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Photoprotective action, antioxidant activity, and toxicity of aqueous extracts of *Campomanesia sessiliflora* O. Berg

Abstract

Campomanesia sessiliflora O. Berg is a medicinal plant that is object of very few studies in the literature. In this context, the antioxidant activity, sun protection factor (SPF) and toxicity in *Artemia salina* Leachs were analysed, as well as the contents of phenolic compounds, flavonoids and tannins in aqueous extracts prepared by infusion and maceration of *C. sessiliflora* leaves. Maceration showed higher levels of phenolic compounds and flavonoids regarding infusion and the two samples had the same tannin content. The LD_{50} was similar for the extracts, both were considered as low toxicity in the test with *A. salina*. Infusion presented a SPF of 9.98, while maceration presented a SPF of 6.74. Maceration presented better contents of secondary metabolites and antioxidant activity and infusion presented a better SPF. The extracts have the potential of incorporation into multifunctional products.

Keywords: Secondary metabolites; *Artemia salina*; Phenolic Compounds.

Acción fotoprotectora, actividad antioxidante y toxicidad de extractos acuosos de *Campomanesia sessiliflora* O. Berg

Resumen

Campomanesia sessiliflora O. Berg es una planta medicinal que tiene pocos estudios en la literatura. En este contexto, se analizó la actividad antioxidante, factor de protección solar (FPS) y toxicidad en lixiviados de *Artemia salina*, así como el contenido de compuestos fenólicos, flavonoides y taninos en extractos acuosos preparados por infusión y maceración de hojas de *C. sessiliflora*. La maceración mostró niveles más altos de compuestos fenólicos y flavonoides, en relación con la infusión, y las dos muestras tuvieron el mismo contenido de taninos. La DL_{50} fue similar para los extractos, considerándose ambas muestras de baja toxicidad en el ensayo con *A. salina*. La infusión presentó un FPS de 9,98, mientras que la maceración tuvo un FPS de 6,74. La maceración mostró niveles más altos de metabolitos secundarios y actividad antioxidante y la infusión mostró un mejor FPS. Los extractos muestran potencial para incorporarse a productos multifuncionales.

Palabras clave: metabolitos secundarios; *Artemia salina*; compuestos fenólicos.

Ação fotoprotetora, atividade antioxidante e toxicidade de extratos aquosos de *Campomanesia sessiliflora* O. Berg

Resumo

Campomanesia sessiliflora O. Berg é uma planta medicinal que apresenta poucos estudos na literatura. Neste contexto, foram analisados a atividade antioxidante, fator de proteção solar (FPS) e toxicidade em *Artemia salina* Leachs, assim como os teores de compostos fenólicos, flavonoides e taninos dos extratos aquosos preparados por infusão e maceração das folhas de *C. sessiliflora*. A maceração apresentou maior teores de compostos fenólicos e flavonoides em relação a infusão e as duas amostras apresentaram o mesmo teor de taninos. O DL_{50} foi semelhante para os extratos, ambas amostras sendo consideradas como baixa toxicidade no ensaio com *A. salina*. A infusão apresentou FPS de 9,98, enquanto que a maceração o FPS foi de 6,74. A maceração apresentou maiores teores de metabólitos secundários e atividade antioxidante e a infusão apresentou melhor FPS. Os extratos apresentam potencial para a incorporação em produtos multifuncionais.

Palavras-chave: Metabólitos secundários; Artemia salina; Compostos fenólicos.

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Introduction

The skin can be subjected to burns, premature aging and harmful neoplasms when exposed to excessive ultraviolet (UV) light, which can trigger skin diseases [1]. Therefore, it is necessary to use photoprotective products that act to protect the skin against this type of radiation [2].

How sunscreens work depends on their chemical composition: Physical filters are composed of inorganic materials that work by scattering and reflecting radiation, whereas organic filters are made up of organic compounds that have an aromatic ring with an ortho electron donor group so that they act by absorbing light through the resonance mechanism, transforming electromagnetic energy into heat or fluorescent light [3].

Despite the importance of using sunscreens, some organic substances can be absorbed, metabolised or accumulated, which can result in skin problems. In this context, natural sunscreens have gained attention due to their diverse biological activities and low toxicity [4].

A common biological model for preliminary toxicity tests is the *Artemia salina* Leachs. It is widely employed due to its low cost, long storage period for dormant cysts, easy handling, and low tolerance to changes in its breeding environment, with the results indicating possible negative effects on human beings [5, 6].

