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Chemical Composition and Photoprotective Potential of Infusion Extract from *Casearia sylvestris* var. *lingua* (Cambess.) Eichler Leaves

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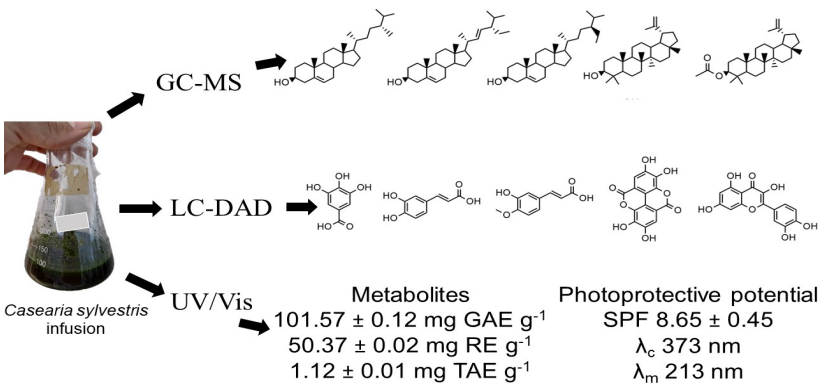
Casearia sylvestris var. *lingua* (Cambess.) Eichler is widely used in traditional medicine to treat diseases. Simultaneously, the growing search for phytocosmetics has culminated in the exploration of plant extracts. In this context, this study aimed to obtain the chemical composition and antioxidant and photoprotective potential of the infusion of *C. sylvestris* var. *lingua* leaves. The techniques of UV/Vis spectroscopy, gas chromatography with mass spectrometry detector (GC-MS) and liquid chromatography with diode array detector (LC-DAD) were used to get the results. The total phenolic content in the infusion of leaves from *C. sylvestris* var. *lingua* was 101.57 mg GAE g^{-1}, flavonoids 50.37 mg RE g^{-1} and tannins 1.12 mg TAE g^{-1}. Quercetin, ferulic acid, gallic acid, ellagic acid, caffeic acid, β-sitosterol, lupeol, lupeol acetate, stigmasterol and campesterol were identified and quantified in the samples. The infusion of C. sylvestris var. *lingua* leaves has potential for application in phytocosmetics and sunscreens, with a sun protection factor of 8.65 ± 0.45, a UVA/UVB ratio of 1.16 and a critical wavelength of 373.

Graphical abstract



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1. Introduction

The photoprotective action in cosmetics aims to protect against ultraviolet (UV) and infrared (IR) radiation, which accelerates the ageing of the skin, producing free radicals that damage DNA, hindering cell replication [1]. Protection against UV radiation occurs through products with a sun protection factor (SPF) and for IR, compounds with antioxidant activity are used [1].

Using extracts in cosmetic preparations is an alternative to this demand that can bring benefits due to phenolic

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compounds in its composition since such compounds have biological activities that help prevent premature ageing [2, 3].

Phenolic compounds and flavonoids are employed in the cosmetic industry and are used as antioxidants, antimicrobials and also as photo protectors [4]. The class of tannins also showed interest in the cosmetic industry, as they act as anti-inflammatory, antiseptic and act as tonic by reducing the size of the skin's pores [1].

The study by Mohamed and Sorour [5] obtained a correlation between the photoprotective activity and the presence of tannins in the extract of 4 medicinal plants (*Pluchea discoridis*, *Lawsonia inermis*, *Aloe vera* and *Eucalyptus camaldulensis*).

Steroids also interest the cosmetics industry, as they are associated with several functions, such as hair and skin conditioners, viscosity regulators and skin protectors [6].

Brazil has a strategic role in the search for extracts rich in secondary metabolites, because of its rich biodiversity [7]. In this context, the Cerrado is the second largest biome in terms of extension, located in the center of the country and formed by pastures, savannas and forests [8]. The study by Santos et al. [9] carried out a survey of plants in a reserved area and identified 89 different species of medicinal plants from 39 different families. In addition, the authors point out that only three of these species are included in the National List of Medicinal Plants of Interest in the Single Health System of Brazil (SUS).

