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Article

Comparation of the Aqueous Extraction Methods of the *Tarenaya Aculeata* Leaves in Relation to the Chemical Composition and Photoprotector, Antibacterial and Antioxidant Potentials

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ABSTRACT

Medicines and cosmetics based on natural products have been in demand since ancient times. This study aimed to compare extracts and fractions of *T. aculeata*, obtained by different extraction methods, in addition to evaluating the antioxidant, antibacterial and photoprotective capacity of the samples. The chemical differentiation of the content different extracts and fractions were evaluated using statistical programs and the levels of phenolic compounds and tannins were evaluated using spectroscopic methods. The results of the statistical analysis showed greater similarity in the extracts obtained by the infusion and decoction methods. The toxicity assay by *Artemia Salina* showed that the aqueous extracts obtained by all methodologies are non-toxic, promising for its use as a cosmetic, and antioxidant activity was more evident for the fractions. Broad-spectrum antibacterial activity was attributed to both extracts and fractions. In order to obtain a sustainable photoprotective product, the aqueous extracts were selected and incorporated into a commercial cream in order to obtain a multifunctional product, the aqueous extract obtained by the infusion method being the one that presented the best sun protection factor results (17.27), with good results of stability.
Keywords: sojinha; mussambê; infusion; decoction.

RESUMO

Medicamentos e cosméticos à base de produtos naturais são procurados desde os tempos antigos. Este estudo teve como objetivo comparar extratos e frações de *T. aculeata*, obtidos por diferentes métodos de extração, além de avaliar a capacidade antioxidante, antibacteriana e fotoprotetora das amostras. A diferenciação química do teor dos diferentes extratos e frações foi avaliada por meio de programas estatísticos e os teores de compostos fenólicos e taninos foram avaliados por métodos espectroscópicos. Os resultados da análise estatística mostraram maior similaridade nos extratos obtidos pelos métodos de infusão e decocção. O ensaio de toxicidade por *Artemia Salina* mostrou que os extratos aquosos obtidos por todas as metodologias são atóxicos, promissores para seu uso como cosmético, sendo a atividade antioxidante mais evidente para as frações. Atividade antibacteriana de amplo espectro foi atribuída aos extratos e frações. Para obtenção de um produto fotoprotetor sustentável, os extratos aquosos foram selecionados e incorporados a

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um creme comercial a fim de se obter um produto multifuncional, sendo o extrato aquoso obtido pelo método de infusão o que apresentou os melhores resultados de fator de proteção solar (17,27), com bons resultados de estabilidade.
Palavras-chave: sojinha; mussambê; infusão; decocção.

1. Introduction

Antioxidant and antimicrobial activities in plants have been studied for a long time, since plant extracts are rich in chemical compounds that can protect humans against cellular oxidation reactions and pathogens (Sengul et al. 2009). Among the potential uses of antioxidants are the prevention of diseases related to oxidative stress in humans and preventing oxidative reactions in cosmetic products (Evans & Packer 2003). While antimicrobial activity has received attention from many researchers due to bacterial resistance that has grown dramatically in recent years, substances that can inhibit pathogens with low toxicity are considered promising candidates for the development of new antimicrobial drugs (Sengul et al. 2009).

The natural antioxidants as sunscreens are a good alternative, which can intensify or improve sun protection factor (SPF) values (He et al. 2020). In addition, when added with antimicrobial properties, these plant extracts can mitigate or even replace the use of synthetic preservatives, inhibiting antimicrobial proliferation (Herman et al. 2019).

The most efficient way to protect the skin against Ultra Violet radiation is the application of an active molecule with properties to absorb or repel UV rays, and among the ideal properties that a sunscreen must contain we can mention: ability to reflect or absorb rays UVA and UVB; property of scavenging antioxidant species, safety stability of the active compound, among others (Prasanth et al. 2020).

The UVA (320-400 nm) region is the most harmful to the skin – it penetrates deeper into the dermis and epidermis, in addition to being able to promote the formation of reactive oxygen species, causing indirect damage to the DNA structure (Raffa et al. 2019). The UVB (290-320 nm) region is responsible for sunburn (Dutra et al. 2004). The UVC region (200 to 290 nm) is fully filtered before reach the skin, not causing damage (Dutra et al. 2004; Raffa et al. 2019).

