b B	

Table 4 – Contents of phenolic compounds, flavonoids, tannins, pH, SPF, and antioxidant potential of aqueous extracts from leaves and stems of *Serjania marginata* Casar. harvested in the spring and autumn at Santa Madalena Farm and Lagoa Grande Settlement in Dourados, MS, Brazil

(conclusion)

	Lagoa Grande Settlement			
Season	**Spring**	**Autumn**	**Spring**	**Autumn**
Leaves/Stems	**Phenolic compounds (mg AGE g^{-1})**		**Flavonoids (mg RE g^{-1})**	
Stems	250.51 ± 16.22 a B		255.51 ± 4.93 a A	
	CV (%): 3.98			
	SPF		**Antioxidant potential (μg/mL^{-1})**	
Leaves	4.56 ± 0.24 b A	8.54 ± 2.37 a A	14.79 ± 0.33a B	13.42 ± 0.33 a A
Stems	2.33 ± 0.20 b B	3.00 ± 0.19 a B	20.95 ± 0.58 a A	14.63 ± 0.57 b A
	CV (%): 6.77		CV (%): 3.59	

Source: Prepared by the Authors (2023). Means followed by the same lowercase letters within rows and uppercase l etters within columns, for each characteristic, do not significantly differ based on the t test at a 5% probability level

The divergence in phenolic compound content among various plant organs is well-documented. These differences can often be attributed to specific functions and responses to environmental factors (Erst *et al*., 2023). The literature underscores the strong correlation between secondary metabolite production and factors such as climatic conditions, circadian cycles, seasonality, plant maturity, geographic location, time of harvesting, and solar radiation exposure (Gobbo-Neto; Lopes, 2007; Rodrigues, 2018). The chemical and physical characteristics of the soil can be added to these abiotic factors. In this regard, it was observed that, in general, the highest levels of phenolic compounds, flavonoids and tannins occurred in SM. This result can be attributed to soil fertility, since even though the soils of the Cerrado are very low (Lambers et al., 2020), one of the SM presented base saturation above 50%, which characterizes it as eutrophic soil (Table 2). For instance, flavonoids, predominantly located in the superficial tissues of plants, serve as ultraviolet filters, shielding plants from the deleterious effects of certain wavelengths (Gobbo-Neto; Lopes, 2007).

Zanatta et al. (2021) emphasized the importance of studying seasonal variations influence on medicinal plants chemical composition particularly to determine if distinct climatic conditions contribute to chemical variations, as observed in *S. marginata* leaves. Souza et al. (2021) reported that certain medicinal plant species tend to produce higher quantities of tannins during the autumn and winter seasons while reducing production during the spring, a period conducive to reproduction (Souza et al., 2021). Seasonality emerges as a determining factor affecting the quantification of bioactive compounds in medicinal plants, thereby implying standardization when employed for medicinal purposes.

3.2 Assessment of antioxidant and photoprotective potentials

Regarding antioxidant potential, aqueous extracts of leaves and stems harvested from SM exhibited similar performance in the spring harvest, resulting in the highest levels of phenolic compounds, flavonoids and tannins (Table 4). However, in the autumn harvest, aqueous extracts of stems displayed greater antioxidant potential. In LG, aqueous extracts from stems harvested in the spring exhibited higher antioxidant potential than leaves, while in the autumn harvest, their antioxidant potentials were comparable. Concerningantioxidant potential,harvesting stems in the spring in LG is recommended (Table 4). These results are consistent with the statement by Zanatta et al. (2021) that seasonality is one of the major environmental factors that influence the synthesis and accumulation of secondary metabolites in medicinal plants. Furthermore, the results shows that all examined aqueous extracts exhibited high antioxidant potential (Table 4), as described by Reynertson, Basile, and Kenelly (2005), who categorize such potential as high when CI50 values fall below 50 μg mL^{-1}. The strong antioxidant activity of many of these metabolites was also found to have beneficial effects on human health.

Heredia-Vieira et al. (2014) reported high antioxidant potential for the hydroalcoholic extract of *S. marginata* leaves, linking this outcome to tannins (proanthocyanins). The authors also associated tannins with the gastroprotective potential of this species. Conversely, other studies attribute the antioxidant, gastroprotective, and anti-inflammatory potential of *S. marginata* leaves to the synergistic effect of tannins and glycosylated flavonoids (Périco *et al*., 2015; Leitão et al., 2022).