Casearia sylvestris Sw. is included in the UHS list. It is a species popularly used as anti-inflammatory, antiviral, antiphonic and for healing [10]. The infusion of leaves from *C. sylvestris* is recommended by the regional council of pharmacy for topical use to treat pain and injuries such as aseptic and healing, as well as internal use to treat dyspepsia, gastritis, and halitosis [11]. However, Castro, Santos, and Cardoso [12] point out that the aqueous extract of *C. sylvestris* is still little explored in the literature. *Casearia sylvestris* var. *lingua* (Cambess.) Eichler is most common in Brazilian Cerrado, with fewer diterpenes compared to the *Casearia sylvestris* var *sylvestris* (common in Atlantic Forest) [13].

This study aimed to determine the chemical composition of the infusion of leaves from *C. sylvestris* var. *lingua*, as well as the sun protection factor to assess the potential for its use in phytocosmetics formulations with photoprotective properties.

2. Results and Discussion

The extraction showed a yield of 23.18%. The extract from the infusion of leaves from *C. sylvestris* var. *lingua* had a higher content of phenolic compounds concerning the levels of flavonoids and tannins (Table 1). This result is consistent, as phenolic compounds represent a larger group of constituents compared to flavonoids and tannins [14].

Buccioli et al. [15] identified flavonoids, catechins and tannins in the aqueous extract of C. sylvestris leaves. Sertié, Carvalho and Panizza [16] identified tannins in the ethanolic and aqueous extracts of *C. sylvestris* leaves, saponins in the aqueous extract and absence of flavonoids in both extracts.

Bueno et al. [17] identified 16 flavonoids from the extract of the leaves of *C. sylvestris* var. *lingua* obtained with a mixture of water:ethanol:isopropanol (5:3:2 v/v). The study by Anhesine et al. [18] studied the species of *Casearia ulmifolia* Vahl ex Vent., *Casearia lasiophylla* Eichler, *Casearia javitensis* Kunth, *Casearia decandra* Jacq, *Casearia grandiflora* Cambess. and *Casearia arborea* (Rich.) Urb. and obtained different flavonoids from the ethanol extracts of the leaves.

Table 1. Phenolic compounds, flavonoids, tannins and DPPH radical inhibition.

	Mean ± SD
Phenolic compounds	101.57 ± 0.12 mg GAE g^{-1}
Flavonoids	50.37 ± 0.02 mg RE g^{-1}
Tannins	1.12 ± 0.01 mg TAE g^{-1}
DPPH radical inhibition	161.29 ± 0.93 µg mL^{-1}

GAE = gallic acid equivalent; RE = rutin equivalent; TAE = tannic acid equivalent; DPPH = 2,2-diphenyl-1-picrylhydrazyl; SD = Standard deviation.

Regarding tannins, Boege [19] reported the presence in the leaves of *Casearia nitida* Jacq. and Weniger et al. [20] also identified tannins in leaves of *Casearia ilicifolia* Vent. The infusion of *C. sylvestris* var. *lingua* leaves also presented tannins (Table I).

The infusion of *C. sylvestris* leaves (Table 1) showed antioxidant potential higher than that reported by Menezes, Schwarz and Santos [21] for the aqueous extract (471.80 µg mL^{-1}), but lower than that obtained for the ethanol extract (5.70 µg mL^{-1}).

Three phytosterols and two triterpenoids were also identified and quantified in the extract from the infusion of *C. sylvestris* var. *lingua* leaves by GC-MS (Figure 1 and Table 2). Such compounds are relevant to producing drugs, also in the composition of functional foods and cosmetics [22].

Table 2. Compounds quantified by GC-MS of the extract from *C. sylvestris* var. *lingua*.