In this sense, plants from the state of Mato Grosso do Sul have stood out due to their chemical and biological potential, especially due to their photoprotective activities and application in phytocosmetics (Castro et al. 2022). The presence of chemical compounds, such as phenolic compounds and tannins, are responsible for numerous biological activities found in medicinal plants (Zhang et al. 2022; Bule et al., 2020). Studies show that polyphenols can protect the skin against UV radiation by absorbing and neutralizing the free radicals generated, in addition to inhibiting the growth of microorganisms, especially bacteria (Lee et al. 2022; Nichols & Katiyar 2010; Efenberger-Szmechtyk et al. 2021). Tannins are polyphenolic compounds most extensively studied due to their *in vitro* antioxidant and antibacterial activities (Sieniawska et al. 2017).

Tarenaya aculeata is a medicinal specie belonging to the genus Cleome (Sánchez-Acebo 2005). There are indications in the literature for the use of its leaves for the treatment of fever and body pain (Lisboa et al. 2017). Besides *T. aculeata* does not present considerable studies related to its indication, the *cleome* genus is known for its medicinal plants, full of biological potential, such as anticancer, antibacterial, anti-inflammatory, as well as countless other activities that can indicate *T. aculeata* a promising species to be studied (Abdullah et al. 2016).

Considering the lack of studies that prove the chemical composition and the medicinal use of this specie, and the use of cosmetics that includes in it is formulation natural sources, this study aimed to evaluate the levels of phenolic compounds and tannins, toxicity, antioxidant, antibacterial and photoprotective activities of aqueous extracts and fractions of *T. aculeata* leaves, and to propose the incorporation of their extracts to a

commercial non-ionic cream and evaluate its stability characteristics for its use as a potential multifunctional product.

2. Material and methods

2.1 Reagents and Equipments

Reagents: Methanol HPLC, Amberlite resin XAD-2, Formic acid, Acetonitrile, Folin-Dennis reagent, Folin-Ciocalteau reagent, sodium carbonate (8 and 20%), gallic acid, tannic acid, 2,2-diphenyl-1-picrylhydrazril. All chemical were purchased from Sigma Aldrich (USA).

Semi-solid pharmaceutical form commercial: water, cetearyl alcohol, cetostearyl alcohol, glyceryl stearate, disodium EDTA, phenoxyethanol, methylisothiazolinone, liquid paraffin, lanolin alcohol, caprylic/capric triglyceride, cyclomethicone and dimethicone (silicone oil).

Equipments: Oven with air circulation (Marconi, Brazil), Knife mill (Marconi, Brazil), Alpha 1-2LD Plus lyophiliser (Martin Christ, Germany), Ultra Performance Liquid Chromatograph (LC-20A Prominence, Japan) coupled with a diode array detector (UPLC-DAD), UV/Vis spectrophotometer (Global Trade Technology, Brazil)

2.2 Plant material and extraction

Leaves of *T. aculeata* species were collected in the municipality of Dourados, MS, Brasil. A exsiccate of the plant was deposited at a herbarium located in the Universidade Federal da Grande Dourados (DDMS 7618) with registration SISGEN number (AD26007).

The leaves were dried (oven with air circulation at 50 °C; 72 hours) and then ground using a knife mill. The different types of extracts were differentiated from each other by the extraction method. For this, the infusions were obtained by inserting the vegetable material in water (initial temperature between 98-100ºC; 30 minutes) in an open container; in the maceration, the leaves were in contact with water for 24 hours, at room temperature (27ºC) and in the decoction, by inserting the plant material in water (controlled temperature of 98 ºC) for approximately 20 minutes, in an open system.

All extracts were prepared at a concentration of 2% (m/v). After the extractions, the extracts were filtered, and a portion was frozen and lyophilized (Alpha 1-2 LD plus, pressure of 0.045 mbar and temperature of -42 ºC), in order to yield the extracts obtained by infusion, maceration and decoction of the leaves (EI, EM and ED, respectively), the other separated one was used in order to obtain the methanolic fractions from each of the extracts. Yields of the aqueous extracts were calculated considering the dry plant mass (g), and for the calculation of fractions, there were considered the starting mass (g) of the extract used to obtain the respective fraction.

The methanolic fractions, obtained from the previously separated aqueous extracts, were processed in a separation column using amberlite resin XAD-2, following the methodology described by Yung An et al. (2016), with adaptations. To obtain the methanolic fractions, 2 L of each of the extracts (EI, EM and ED) were eluted in a glass column with dimensions of 25 x 3 cm, packed with 100g of amberlite XAD-2 in methanol. The collected fraction was dried in a laminar flow hood in order to obtain the methanolic fractions of the leaves (FI, FM and FD).