In photoprotective evaluation, the sun protection factor (SPF) was highest in the aqueous extracts of leaves harvested from SM, regardless of the harvesting time. In LG, the highest SPF was found in the aqueous extract of leaves harvested in the autumn (Table 4). The photoprotective activity of plants is associated with safeguarding against ultraviolet radiation, which can exert harmful effects on plant tissues. Consequently, plants synthesize substances as a protective mechanism against such effects (Silva Júnior et al., 2022). Tannins and flavonoids are integral to this protective function, and these chemical classes are found in numerous *Serjania* species. In *S. marginata*, these compounds are described in its leaves (Heredia-Vieira *et al*., 2014; Moreira et al., 2019), while *S. erecta* features them in leaves, stems, and roots (Gomig et al., 2008; Cardoso et al., 2013), and *S. schiedeana* possesses them in its stems (Salinas-Sánchez et al., 2017).

The Brazilian Health Regulatory Agency (ANVISA), through RDC no. 30 of July 1, 2012, mandates that cosmetic products or formulations intended for use as photoprotectors must possess an SPF of 6 or higher (Resolução – RDC nº 30, 2012). This resolution substantiates the findings obtained for the aqueous extracts of S. marginata leaves harvested in SM, regardless of harvesting time, and those harvested in LG in the autumn, indicating the potential therapeutic use of the species.

In terms of the correlation between SPF and the chemical composition of *S. marginata*, both for leaves and stems, a strong positive correlation exists between SPF and levels of phenolic compounds, flavonoids, and tannins, except leaves with phenolic compounds, which display a moderate correlation, and tannins, which demonstrate a negative correlation. Conversely, the antioxidant potential of leaves and stems exhibits a negative correlation with phenolic compounds and flavonoids, with stems demonstrating a very strong correlation. Regarding tannins, a negative correlation is observed for stems and a positive one for leaves (Table 5).

Table 5 – Pearson correlation analysis of phenolic compounds, flavonoids, and tannins with Sun Protection Factor (FPS) and antioxidant potential of aqueous extracts from leaves and stems of *Serjania marginata* Casar

Correlation	Leaves	Stems
SPF X Phenolic compounds	0.60	0.72
SPF X Flavonoids	0.76	0.84
SPF X Tannins	-0.32	0.76
Antioxidant X Phenolic compounds	-0.11	-0.96
Antioxidant X Flavonoids	-0.54	-0.90
Antioxidant X Tannins	0.22	-0.43

Source: Prepared by the Authors (2023)

The significance of the strong correlation between phenolic compounds and flavonoids with antioxidant potential resides in the fact that these metabolites play crucial roles in scavenging free radicals, a function essential for treating and preventing diseases. Barros et al. (2018) expound upon the beneficial biological properties facilitated by phenolic compounds, positing that these compounds possess therapeutic potential for wound treatment, alleviating symptoms associated with skin diseases, and even impeding the progression of such conditions. Natural antioxidants are integral to counteracting aging and are linked to SPF, making them suitable for topical and oral administration (Gallina, 2021).

3.3 Assessment of antimicrobial activity

All assessed aqueous extracts demonstrated antibacterial activity (Table 6), with no significant differences in Minimum Inhibitory Concentration (MIC) values based on seasons, harvesting locations, or plant organs. However, the aqueous extracts exhibited strong activity against the bacteria *S. epidermidis* and *S. aureus*, while against other bacteria, their activity was moderate. The positive control (tetracycline) displayed an MIC of 3.75 μg mL^{-1}. As per Bernardi et al. (2017), plant extracts with MIC values below 100 μg mL^{-1} have high antimicrobial activity. In contrast, those with MIC values between 100 and 500 µg mL^{-1} are classified as active.

Table 6 – Antimicrobial activity of aqueous extracts from leaves and stems of *Serjania marginata* Casar. Harvested at Santa Madalena Farm (SM) and Lagoa Grande Settlement (LG) in two seasons of the year. Caption: Spr. = spring; Aut. = autumn; MIC = minimum inhibitory concentration (µg mL^{-1})

Bacteria	**Santa Madalena Farm MIC (µg mL^{-1})**				**Lagoa Grande Settlement MIC (µg mL^{-1})**			
	Leaves		**Stems**		**Leaves**		**Stems**	
	Spr.	**Aut.**	**Spr.**	**Aut.**	**Spr.**	**Aut.**	**Spr.**	**Aut.**
Burkholderia cepacia	125	125	125	125	125	125	125	125
Enterococcus faecalis	125	125	125	125	125	125	125	125
Escherichia coli	125	125	125	125	125	125	125	125
Pseudomonas aeruginosa	125	125	125	125	125	125	125	125
Staphylococcus epidermidis	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Staphylococcus aureus	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Staphylococcus saprophyticus	125	125	125	125	125	125	125	125