Number	**Compound**	**Mean (mg g^{-1}) ± SD**
1	Campesterol	4.30 ± 0.01
2	Stigmasterol	4.40 ± 0.02
3	β-Sitosterol	17.70 ± 0.02
4	Lupeol	12.56 ± 0.01
5	Lupeol acetate	12.56 ± 0.03

SD = Standard deviation.

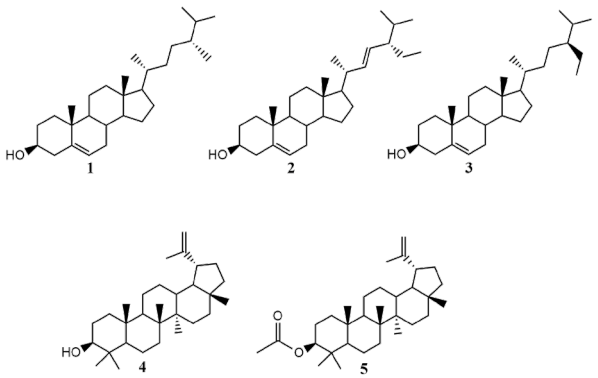

Fig. 1. Chemical structures of the compounds identified in the infusion of *C. sylvestris* var. *lingua* leaves by GC-MS. Font: Author (2022).

According to a review by Fernandes and Cabral [22], β-sitosterol, stigmasterol and campesterol are among the most common phytosterols in plant extracts. Of these compounds, β-sitosterol was the most abundant compound among the quantified compounds (Table 2). The presence of β-sitosterol has already been identified in the leaves of *C. ulmifolia*, *C. lasiophylla*, *C. javitensis*, *C. decandra*, *C. grandiflora* and *C. arborea* [18]. β-sitosterol is associated with anti-inflammatory activity [23].

Phytosterols are associated with increased membrane fluidity through the desaturation of fatty acids in the skin, besides helping to delay skin ageing [23]. Thus, the topical application of phytosterols helps in the recovery of the skin's protective barrier [24], indicating that the aqueous extract of *C. sylvestris* var. *lingua* has potential for studies of photoprotective phytocosmetic formulations.

In the analysis by LC-DAD, five phenolic compounds were identified (Figure 2), quercetin was the most abundant in the sample (Table 3). Quercetin has a photoprotective effect, showing a sun protection factor (SPF) like standards when incorporated in oil-in-water emulsions, up to a concentration of 10% (m/m), also protects the UVA region [25], presenting the advantage of increasing the stability of common sunscreens due to its antioxidant capacity [26].

Table 3. Chemical composition identified from the LC-DAD of the extracts (mg g^{-1} ± SD).

Number	Compound	Mean (mg g^{-1}) ± SD
1	Gallic acid	10.38 ± 0.01
2	Caffeic acid	5.68 ± 0.02
3	Ferulic acid	22.12 ± 0.01
4	Ellagic acid	8.13 ± 0.01
5	Quercetin	51.56 ± 0.02

SD: Standard deviation

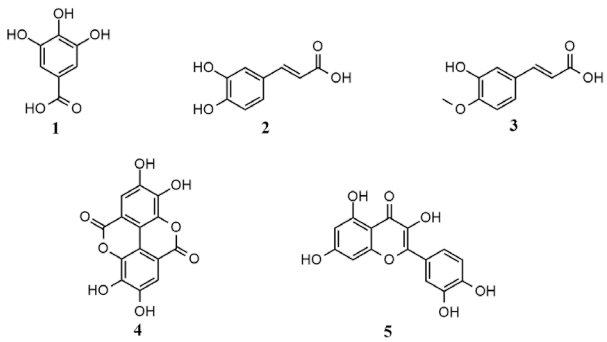

Fig. 2. Chemical structures of the compounds identified in the infusion of *C. sylvestris* var. *lingua* leaves by LC-DAD. Font: Author (2022).

Silva et al. [27] have isolated two gallic acid derivatives from the leaves of *C. sylvestris*. The research by Bueno et al. [28] analyzed the composition of *C. sylvestris* leaves collected in different regions and reported that samples from the Atlantic Forest have higher levels of clerodane-type diterpenes, while samples from the Cerrado have a predominance of phenolic compounds and other secondary metabolites.