2.3 Chemical composition:

Chromatographic analysis using UPLC-DAD

To carry out the chromatographic analyses, it was used a previously methodology described by Castro et al. (2023) for the analysis of aqueous extracts in teas. For this, an Ultra Performance Liquid Chromatograph (LC-20A Prominence, Japan) coupled with a diode array detector (UPLC-DAD) was used. The column used was a Shimadzu's Shim-pack XR-ODS column (2.2 μm particles and 2 mm x 75 mm size) with oven at 35ºC . The injection volume used was 10 μL, and the flow was 0.35 mL min^{-1}. A gradient was used employing water with 0.1% formic acid (A) and acetonitrile (B). The analysis conditions were 0-3 min (5-12%), 3-7 min (12-13%), 7-7.5 min (13-35%), 7.5-8.25 min (35-20%), 8.25 -9 min (20-10%), 9-9.5 (10-5%) and 9.5-12 (5% B). Injections were performed in triplicate.

Based on the chromatographic profile obtained, 14 peaks present in the samples were selected for multivariate comparison of the chemical composition, and present in the retention times: 1.18, 1.52, 4.36, 4.5, 5.11, 5.5, 5.88, 6.16, 6.29, 6.56, 6.78, 6.92, 10.57 and 10.9 min. The quantification of the area was performed at a wavelength of 300 nm, aiming to optimize the response of the selected peaks.

Content of phenolic compounds and tannins

In order to evaluate the content of phenolic compounds and tannins, a stock solution at a concentration of 1000 μg mL^{-1} of the extracts and fractions were prepared for the tests. The content of phenolic compounds was evaluated according to the methodology proposed by Djeridane et al. (2006), and for this, 100 μg of the stock solution of each of the samples were mixed with the different reagents and reacted for 30 minutes. Their absorbances were recorded at a wavelength equal to 760 nm, and the results expressed in mg of gallic acid per g of lyophilized extract (mg GAE g^{-1}), used as a sample standard. The total tannin content was determined according to the methodology described by Pansera et al. (2003). The different solutions were reacted with Folin-Denis reagent and 8% sodium carbonate for 2 hours. After the reaction time has elapsed, the reading was performed at a wavelength equal to 725 nm. Tannic acid was used as the sample standard and results were expressed as mg of tannic acid equivalent per g of lyophilized extract or fraction freeze-dried (mg TAE g^{-1}). Water was used as a blank in all analyses. All analyzes were performed in triplicate.

2.4 Toxicity test

To perform the toxicity test, the methodology proposed by Meyer et al. (1982), with adaptations was used. For survival analysis, a stock solution of each extract and fractions, was prepared at a concentration of 1500 μg mL^{-1}, and dilutions at concentrations ranging from 0 to 1000.0 μg mL^{-1}. In test tubes containing 10 mL of each of the solutions, 10 nauplii of *A. salina* were transferred, which were later counted according to their survival after 24 hours of exposure. For control, a saline water solution was used. After survival results were obtained, a graph was plotted considering the number of dead nauplii by the concentration of the extract/fraction (μg mL^{-1}). Once the graphs were obtained, it was possible to calculate the values of the linear and angular coefficients a and b, to calculate the mean lethal concentrations (LC_{50}). All tests were performed in triplicate.

2.5 Antioxidant activity

Stock solutions of each of the extracts (EI, EM and ED) and fractions (FI, FM and FD) were prepared at a concentration of 500 μg mL^{-1} using a 50% solution (water/methanol) as solvent. Dilutions were prepared at concentrations of 240, 120, 60 and 30 μg mL^{-1}. The antioxidant activity was evaluated by the DPPH free radical

method (2,2-diphenyl-1-picrylhydrazryl), proposed by Kumaran and Joel Karunakaran (2006). The solutions prepared were reacted with the DPPH solution and read at a wavelength equal to 517 nm, using water as a blank, and the results were presented in minimum inhibitory concentration (IC_{50}).

2.6 Evaluation of antibacterial activity

The evaluation of antibacterial activity was performed according to the methodology described by Bernardi et al. (2017). The different extracts and fractions were evaluated at concentrations from 15.62 to 1000 μg mL^{-1}, and evaluated against strains of bacteria of the species *Burkholderia cepacia* (NEWP 0059), *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC 38731), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus epidermidis* (ATCC12228), *Staphylococcus aureus* (ATCC 25232) and *Staphylococcus saprophyticus* (ATCC15305). As a positive control, it was used tetracycline, at a concentration of 15 μg mL^{-1} and distilled water were used as a negative control. All analyzes were performed in triplicate.

2.7 Analysis of absorbance in the UV region and photoprotective potential

The extracts were prepared using a 200 μg mL^{-1} solution (obtained from the dilution of stock solutions) and the exploratory scans of the solutions were recorded at wavelengths between 200 and 600 nm, with a 1nm interval, using a UV/Vis spectrophotometer (Global Trade Technology, Brazil). To calculate the critical wavelength (λc), the intervals from 290 nm to 600 nm were selected and the graph area was calculated and integrated, using the OriginPro 2018 software version 9.5.