Plant extracts have shown great potential for being used as natural sunscreens, due to the presence of secondary metabolites [7]. Phenolic compounds are secondary metabolites that help in the defense of the plant and are constituted by two aromatic rings that have one or more hydroxyls (electron donor groups) [8] whose presence has been associated with the photoprotective action of plant extracts, mainly molecules from the subgroups of flavonoids and tannins [9].

In addition to natural sunscreens having a structure and activity similar to synthetic ones, they may also have antioxidant activity [3]. Radiation in the UV-B region that manages to reach the skin causes the production of reactive oxygen species (ROS) and reduces the skin's natural production of antioxidants. In this regard, the presence of antioxidants in products applied to the skin can combat ROS, preventing premature aging and cancer formation [10]. It is estimated that 90% of skin cancer cases are melanoma, of which 86% are related to exposure to UV radiation [11].

Therefore, the addition of extracts in cosmetics has been studied, considering that there is also a reduction in production costs and the obtaining of products that have multifaceted effects [12], and also considering that for these products to proclaim the photoprotective effect, they must have a SPF greater than six to proclaim the sun protection effect [13].

In this sense, Brazil has great potential for exploring extracts for this application, according to Lima *et al.* [14], 15 to 20% of the world's biodiversity is concentrated in Brazilian territory, containing two of the nineteen global biodiversity hotspot territories. The Cerrado (Brazilian savannah) is one of these, covering 23% of the national territory and presenting the highest level of plant diversity and endemism among the savannahs [15].

The *Campomanesia* genus has species relevant to this biome [16], with its fruit being considered a symbol of the state of Mato Grosso do Sul as of November 2017, by way of law 5.082, which authorises the use of the fruit of this genus in the touristic dissemination of the state of Mato Grosso do Sul [17]. Studies have evidenced the presence of phenolic compounds and antioxidant activity in several species of this genus [18].

Campomanesia sessiliflora O. Berg species is one of these, regarding which however, there are few studies in the literature [16]. *C. sesiliflora* is a tree or shrub, with a height between five and 17 meters [19], popularly known as guabirobeira-verde [20]. In the research by Catelan *et al.* [21], it was shown that the ethanol extracts of *C. sessiliflora* leaves have photoprotective activity, however there are as yet no studies related to the aqueous extracts.

For the preliminary analysis of sunscreens, spectrophotometric methods are applied [3]. The sun protection factor (SPF) can be calculated by means of empirical calculations based on absorption in the UVB region [21], whereas the critical wavelength is a parameter that determines whether the sunscreen has ample protection [3, 13].

Considering the above information, this research aimed to evaluate the contents of phenolic compounds, flavonoids and tannins, the SPF and the toxicity in *A. salina* of the aqueous extracts of *C. sessiliflora* leaves obtained by maceration and infusion.

Materials and Methods

Reagents

Methanol HLPC (Sigma-Aldrick, USA), Folin-Dennis reagent (Sigma-Aldrick, USA), Folin-Ciocalteau reagent (Sigma-Aldrick, USA), aluminum chloride (Sigma-Aldrick, USA), 2,2-diphenyl-1-picrylhydrazril (Sigma-Aldrick, USA).

Equipments

Wiley mill (Marconi, Brazil), Alpha 1-2LD Plus lyophiliser (Martin Christ, Germany), UV/Vis spectrophotometer (Global Trade Technology, Brazil)

Plant material and preparation of extracts

C. sessiliflora O. Berg leaves were collected in Dourados-MS, at the Medicinal Plants Garden of the Federal University of Grande Dourados. The leaves were dried in an air circulation oven at 37 ± 2 °C and ground in a Willey type mill with a 10-mesh sieve. Subsequently, the sample was packaged, labeled, and stored at a temperature of 4 °C.

To obtain the infusion extracts (IE), the powder from the leaves was mixed with water at a concentration of 20 $g \cdot L^{-1}$, with an initial temperature between 95-100 °C for 10 min in a closed container [16]. In maceration extraction (ME), the leaves were in contact for 24 h at room temperature (25 °C). Afterwards, the extracts were filtered and lyophilised (using the parameters of vacuum 0.045 mbr and temperature of -42 °C) and stored in hermetically sealed flasks. The extracts were solubilised at a concentration of 1 $mg \cdot mL^{-1}$ in distilled water for the tests of phenolic compounds, flavonoids, tannins, saponins and antioxidant activity and sun protection factor, while for toxicity in *A. salina* the initial concentration was 2 $mg \cdot mL^{-1}$.