Despite the absence of antioxidant activity in the extract, the presence of phenolic compounds, flavonoids and tannins indicates that the extract may have other applicabilities. Within this context, the sun protection factor of the extract was investigated.

The radiation in the UV region is divided into UVA (320 to 400 nm), UVB (290 to 320 nm) and UVC (200 to 290 nm) [29]. UVA radiation has great penetration into the skin causing tanning, photoaging and skin cancer, UVB radiation has medium penetration and promotes erythema, skin ageing and skin cancer and UVC radiation does not reach the earth's surface due to the ozone layer [1]. Scanning in the UV region indicated that the extract absorbs in the UVB region (peak in 388 nm) (Figure 3). It can be seen that most of the absorption of the infused extract of C. sylvestris var. lingua leaves occurs in the UVC region, with the maximum wavelength at 213 nm (Figure 3).

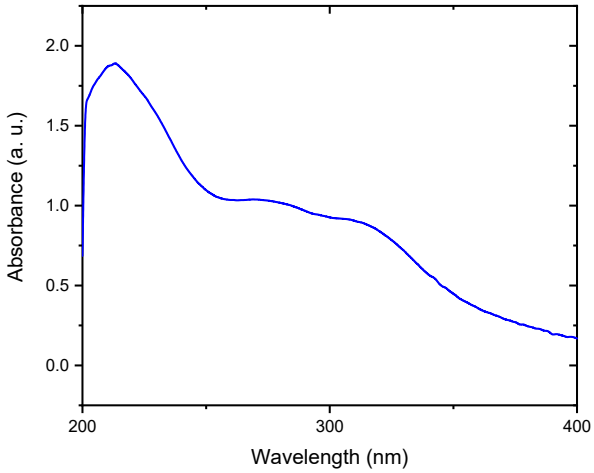

Fig. 3. Scan of *C. sylvestris* var. *lingua* infusion absorbance in the UV region. Font: Author (2022).

The FPS of the sample was 8.65 ± 0.45. According to ANVISA [29], products with SPF greater than 6 can be considered sunscreens, with values between 6.0 and 14.9 is indicated for skin that is not very sensitive to sunburn. As for the preparation of multifunctional cosmetics, the requirement is a minimum SPF of 2 [29], indicating that the extract also has great potential for this application. Another prerequisite for a product to be considered photoprotective is to have a critical wavelength (λ_c) greater than 370 nm [29], and the sample presented λ_c of 373. The λ_c determines the distribution of solar absorption across the region of UVA and UVB, thus a sunscreen with λ_c presents more uniform protection between the different wavelengths [30].

The UVA/UVB ratio serves as a parameter to verify the photoprotective action against UVA radiation [30]. The values of this parameter are classified by the Boot's Star Rating system, where values above 0.9 are considered “ultra” and receive 5 stars [30]. The sample had a UVA/UVB ratio of 1.16. It is required that sunscreens present UVA protection equivalent to at least 1/3 of the UVB [29]. In this sense, the infused extract of *C. sylvestris* var. *lingua* leaves is promising.

The high UVA/UVB ratio is related to the high λ_c, as the λ_c indicates a well-distributed absorption between UVA and UVB radiation. In this sense, the studied extract has the potential for the development of photo protectors and multifunctional cosmetics that act in the UVA and UVB regions.

3. Material and Methods

3.1 Plant material

The leaves of *C. sylvestris* var. *lingua* were harvested manually at the Campus of the Federal University of Grande Dourados, located in the municipality of Dourados-MS, Brazil and a specimen was deposited (DDMS 6409) in the herbarium of UFGD, MS, Brazil. The plant material was registered in SISGen under code A72622B.

3.2 Extract preparation

The leaves were ground in a mill (Wiley mill, Marconi) at a particle size of 10 mesh. The powder was stored in dark glass and frozen at -20 ºC.