To determine the SPF of the solutions, the samples were read in a UV/Vis spectrophotometer between 290 and 320 nm, with an interval of 5 nm. As a blank of the analysis, distilled water is used for the extracts and fractions samples, and methanol for the incorporations. The absorbances obtained were substituted in the Equation (Mansur et al. 1986), as follows:

$$SPFspectrophotometric = CF\ X \sum_{290}^{320} EE\ (\lambda)\ \text{x I}\ (\lambda)\ \text{x Abs}\ (\lambda)$$

where the CF= correction factor; EE= Erythemal effects spectrum; I= Solar intensity spectrum and ABS= absorbance of sunscreen sample. The values of EE x I are constant values that were determined by Sayre et al. (1979).

2.8 Incorporation of the commercial cream and stability analysis of the formulations

EI, EM and ED were incorporated into the commercial cream, at a concentration of 1%, in ordem to obtain the respective incorporated products: incorporated infused extract (EII), incorporated maceration extract (EMI) and incorporated decoction extract (EDI).

After the incorporations, the samples were diluted in methanol, in order to obtain concentration of 200 μg mL^{-1}, according to the methodology described by Dutra et al. (2004). To calculate the SPF made as previously described (3.6). The reading of the SPF potential of the samples was performed daily for 7 days in order to evaluate their stability parameters, however, other factors were also evaluated, such as organoleptic analyzes. The evaluation of the organoleptic properties was based on visualization, to be considered any change in color, odor or appearance and to analyze the pH, an indicator paper strip (Fusion).

2.9 Statistical analysis

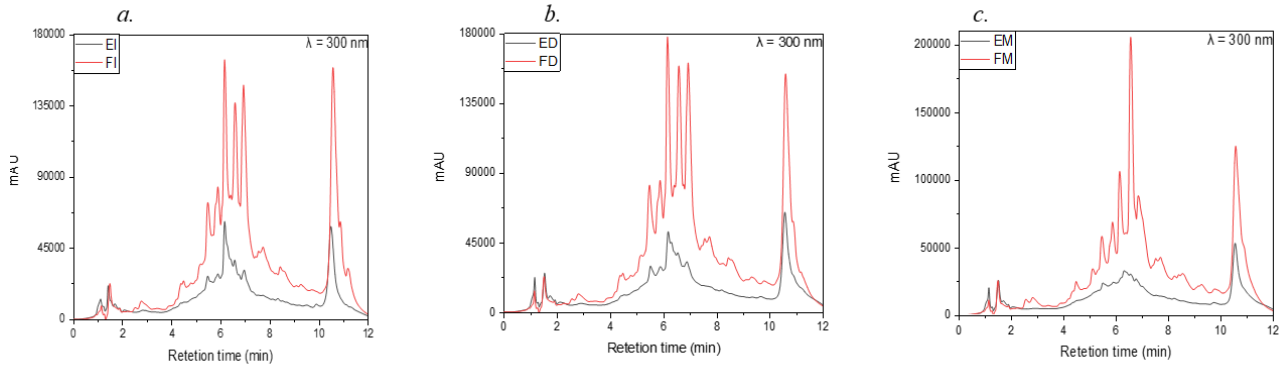
Differences in the content of phenolic compounds and tannins in the extracts and fractions of *T. aculeata*, were analyzed by one-way analysis of variance (ANOVA) and Tukey a posteriori ($p<0.05$). These were performed using the free software Bioestat 5.0 (Ayres et al. 2007).

Seeking to evaluate the influence of the form of extraction (extract or fraction) and the type of sample preparation employing chromatographic analysis, we performed a multivariate analysis of variance based on distance (permanova) using the "adonis2" function where the similarity in chemical composition was estimated using the Euclidean distance using the "vegdist" function. The significance of the difference between the groups was tested through 999 permutations in the "vegan" package (Oksanen et al. 2022). We also performed a principal coordinate analysis (PCoA) also using the Euclidean distance and the "cmdscale" function with two axes. All analyzes were performed in the R environment (R Core Team 2022).

3. Results and discussion

The yields of aqueous extracts obtained by the methods of infusion, maceration and decoction were 22.9 ± 0.9%, 24.3 ± 0.7% and 23.8 ± 0.5%, respectively in relation of the mass plant and mass obtained of the extracts, while the fractions were 8.7 ± 0.4%, 9.3 ± 0.8% and 10.5 ± 0.7%, considering the initial mass (g) of the aqueous extracts in relation of the mass obtained of the fractions.