Source: Prepared by the Authors (2023)

S. marginata has previously been acknowledged for its antimicrobial potential. The hydroalcoholic extract of its leaves has shown efficacy against *Helicobacter pylori*, *S. aureus* (MIC 125 µg ml^{-1}), *E. coli* (MIC 250 µg ml^{-1}), *Salmonella setubal* (MIC 250 µg mL^{-1}), and *Candida albicans* (MIC 250 µg mL^{-1}) (Périco et al., 2015).

Other species within the *Serjania* genus have also exhibited antimicrobial potential. Ethanolic extracts from leaves and stems of *S. lethalis* displayed antibacterial activity against both susceptible and resistant strains of *S. aureus* (MIC ≤100 µg mL^{-1}) (Lima *et al*., 2006). For *S. erecta*, ethanolic extracts from leaves and stems inhibited the growth of *Mycobacterium tuberculosis*, with MIC values of 128 and 256 µg mL^{-1}, respectively, and were effective against S. *aureus, P. aeruginosa, S. setubal, C. albicans, Saccharomyces cerevisiae*, and *E. coli*, with MIC values ranging from 5 to 25 µg mL^{-1} (Cardoso et al., 2013).

3.4 Toxicity assessment

None of the aqueous extracts of *S. marginata* assessed in this study demonstrated toxicity towards *A. salina* at the concentrations tested. The selected toxicological model, widely employed in natural product studies, utilizes *A. salina* due to its simple anatomy and short life cycle. Moreover, it offers a quick and cost-effective testing method, particularly advantageous for preliminary toxicological assessments involving multiple samples (Ntungwen et al., 2020).

Both aqueous and hydroalcoholic extracts of *S. marginata* leaves have previously been determined to be non-toxic *in vivo* tests involving a single-dose oral administration (2,000 mg kg^{-1}) (Périco et al., 2015; Moreira et al., 2019).

4 FINAL REMARKS

A higher concentration of phenolic compounds was observed in aqueous extracts derived from stems of *S. marginata*, regardless of harvesting time, when harvested in SM, or in leaves harvested in the autumn in LG. As for flavonoids, the most elevated levels were detected in the aqueous extracts of leaves, irrespective of harvesting time, when harvested in SM, or in the autumn in LG. Regarding tannins, the aqueous extracts of leaves, when harvested in the spring season at any location, exhibited the highest concentrations.

The highest levels of phenolic compounds, flavonoids and tannins occurred, in general, in leaves and stems harvested in SM, probably due to base saturation above 50%, which characterizes it as eutrophic soil.

Geographic origin and seasonality were found to not influence the antibacterial activity of aqueous extracts derived from the leaves and stems of *S. marginata*. None of the aqueous extracts displayed toxicity against *A. salina* at the tested concentrations.

The aqueous extract of *S. marginata* leaves harvested in SM at any time of the year and the extract of stems harvested in the autumn in LG exhibited the highest Sun Protection Factor (SPF). Meanwhile, the aqueous extract of stems harvested in the spring season in LG demonstrated superior antioxidant potential.

These findings serve as a reference for selecting the optimal time to harvest *S. marginata* leaves and stems based on the constituent of interest and for using the plant and its constituents for antimicrobial and antioxidant medicinal purposes, as well as for formulating topical products such as sunscreen.

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REFERENCES

Alves J. M. (2015). *Características de plantas de Serjania marginata Casar. propagadas e cultivadas em substratos à base de resíduos orgânicos e fósforo* (Master's Dissertation in General Biology). Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, Repositório Institucional da UFGD, Dourados, Mato Grosso do Sul, Brasil. Recuperado de https://repositorio.ufgd.edu.br/jspui/handle/prefix/703

Barros, N. R., Heredia-Vieira, S. C., Borges, F. A., Benites, N. M., Reis, C. E. D., Miranda, M. C. R., Cardoso, C. a. L., & Herculano, R. D. (2018). Natural rubber latex biodevice as controlled release system for chronic wounds healing. *Biomedical Physics & Engineering Express*, *4*(3), 035026. Recuperado de https://doi.org/10.1088/2057-1976/aab33a

Bernardi, R. C., Santos-Junior, L. C., Guimarães, I. C., Macorini, L. F. B., Antonialli-Junior, W. F., & Cardoso, C. A. L. (2017). Screening do potencial da peçonha da formiga *Odontomachus chelifer* (Fowler, 1980) como fonte de agentes terapêuticos. *Interbio*, *11*, 55-62.