The preparation of the infusion was carried out as described by Castro et al. [31] with modifications. For this purpose, distilled water (95 ºC) was added to the crushed leaves at a concentration of 20 g L^{-1} for a contact period of 30 minutes in a closed container. After the specified time, the extract was filtered and lyophilized in an Alpha 1-2LD Plus lyophilizer (Martin Christ). The extract was stored in glass flasks at a temperature of -20°C.

3.3 Phenolic, flavonoids and tannins contents

For the analysis of the phenolic compounds, flavonoids and tannins the extract was solubilized in ultrapure water at the concentration of 1 mg mL^{-1}. The absorbance in the tests was determined by spectrophotometry (Global Trade Technology, Brazil).

The total phenolic content was determined using Folin–Ciocalteu's reagent as described by Djeridane et al. [32]. Gallic acid (GA) was used as a standard to construct an analytical curve, and the result was expressed in mg of gallic acid equivalent (GAE) per g of lyophilized extract. The flavonoid content was also based on Djeridane et al. [32] work. For the flavonoid concentration, an analytical curve was performed using rutin as standard. The result was expressed in mg of rutin equivalent (RE) per g of lyophilized extract. The tannin content was determined using Folin from the methodology proposed by Ibe et al. [33], with tannic acid as the standard to determine the concentration of tannins. The result was expressed in mg of tannic acid equivalent (TAE) per g of lyophilized extract. The analyses were performed in triplicate.

3.4 Chromatographic analysis by GC-MS

For GC-MS analysis, 1 mg of the extract was added to 200 µL of ultrapure water and 200 µL of hexane, after phase formation the hexane fraction was separated from the aqueous fraction. To the aqueous fraction was added 200 µL of hexane and the process was repeated. After the two extractions, the hexane fractions were dried and suspended in 200 µL hexane and the solution was filtered in the 0.45 µm ultrafilter.

The sample was also evaluated by gas chromatography with a mass spectrometry detector (GC-MS). The GC-MS analysis was performed using a GC-2010 chromatographer (Plus, Shimadzu, Kyoto), equipped with a mass spectrometry detector (GC-MS Ultra 2010), using LM-5 (15 m length x 0.2 mm id, and 0.2 µm thick film). The analysis followed these conditions: helium gas (99.999% and flow rate 1 mL min^{-1}), 1 µL of injection volume, split (1:20), furnace initial temperature 150 ˚C and heating at 150 ˚C to 280 ˚C at 15 ˚C min^{-1} and hold at 280 ˚C for 15 min. The injector temperature was 280 ˚C and the quadrupole detector temperature was 290 ºC. The MS scanning parameters included an electron impact ionization voltage of 70 eV, a mass range of *m/z* 45-600 and a scanning interval of 0.3s. The identifications were performed by comparing the mass spectra with the NIST21 and WILEY229 libraries.

Standards of stigmasterol, campesterol, β- sitosterol, lupeol and lupeol acetate (Sigma, ⩾98%) were prepared in hexane at the concentration of 1000 µg mL^{-1}. The concentrations of compounds were determined by external calibration. The linearity for standards was assessed for 5 concentration ranges. The respective coefficients of determination (r2) were 0.9996 for stigmasterol, campesterol, β- sitosterol and lupeol and 0.9994 for lupeol acetate. The analyses were performed in triplicate.

3.5 Chromatographic analysis LC-DAD

The extract (1 mg mL^{-1}) was solubilized in ultrapure water and filtered in the 0.45 µm ultrafilter and analyzed in liquid chromatography (LC-DAD Shimadzu, Kyoto) with the aid of a diode array detector (DAD) which was monitored between 200 and 800 nm. The column was ODS HYPERSIL (C-18, 150 mm long x 4.6 mm diameter, Thermo Electron Corporation).