The chromatographic profile indicated that the fractions showed a higher content of chemical constituents, compared to the extracts, in the three preparation forms evaluated (Fig. 01), allowing us to conclude that the use of the Amberlite resin XAD-2 provided a concentration of the phytochemical constituents of the fractions. Furthermore, the preparation method used also caused chemical changes in the extracts and fractions of *T. aculeata* ($F = 10.049$; $p<0.001$). In fact, the use of different extraction methods in the aqueous preparations can result in chemical differences, in this sense, the choice of method of extracting the compounds is relevant in order to extract thermolabile compounds (Bitwell et al. 2023; Delgado et al. 2019). The differences observed in the chromatographic profiles due to the fact that the decoction and infusion methods use heating, thus favoring the extraction of non-thermolabile compounds, while the maceration preparation has a higher content of thermolabile compounds, similar result was found by Castro et al. (2021), who observed a grater efficiency when extracting secondary metabolites from *C. sessiliflora* leaves (phenolic compounds and flavonoids) compared to infusion.



EI: Extract prepared by infusion; **FI:** Fraction prepared by infusion; **EM:** Extract prepared by maceration; **FM:** Fraction prepared by maceration; **ED:** Extract prepared by decoction; **FD:** Fraction prepared by decoction.

Fig 01. Chromatograms of the *T. aculeta* extracts and fractions obtained by the preparations of *a.* infusion *b.* maceration and *c.* decoction.

Still based on the chromatographic profile, the statistical analysis showed greater relevance in the chromatographic profile of the samples associated with the fractions ($F = 121.873$; $p<0.001$), indicating that the fractionation using the amberlite resin XAD-2 resulted in most of the chemical difference between the samples. As the PCoA analysis is associated with the Euclidean distance in the samples, so that the combination of the two axes provides an overview of the similarity or dissimilarities between the samples, it is possible to observe a proximity of the samples from axis 1 and a separation of the maceration of the axis 2 (Fig. 02), been possible to verify that the fractionation using maceration presented the greatest differentiation in the chromatographic profile, possibly as a result of the concentration of thermolabile compounds less present in the decoction and infusion.

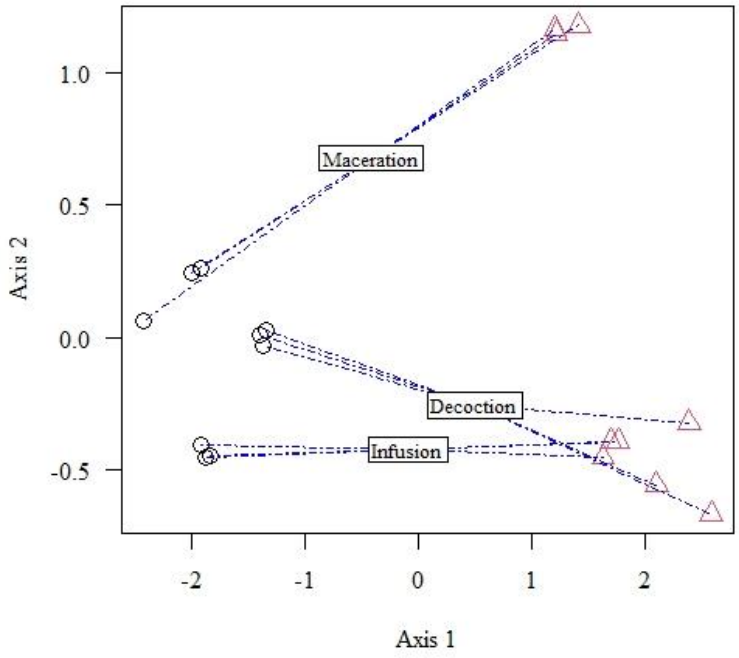


Fig. 02 – Principal Coordinate Analysis (PCoA) of extracts (circle) and fractions (triangle) of *T. aculeta*, in the three different methods of extraction of preparations.

The concentration of chemical constituents associated with fractionation using amberlite resin XAD-2 was also observed in the analysis of phenolic compounds, in which the results showed that the fractions presented higher amounts of phenolic compounds compared to the extract (Table 01). In fact, the use of the amberlite resin XAD-2 column has been described in the literature for the purpose of preparing, isolating and purifying phenolic compounds, which may have resulted in a higher content of these constituents in the fractions (Lason-Rydel et al. 2021).

The statistical analysis regarding the phenolic compounds showed a statistical difference in 5 of the 6 analyzed treatments (EI, EM, ED, FI, FM and FD), and the statistical similarity was only between the EM and ED samples (Table 01). This statistical similarity between the EM and ED samples shows that these samples present similar amounts of phenolic compounds, which must have been caused due to the extraction of thermolabile compounds from this extraction methods. Statistical differences in the remaining samples show that the preparation method strongly alters the content of phenolic compounds.