Bourdy, G., Michel, L. C., & Roca-Coulthard, A. (2004). Pharmacopoeia in a shamanistic society: the Izoceño-Guaraní (Bolivian Chaco). *Journal of Ethnopharmacology*, *91*(2–3), 189–208. Recuperado de https://doi.org/10.1016/j.jep.2003.09.013

Cardoso, C. A. L., Coelho, R. G., Honda, N. K., Pott, A., Pavan, F. R., & Leite, C. Q. F. (2013). Phenolic compounds and antioxidant, antimicrobial and antimycobacterial activities of *Serjania erecta* Radlk. (Sapindaceae). *Brazilian Journal of Pharmaceutical Sciences*, *49*(4), 775–782. Recuperado de https://doi.org/10.1590/s1984.825.0201300.040.0017

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, *97*(4), 654–660. Recuperado de https://doi.org/10.1016/j.foodchem.2005.04.028

Dutra, E. A., Oliveira, D. A. G. C., Kedor-Hackmann, E. R. M., & Santoro, M. I. R. M. (2004). Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. *Revista Brasileira De Ciências Farmacêuticas*, *40*(3), 381–385. https://doi.org/10.1590/s1516.933.2200400.030.0014

Embrapa. (2020). *Guia Clima – Estação Embrapa Agropecuária Oeste*. Dourados: Autor. Recuperado de https://clima.cpao.embrapa.br/?lc=site/banco-dados/base_dados.

Erst, A. A., Kotsupiy, O. V., Erst, A. S., & Kuznetsov, A. A. (2023). Individual Differences in Growth and in Accumulation of Secondary Metabolites in *Rhodiola rosea* Cultivated in Western Siberia. *International Journal of Molecular Sciences*, *24*(14), 11244. https://doi.org/10.3390/ijms241411244

Reflora. Flora e Funga do Brasil. (2023). *Serjania in Flora e Funga do Brasil*. Jardim Botânico do Rio de Janeiro. Recuperado de: https://floradobrasil.jbrj.gov.br/FB20963

Gallina, P. R. (2021). Fitocosméticos antioxidantes: utilização de fitoativos como recurso para tratamentos antienvelhecimento. *Revista Brasileira de Práticas Integrativas e Complementares em Saúde*, *1*(2), 31–38. Recuperado de https://www.revistasuninter.com/revistasaude/index.php/revista-praticas-interativas/article/view/1245

Gobbo-Neto, L., & Lopes, N. P. (2007). Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, *30*(2), 374–381. Recuperado de https://doi.org/10.1590/s0100.404.2200700.020.0026

Gomig, F., Pietrovski, E. F., Guedes, A., Dalmarco, E. M., Calderari, M. T., Guimarães, C. L., Pinheiro, R. M., Cabrini, D. A., & Otuki, M. F. (2008). Topical anti-inflammatory activity of *Serjania erecta* Radlk (Sapindaceae) extracts. *Journal of Ethnopharmacology*, *118*(2), 220–224. Recuperado de https://doi.org/10.1016/j.jep.2008.03.017

Heredia-Vieira S. C., Falco M. E., Motti P. R., Porto, K. R.A., Roel A. R., Vieira M. C., Zárate, N. A. H., Vilegas, W., & Cardoso, C. A. L. (2017). Bioprospecting *Serjania marginata* Casar.: chemical and larvicidal evaluation and agricultural productivity. In M. C. Soares & M. A. G. Jardim (Org.), *Natural resources in wetlands from Pantanal to Amazonia* (pp.182-205). Museu Paraense Emilio Goeldi. Belém: Museu Paraense Emílio Goeldi.