Contents of phenolic compounds, flavonoids, and tannins

All analyses were performed in triplicate. The content of phenolic compounds was determined based on the Folin-Ciocalteau colorimetric method [22]. The concentration of phenolic compounds was calculated using an analytical curve, using gallic acid as a standard at concentrations of 10, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 $\mu g \cdot mL^{-1}$. The slope (a = 0.0009) and linear coefficient (b = 0.0014) were obtained by linear regression. The result was expressed in mg of gallic acid per g of lyophilised extract (mg GAE $\cdot g^{-1}$).

The determination of flavonoids followed the methodology proposed by Djeridane *et al.* [22]. To calculate the flavonoid concentration, an analytical curve was drawn using rutin as a standard, the concentrations of 10, 20, 30, 40 and 50 $\mu g \cdot mL^{-1}$ were employed, using linear regression. The values of slope (a = 0.0019) and linear coefficient (b = 0.0105) were obtained, and the result was expressed in mg of rutin per g of lyophilised extract (mg $RE \cdot g^{-1}$).

The tannin content was determined using the Folin-Denis spectrophotometric method, with tannic acid as reference [23]. To calculate the concentration of tannins, an analytical curve was made using tannic acid at concentrations of 0.1, 10, 20, 30, 40 and 50 $\mu g \cdot mL^{-1}$, obtaining the values of the slope (a = 0.0017) and linear coefficient (b = 10.14). The result was expressed in mg of tannic acid per g of lyophilised extract (mg $TAE \cdot g^{-1}$).

Antioxidant activity of the 2,2-diphenyl-1-picrylhydrazril radical

The antioxidant activity of the extracts was evaluated by the free radical DPPH method (2,2-diphenyl-1-picrylhydrazril) [24]. The experiment was carried out in the dark, with a controlled temperature (25 ± 1ºC) and all tests were carried out in triplicate [24].

The results are presented in minimal inhibitory concentration. The IC_{50} expresses the minimum concentration of antioxidant necessary to reduce the initial concentration of DPPH by 50% [25]. Based on the sequestering activity obtained from the different sample dilutions, a graph was plotted with the DPPH reduction % on the Y axis and the concentration of extracts (μg mL^{-1}) on the X axis in order to obtain the concentration of the sample capable of reducing DPPH by 50 %. To calculate the IC_{50}, the extracts were prepared in distilled water in dilutions at concentrations of 20, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 μg·mL^{-1}.

Toxicity in A. salina

The toxicity in *A. salina* test was performed with aqueous extracts according to the methodology described by Meyer *et al.* [26], with modifications. The *A. salina* cysts were incubated for 48 h in a solution of synthetic sea salt (20 g·L^{-1}) and 0.7 g·L^{-1} of sodium bicarbonate (pH: 8), with constant lighting (60 W) and aeration. The evaluation was performed at concentrations of 2000, 1000, 500, 250 and 100 μg·mL^{-1} in saline solution, determining the lethal dose (LD_{50}). For each concentration tested, three replicates were performed with ten larvae belonging to the second stage with saline solution as a negative control, which followed the same procedure described for the samples. At the end of the 24 h of incubation, the individuals' deaths were counted and the LD_{50} calculated. To determine the lethal dose for 50%, the concentrations were used based on the mortality percentage. The analyses were performed in triplicate.

Spectrophotometric analysis of the photoprotective action

The exploratory scanning by ultraviolet and visible spectrometry occurred at wavelengths between 200 nm and 600 nm with an interval of 1 nm [21] in a UV/Vis spectrophotometer. The extracts were prepared at a concentration of 1 mg·mL^{-1} diluted in water.

With the data obtained from the scan for the range between 290 nm and 400 nm, the critical wavelength (λc) was calculated by integrating the spectrum area and determining the wavelength equivalent to 90% of the spectrum area [13, 3] by the use of Orgin Pro 2018 version 9.5 software.

To determine the SPF of the solutions, the *in vitro* spectrophotometric method was used [27]. Samples at the initial concentration of 1 mg·mL^{-1} were diluted in water to a concentration of 0.2 mg·mL^{-1} and then readings were taken in a UV/Vis spectrophotometer registering between 290 and 320 nm, with an interval of 5 nm, using distilled water as a blank. The calculation of the SPF was performed as described by Dutra et al. [27].