Table 01. Content of phenolic compounds and tannins in aqueous extracts and fractions of leaves of the *T. aculeata*.

Extract	Phenolic compounds (mg/g)	Tannins (mg/g)
EI	110.80 ± 2.03[b]	47.25 ± 3.02[a]
FI	280.13 ± 3.46[c]	47.51 ± 3.14[a]
EM	151.47 ± 6.40[a]	45.81 ± 0.46[a]
FM	318.80 ± 3.71[d]	46.62 ± 4.01[a]
ED	146.13 ± 3.08[a]	48.66 ± 1.60[a]
FD	295.47 ± 5.03[e]	51.37 ± 1.89[a]

Values expressed as mean ± standard deviation. **EI:** Extract prepared by infusion; **FI:** Fraction prepared by infusion; **EM:** Extract prepared by maceration; **FM:** Fraction prepared by maceration; **ED:** Extract prepared by decoction; **FD:** Fraction prepared by decoction. Averages followed by different letters in the column, differ by Tukey test.

Among the three extraction methods evaluated (EI, EM and ED), EM was the one that presented the highest levels of phenolic compounds. Similarly, FM presented the highest levels among the three fractionations evaluated, which allows us to conclude that maceration is the best extraction technique in order to extract this class of compounds (Table 01). Castro et al. (2021) found similar results when evaluating the extraction efficiency of chemical compounds from *Campomanesia sessiflora,* and when evaluating the content of phenolic compounds in extracts prepared by infusion and maceration, the highest content was found for extracts prepared by maceration

Among the few studies published about the chemical composition of *T. aculeata* until this moment, we can mention the study by Mendonça et al. (2020), who identified the presence of phenolic compounds and flavonoids in the ethanolic extract of its leaves and Garcia et al. (2010) that identified eight substances in the essential oil of the aerial parts, leaves and stems, with epi-α- cardinol being the majority. This is, in fact, the first study to evaluate the presence of these constituents in aqueous extracts of this species.

Furthermore, the chemical composition has already been described in the leaves extracts in other species of the genus. Rodrigues et al. (2019) identified the presence of 9 phenolic compounds in the aqueous and methanolic extracts of *T. spinosa*. Santos et al. (2019) identified 8 phenolic compounds in the aqueous extracts of *T. spinosa* and 9 in the ethanolic extracts and Bezerra et al. (2019) found 9 phenolic compounds in the ethanol extracts of this species, of which only 6 were present in the aqueous extract.

The statistical ANOVA analysis showed no statistical difference for tannins levels ($p = 0.17$). The presence of this class of compounds in ethanol extracts from leaves of *T. aculeata* has already been identified in a phytochemical screening carried out by Mendonça et al. (2020) and also in a study carried out by Silva et al. (2016) in extracts obtained by different organic solvents (cyclohexane, chloroform, ethyl acetate and methanol) from leaves and roots of *T. spinosa*.

Previous studies have already evaluated the toxicological effect of *T. aculeata*. Mendonça et al. (2020) found high toxicity for the ethanol extracts of the leaves of this species, and therefore emphasized that this result deserves attention. However, in our study, the aqueous extracts of *T. aculeata* were considered non-toxic, with LC > 1500 μg mL^{-1}. On the other hand, the fractions showed high toxicity, with LC < 25 μg mL^{-1}. The

calculation of the LC_{50} was thus determined because, for the extracts, the mortality of *A. salina* nauplii was greater than 50% at the lowest concentration tested, while for the fractions, the mortality of *A. salina* nauplii was greater than 50% at the lowest concentration tested. An extract is considered non-toxic when $LC_{50} > 1000$ μg mL^{-1} to highly toxic when $LC_{50} < 100$ μg mL^{-1} (Sandoval et al. 2020).

Studies indicate that when an extract has an LC_{50} below 1000 μg mL^{-1}, it can be considered a potential antitumor agent (Arcanjo et al. 2012; Dass et al. 2021), with a high probability of biosynthesizing anticancer products, since the fractions evaluated in our study (FI, FM and FD) present favorable conditions for its use as an antitumor agent, requiring more robust studies to evaluate these fractions. After evaluating the toxicological profile of the extracts and fractions of the leaves of *T. acuelata*, we performed the analysis of the antioxidant, antimicrobial and photoprotective potentials of the aqueous extracts.