Heredia-Vieira, S. C., Simonet, A. M., Vilegas, W., & Macías, F. A. (2014). Unusual C,O-Fused Glycosylapigenins from *Serjania marginata* Leaves. *Journal of Natural Products*, *78*(1), 77–84. Recuperado de https://doi.org/10.1021/np500715x

Kumaran, A., & Karunakaran, R. J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food Chemistry*, *97*(1), 109–114. Recuperado de https://doi.org/10.1016/j.foodchem.2005.03.032

Lambers, H., Costa, P. B., Oliveira, R. S., & Silveira, F. A. O. (2020). Towards more sustainable cropping systems: lessons from native Cerrado species. *Theoretical and Experimental Plant Physiology*, *32*, 175–194. Recuperado de https://doi.org/10.1007/s40626.020.00180-z

Leitão, M. M., Radai, J. A. S., Macorini, L. F. B., Fraga, T. L., Heredia-Vieira, S. C., Cardoso, C. a. L., Arena, A. C., & Kassuya, C. a. L. (2022). *Serjania marginata* Casar. Hydroalcoholic Extract Reduced Cytokine and Inflammatory Parameters in Experimental Models of Inflammation and Infection in Mice. *Pharmacognosy Research*, *14*(2), 127–134. Recuperado de https://doi.org/10.5530/pres.14.2.18

Leitão, M. M., Silva-Filho, S. E., Arena, A. C., Heredia-Vieira, S. C., Cardoso, C. a. L., & Kassuya, C. a. L. (2023). Antinociceptive and anti-inflammatory properties of aqueous extract obtained from *Serjania marginata* Casar leaves. *Journal of Ethnopharmacology*, *304*, 116018. Recuperado de https://doi.org/10.1016/j.jep.2022.116018

Lima, M. R. F., Luna, J. S., Santos, A. F. D., Andrade, M. C. C., Sant'Ana, A. E. G., Genet, J., Marquez, B., Neuville, L., & Moreau, N. (2006). Anti-bacterial activity of some Brazilian medicinal plants. *Journal of Ethnopharmacology*, *105*(1–2), 137–147. Recuperado de https://doi.org/10.1016/j.jep.2005.10.026

Majtan, J., Bucekova, M., Kafantaris, I., Szweda, P., Hammer, K., & Mossialos, D. (2021). Honey antibacterial activity: A neglected aspect of honey quality assurance as functional food. *Trends in Food Science & Technology*, *118*, 870–886. Recuperado de https://doi.org/10.1016/j.tifs.2021.11.012

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, J. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, *45*(5), 31–34. Recuperado de https://doi.org/10.1055/s-2007-971236

Ministéio da Saúde. (2008). *Portaria Interministerial n. 2.960 de 9 de dezembro de 2008.* Aprova o Programa Nacional de Plantas Medicinais e Fitoterápicos e cria o Comitê Nacional de Plantas Medicinais e Fitoterápicos. Recuperado de https://bvsms.saude.gov.br/bvs/saudelegis/gm/2008/pri2960_09_12_2008.html.

Ministério da Saúde. (2012). *Resolução – RDC nº 30, de 1º de junho de 2012*. Aprova o Regulamento Técnico Mercosul sobre Protetores Solares em Cosméticos e dá outras providências. Recuperado de https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2012/rdc0030_01_06_2012.html#:~:text=Aprova%20o%20Regulamento%20T%C3%A9cnico%20Mercosul,Regulamento%20aprovado%20pelo%20Decreto%20n.

Moreira, S. S., Tamashiro, L. K., Jorge, B. C., Balin, P. S., Heredia-Vieira, S. C., Almeida, G. L., Cardoso, C. a. L., Kassuya, C. a. L., & Arena, A. C. (2019). Toxicological safety evaluation in acute and 28-day studies of aqueous extract from Serjania marginata Casar. (Sapindaceae) leaves in rats. *Journal of Ethnopharmacology*, *231*, 197–204. Recuperado de https://doi.org/10.1016/j.jep.2018.11.024

Napolitano, D. R., Mineo, J. R., Souza, M. A., Paula, J. E., Espindola, L. S., & Espindola, F. S. (2005). Down-modulation of nitric oxide production in murine macrophages treated with crude plant extracts from the Brazilian Cerrado. *Journal of Ethnopharmacology*, *99*(1), 37–41. Recuperado de https://doi.org/10.1016/j.jep.2005.01.059

Ntungwe, E. N., Domínguez-Martín, E. M., Roberto, A., Tavares, J., Isca, V. M. S., Pereira, P., Cebola, M., & Rijo, P. (2020). Artemia species: An Important Tool to Screen General Toxicity Samples. *Current Pharmaceutical Design*, *26*(24), 2892–2908. Recuperado de https://doi.org/10.2174/138.161.2826666.200.406083035