The flow rate and the injection volume were respectively 1 mL min^{-1} and 10 µL. All the chromatographic analyses took place at a temperature of 25 ˚C. The eluent A was composed of a binary mobile phase of water with 6% acetic acid and 2 mM of sodium acetate, and the eluent B, composed of acetonitrile and the following gradient was applied: 0 min 5 % B; 20 min 15 % B; 30 min 60 % B; and 40 min 100 % B. Standards of caffeic acid, ellagic acid, vanillic acid, sinapic acid, ferulic acid and gallic acid, rutin, luteolin, apigenin, naringin, kaempferol, and quercetin were used (Sigma, 98%), prepared in methanol-water at a concentration of 1000 µg mL^{-1}. Standards were easily identified and quantified based on their absorption spectra in the UV region and in retention time. The linearity for standards was assessed for 5 concentration ranges. The respective coefficients of determination (r^2) were 0.9994 for caffeic acid, ellagic acid, sinapic acid, vanillic acid, ferulic acid and gallic acid and 0.9996 for rutin, luteolin, apigenin, naringin, kaempferol, and quercetin.

3.6 Determination of antioxidant activity 2,2-diphenyl-1-picrylhydrazril (DPPH) inhibition

The extract was prepared at a concentration of 1 mg mL^{-1} for this analysis was performed in triplicate.

The antioxidant activity of the extract was evaluated by the free radical DPPH method (2,2-diphenyl-1-picrylhydrazril). The sample (0.1 mL) was added to 3 mL of 0.004% DPPH in methanol in contact for 30 minutes in dark, with a controlled temperature (25 ± 1ºC). The results are presented in inhibition concentration [34], after dilution for test.

3.7 Determination of the Sun Protection Factor (SPF), Critical Wavelength (λc) and UVA/UVB ratio

First, an exploratory scan between 200 and 400 nm was performed in a UV/Vis spectrophotometer with a quartz cuvette. The extract was diluted at a concentration of 0.2 mg mL^{-1}. The absorbance data obtained were used to calculate the sun protection factor (SPF), critical wavelength (λ_c) and UVA/UVB ratio.

To determine the SPF, it was used the absorbance between 290 and 320 nm using equation 1 in the calculation as described by Mansur et al. [35]. The multiplication values of the erythematogenous effect (EE_λ X I_λ) and light intensity

(Abs_λ) (Table 4) are described by Sayre et al [36]. The correction factor (CF) used was 10.

$$SPF = CF \times \sum_{290}^{320} EE_\lambda \times I_\lambda \times Abs_\lambda \quad (1)$$

Table 4. Erythematogenous effect used in SPF calculation.

Wavelength (nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Source: Sayre et al. [36]

The calculation of λ_c was performed by integrating the absorption area of the extract from 290 to 400 nm and determining the wavelength corresponding to 90% of the area, as described by Aguiar and Novelli [3].

The UVA/UVB ratio was performed using equation 2 as described by Velasco et al. [30].

$$\frac{UVA}{UVB} = \frac{\int_{320\,nm}^{400\,nm} A_\lambda \cdot d\lambda}{\int_{290\,nm}^{320\,nm} A_\lambda \cdot d\lambda} \quad (2)$$

4. Conclusions

This study explores for the first time the potential of *C. sylvestris* var. *lingua* to obtain a photoprotective extract. The extract obtained by infusion of leaves from *C. sylvestris* var. *lingua* has potential for applications in phytocosmetics due to the presence of phenolic compounds, phytosterols and good sun protection factor. It was also observed a critical wavelength and UVA/UVB ratio that indicate a possible application as a sunscreen and multifunctional cosmetics with action in the UVA region.

Based on the results obtained, further studies are suggested addressing cosmetic formulations for photoprotection with this species, as well as its cutaneous toxicity and in vivo photoprotection.

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Author Contributions

Claudia Andrea Lima Cardoso: Formal Analysis, Methodology, Resources and Writing – review & editing. Thiago Luis Aguayo de Castro: Formal Analysis and Writing – original draft. André Luís Duarte Goneli: Resources and Writing – review & editing. Maria Helena Verdan: Formal Analysis.

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