The results in the antioxidant analysis are presented in table 02. The best antioxidant activity results were attributed to the fractions, especially to FI, with IC_{50} of 13.40 μg mL^{-1}, followed by FD, with IC_{50} of 32.99 μg mL^{-1} (Table 02). Antioxidant activity is considered active when $IC_{50} < 50$ μg mL^{-1}, moderately active when IC_{50} is between 50 and 100 μg mL^{-1}, little active between 100 and 200 μg mL^{-1} and inactive for values above 200 μg mL^{-1} (Reynertson et al. 2005).

Table 02. Antioxidant potential and critical wavelength in aqueous extracts and fractions of leaves of the *T. aculeata*

Sample	IC_{50} (μg mL^{-1})	λc (nm)
EI	278.03 ± 18.23	375.67 ± 0.58
FI	13.40 ± 6.45	374.00 ± 1.73
EM	> 500	380.00 ± 0.01
FM	51.66 ± 4.85	373.00 ± 0.01
ED	257.57 ± 2.60	376.00 ± 1.00
FD	32.99 ± 1.08	374.33 ± 2.31

Values expressed as mean ± standard deviation. **IC_{50}:** Inhibitory concentration; **λc:** Wavelength. **EI:** Extract prepared by infusion; **FI:** Fraction prepared by infusion; **EM:** Extract prepared by maceration; **FM:** Fraction prepared by maceration; **ED:** Extract prepared by decoction; **FD:** Fraction prepared by decoction.

Bezzera et al. (2019) found antioxidant activity for infusions of *T. spinosa* with an IC_{50} value equal to 445.8 μg mL^{-1}. In this study, the infusion presented IC_{50} 278.03 μg mL^{-1} compared to the three preparations evaluated in the extracts, EI and ED showed lower antioxidant potential. However, all the three preparations are considered inactive, according to the classification proposed by Reynertson et al. (2005).

Antimicrobial activity is the most reported biological activity in studies involving the vegetal species. Ethanol extracts of *T. aculeata* showed antimicrobial activity against five bacteria and two fungi (Nascimento et al. 1990). In addition, *T. spinosa* showed antimicrobial activity against 12 bacteria and 5 yeasts, in a study by Silva et al. (2016), in addition to relevant antibacterial activity against *Staphylococcus aureus* (MIC= 512 μg mL^{-1}), in the aqueous extracts of leaves, in the study by Santos et al. (2019).

Table 03. Minimum Inhibitory Concentration (MIC) in aqueous extracts and fractions of leaves the of *T. aculeata.*

	MIC (µg mL^{-1})					
Microorganism	**EI**	**FI**	**EM**	**FM**	**ED**	**FD**
Burkholderia cepacia	250	125	250	125	250	125
Enterococcus faecalis	250	125	250	125	250	125
Escherichia coli	125	62.5	125	62.5	125	62.5
Pseudomonas aeruginosa	125	31.25	125	62.5	125	62.5
Staphylococcus epidermidis	250	125	250	125	250	125
Staphylococcus aureus	250	125	250	125	250	125
Staphylococcus saprophyticus	500	500	500	500	500	500

EI: Extract prepared by infusion; **FI:** Fraction prepared by infusion; **EM:** Extract prepared by maceration; **FM:** Fraction prepared by maceration; **ED:** Extract prepared by decoction; **FD:** Fraction prepared by decoction.