Pansera, M. R., Santos, A. C. A., Paese, K., Wasum, R., Rossato, M., Rota, L. D., Pauletti, G. F., & Serafini, L. A. (2003). Análise de taninos totais em plantas aromáticas e medicinais cultivadas no Nordeste do Rio Grande do Sul. *Revista Brasileira de Farmacognosia*, *13*(1), 17–22. Recuperado de https://doi.org/10.1590/S0102-695X200.300.0100002

Perico L. L., Heredia-Vieira S. C., Beserra F. P., Santos R. C., Weiss M. B., Resende F. A., Ramos, M. A. S., Bonifácio, B. V., Bauab, T. M., Varanda, E. A., Gobbi, J. I. F., Rocha, L. R. M., Vilegas, W., & Hiruma-Lima, C. A. (2015). Does the gastroprotective action of a medicinal plant ensure healing effects? An integrative study of the biological effects of *Serjania marginata* Casar. (Sapindaceae) in rats. *Journal of Ethnopharmacology*, *172*, 312–324. Recuperado de https://doi.org/10.1016/j.jep.2015.06.025

Reynertson, K. A., Basile, M. J., & Kennelly, E. J. (2005). Antioxidant Potential of Seven Myrtaceous Fruits. *Ethnobotany Research & Applications*, *3*, 25-36. Recuperado de https://scholarspace.manoa.hawaii.edu/items/1c98267f-fb65-4f97-822e-9de57d996b79

Rodrigues, A. T. (2018). *Farmaconosia*. Londrina: Editora e Distribuidora Educacional.

Saeg. (2007). *SAEG: Sistema para Análises Estatísticas – Versão 9.1.* Fundação Arthur Bernardes. Viçosa, MG: Autor.

Salinas-Sánchez, D., Jiménez-Ferrer, E., Sánchez-Sánchez, V., Zamilpa, A., González-Cortazar, M., Tortoriello, J., & Herrera-Ruiz, M. (2017). Anti-Inflammatory Activity of a Polymeric Proanthocyanidin from *Serjania schiedeana*. *Molecules*, *22*(6), 863. Recuperado de https://doi.org/10.3390/molecules22060863

Serpeloni, J. M., Specian, A. F. L., Ribeiro, D. L., Tuttis, K., Heredia-Vieira, S. C., Vilegas, W., Martínez-López, W., Varanda, E. A., & de Syllos Cólus, I. M. (2021). Selective anticancer effects of *Serjania marginata* Casar. extract in gastric cells are mediated by antioxidant response. *Environmental Toxicology*, *36*(8), 1544–1556. Recuperado de https://doi.org/10.1002/tox.23151

Silva Junior, N. M., Lima, L. S., Bandeira, M. L. S. F., Costa, J. A. S., & Leite, A. B. (2022). Avaliação do potencial antioxidante e fotoprotetor em plantas coletadas na Mata Atlântica do sul da Bahia. *Ciência E Natura*, *44*, e62. Recuperado de https://doi.org/10.5902/2179460x66491

Souza, A. P., Mota, L. L., Zamadei, T., Martin, C. C., Almeida, F. T., & Paulino, J. (2013). Classificação climática e balanço hídrico climatológico no estado de Mato Grosso. *Nativa*, *1*(1), 34-43. Recuperado de https://doi.org/10.31413/nativa.v1i1.1334

Souza, J. B., Azevêdo, T. K. B., Pimenta, A. S., Gomes, J. P. S., Meza Filho, J. G. U., & Silva, B. R. F. D. (2021). Seasonality of the bark tannins content of five-year-old Acacia mangium trees grown in northeast Brazil. *Revista Árvore*, *45*, e4523. Recuperado de https://doi.org/10.1590/1806.908.820210000023

Tabaldi, L. A., Vieira, M. D. C., Zárate, N. A. H., Silva, L. R. D., Gonçalves, W. L. F., Pilecco, M., Formagio, A. S. N., Gassi, R. P., & Padovan, M. P. (2012). Cover crops and their effects on the biomass yield of *Serjania marginata* plants. *Ciência Rural*, *42*(4), 614–620. Recuperado de https://doi.org/10.1590/s0103.847.8201200.040.0006

Zanatta A. C., Vilegas W, & Edrada-Ebel R. (2021). UHPLC-(ESI)-HRMS and NMR-based metabolomics approach to access the seasonality of *Byrsonima intermedia* and *Serjania marginata* From Brazilian Cerrado Flora Diversity. *Frontiers in Chemistry*, 9, 1-17. Recuperado de https://doi.org/10.3389/fchem.2021.710025

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