Results and Discussion

The maceration extraction method showed greater efficiency in extracting secondary metabolites from the leaves of *C. sessiliflora* (Table 1), with higher levels of flavonoids and phenolic compounds as compared to infusion. Tannin contents were similar in both samples (Table 1).

Table 1. Quantification of secondary metabolites and toxicity of the aqueous extracts from *C. sessiliflora* leaves.

Analysis	Infusion	Maceration
Phenolic Compounds (mg GAE·g^{-1} ± SD)	106.50 ± 10.10	148.64 ± 1.01
Flavonoids (mg RE·g^{-1} ± SD)	47.58 ± 0.20	63.39 ± 0.33
Tannins (mg TAE·g^{-1} ± SD)	0.22 ± 0.01	0.22 ± 0.01
$_{LD\,50}$ in *Artemia salina* (μg·mL^{-1} ± SD)	778.55 ± 1.74	786.03 ± 2.19

GAE = Gallic acid equivalent; RE = Rutin Equivalent; TAE = Tannic acid equivalent; LD_{50} = Lethal Dose; SD = Standard deviation.

The greater efficiency in extraction by maceration may be associated with the thermosensitivity of some phenolic compounds [28], as well as the swelling effect of the leaves that facilitates the migration of the compounds to the solvent [29].

Catelan *et al.* [21] quantified the secondary metabolites of *C. sessiliflora* leaves for extraction using ethanol, obtaining 299.79 mg RE·g^{-1} of flavonoids and 435.67 mg GAE·g^{-1} of phenolic compounds, values higher than those obtained for aqueous extracts (Table 1), indicating that the solvent can influence metabolite contents.

Phytochemical analyses indicate the presence of tannins in the leaves of *Campomanesia xanthocarpa O. Berg* [30], and *Campomanesia guazumifolia (Cambess.) O. Berg* [31].

LD_{50} greater than 1000 μg mL^{-1} indicates that the compound is non-toxic, concentrations between 500 and 1000 μg·mL^{-1} indicate low toxicity, 100 to 500 μg·mL^{-1} show moderate toxicity and below 100 μg ·mL^{-1} is related to high toxicity [32]. Both forms of preparation had low toxicity.

The hexane extract of the *Campomanesia pubescens* (DC) O. Berg leaves presented a LD_{50} of 2200 μg·mL^{-1} [33], whereas the hexane extracts of the *C. adamantium* (Cambess.) O. Berg leaves did not present toxicity [34].

Senigali *et al.* [6] analysed the toxicity of other Cerrado plants regarding the *A. salina* crustacean, where it was found that the aroeira presents moderate toxicity. Ramos *et al.* [34] also evaluated the toxicity of hexane extracts in *Artemia sp.* and they found that there are no signs of toxicity in concentrations below 2 mg·mL^{-1}.

The infusion of *C. sessiliflora* leaves was studied by Castro et al. [16] in the biological model of *Allium cepa* L., and it was observed that the extracts do not induce mutagenicity and cell death in concentrations equal to or less than 0.5 mg·mL^{-1}. The hydroethanolic extracts of the leaves of *C. xanthocarpa* [35] and *C. pubescens* [36] did not show chronic toxicity in rats. There are as yet no studies in rats related to *C. sessiliflora*, however the results obtained in *A. cepa* [16] and in *A. salina* (Table 1) have been promising regarding the safety of consumption.

According to ANVISA [13], electromagnetic radiation in ultraviolet is between 200 and 400 nm and is divided into three parts: AV-A (320-340 nm), UV-B (290-320 nm) and UV-C (200 -290 nm). Scanning in the UV/Vis region of the infusion of *C. sessiliflora* leaves indicated a maximum absorption of 244 nm, while maceration showed a maximum absorption of 243 nm (Figure 1), both in the UV-C region.

λc is associated with the level of sun protection [37]. According to ANVISA [13], the minimum λc for sunscreens is 370 nm. The two samples are within the range (Table 2), indicating that the extract has a wide radiation absorptivity in the UV region.

The antioxidant activity helps protect the skin by combating reactive oxygen species formed by UV radiation [10]. The extract obtained by maceration required lower concentrations to inhibit 50% of DPPH radicals compared to the extract obtained by infusion (Table 2). This result may be associated with a higher concentration of phenolic compounds in the maceration (Table 1).

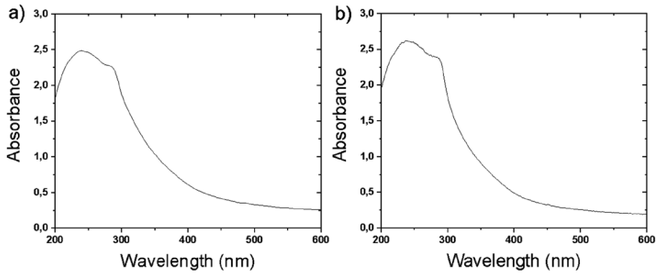

Figure 1. Scanning spectra of *C. sessiliflora* leaf extracts obtained by maceration (a) and infusion (b).

Table 2. Sun protection factor, critical wavelength, and antioxidant activity of aqueous extracts of ***C. sessiliflora*** leaves.

Analysis	Infusion	Maceration
SPF	9.98	6.74
λc (nm)	386	381
IC_{50} (μg·mL^{-1} ± SD)	54.30 ± 0.20	52.37 ± 0.51

SPF = Sun protection factor; λc = Critical wavelength; IC_{50} = Mean inhibitory concentration of the DPPH radical; SD = Standard deviation.

Reynertson *et al.* [38] classified the mean inhibitory concentration against DPPH as highly active to antioxidant activity for values below 50 μg·mL^{-1}, moderately active for values between 50 and 100 μg·mL^{-1}, somewhat active between 100 and 200 μg· mL^{-1} and inactive at values above 200 μg·mL^{-1} for the DPPH radical test [38]. In this sense, the two extracts showed moderately active antioxidant activity (Table 2).

The extract obtained with methanol:water 50:50 (*v/v*) of the *Byrsonima crassifolia* (L.) Rich fruits presents antioxidant activity with a phenolic compounds content of 334.37 mg·g^{-1} [39]. The ethanol extracts of the *Amburana cearensis* AC Smith bark and leaves of *Croton sonderianus* Muell and *Sida galheirensis* Ulbr contain IC_{50} of 54.88 μg·mL^{-1}, 51.40 μg·mL^{-1} and 57.41 μg·mL^{-1} respectively, with values similar to those analysed extracts [4]. The infusion of *C. sessiliflora* leaves presented antioxidant activity at different times of the year [40]. The extracts obtained by decoction of *C. xanthocarpa* leaves showed IC_{50} against the DPPH 38.47 μg·mL^{-1} [41].

According to Agência Nacional de Vigilância Sanitária (ANVISA) [13], cosmetics with a SPF greater than two can be considered as multifunctional products, in this context, the extracts are promising for this purpose when considering the SPF obtained and the observed antioxidant activity (Table 2). The extracts obtained by maceration or infusion of *C. sessiliflora* leaves also presented a photoprotective effect, being classified as low sun protection, and may be applicable for skin that is not very sensitive to sunburn (Table 3).

Table 3. SPF usage classification table according to skin type. Adapted from ANVISA [13].

SPF	Category	Indication of use
6.0-14.9	Low protection	Skin insensitive to sunburn
15.0-29.9	Medium protection	Skin slightly sensitive to sunburn
30.0-49.9	High Protection	Skin very sensitive to sunburn
50-100	Very high protection	Skin extremely sensitive to sunburn

SPF = Sun protection factor.

The ANVISA and the Food and Drug Administration (FDA) determine that extracts must contain a minimum SPF of six for *in vivo* tests to be conducted [13, 42].

Different fractions (*n*-hexane, hydromethanolic ethyl acetate and butyl hydroxytoluene) of the ethyl extract of *Campomanesia guaviroba* (DC.) Kiaersk have already been analysed via L929 fibroblasts for viability against UV-B radiation, where an increase in viability was observed, with emphasis on the ethyl acetate extract [10].

In this context, aqueous extracts are promising, using a solvent with low impact on extraction and presenting enough SPF for *in vivo* analysis and formulations of multifunctional products.

Conclusions

Both infusion and maceration of the *C. sessiliflora* leaves presented photoprotective action, which can be associated with the presence of secondary metabolites such as flavonoids and tannins. The extracts from *C. sessiliflora* are promising for applications in formulations of multifunctional products and natural cosmetics based on photoprotective and antioxidant activity and also present low toxicity in the *A. salina* test. The observed SPF justifies future *in vivo* studies and brings a novel perspective of the *C. sessiliflora* potential in the elaboration of photoprotective products.

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