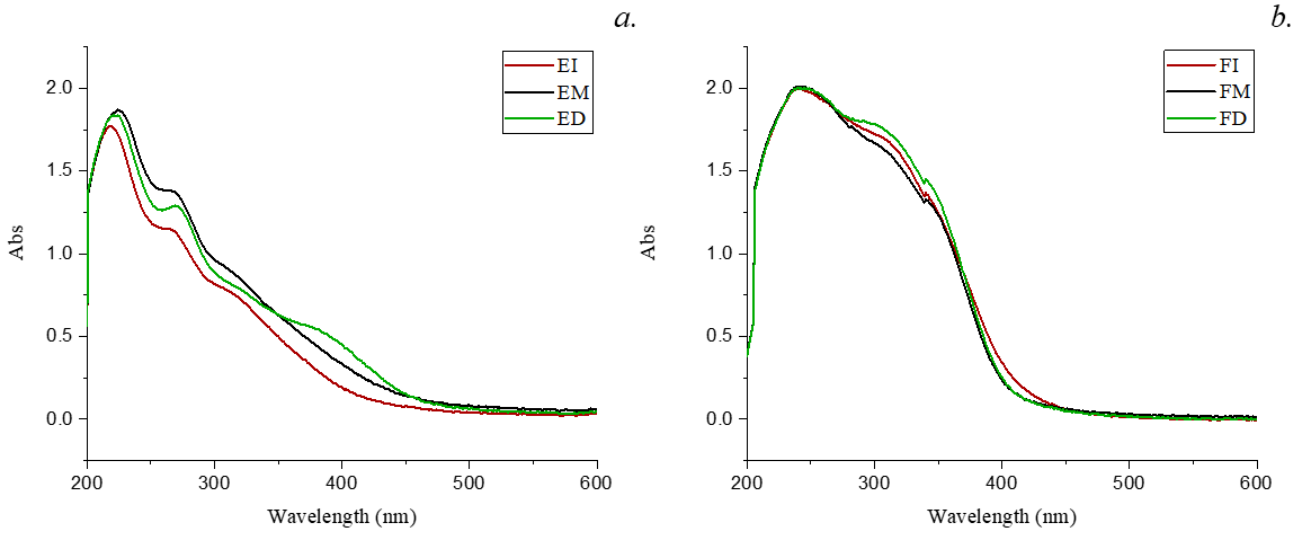
These results corroborate those found in our study, where the extracts and fractions of *T. aculeata* showed antibacterial activity against all 7 bacterial strains tested, evidencing a broad spectrum of action (Table 03). The fractions showed even more promising results, presenting MIC values higher than those of their respective extract. However, the extractive method did not cause significant changes in the inhibition values for the extracts for most fractions. The only change found was for the bacterium *Pseudomonas aeruginosa* which presented MIC: 31.25µg mL^{-1} for EI and 62.50 µg mL^{-1} for FM and FD, which is still the best result obtained in antibacterial activity obtained in our study (Table 03).

Samples with antioxidant and antimicrobial potential are important in cosmetic formulations. In this sense, natural actives are interesting alternatives for multifunctional products, since their constituents are complex matrices that have several components that may have different mechanisms of action, providing the product more than one function (Almeida 2013). The absence of toxicity is an important factor in the choice of actives inserted in the formulation. Thus, the aqueous extracts of the leaves were selected for analysis of the photoprotective potential.

Electromagnetic radiation is divided into UVA (320 to 400 nm), UVB (290 to 320 nm) and UVC (200-290 nm) regions. While the UVB region can cause harmful damage to human skin, such as erythema, skin photoaging and cancer induction, the UVA region is capable of promoting the formation of reactive oxygen species, being the most harmful to the skin, since it penetrates deepier into the dermis and epidermis (Hupel & Poupart 2011; Raffa et al. 2019). In this sense, photoprotective substances have been incorporated into commercial products in order to attenuate the chance of incidence of electromagnetic radiation (Dutra et al. 2004).

The extracts and fractions of *T.aculeata* showed electromagnetic absorption in the regions of 200 to 400 nmm (Fig. 3), which allows us to predict that the extracts and fractions are promising for their use as a sunscreen.

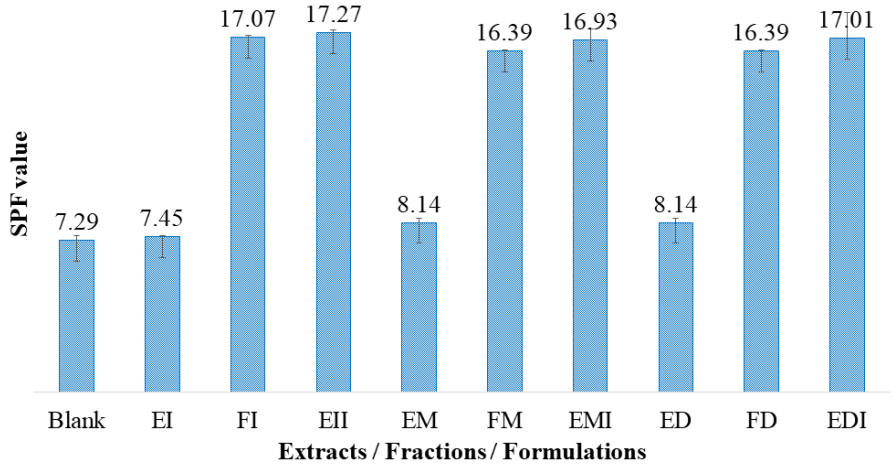
Differences between extractive methods were not substantial, with the greatest differences attributed to fractions (Fig 3b). The higher absorption may be due to the greater presence of phenolic compounds present in the extract (Table 1), since phenolic compounds have aromatic rings that absorb between the wavelength range between 240 and 550 nm (Morocho-Jácome et al. 2021; Mansuri et al. 2021).



EI: Extract prepared by infusion; **FI:** Fraction prepared by infusion; **EM:** Extract prepared by maceration; **FM:** Fraction prepared by maceration; **ED:** Extract prepared by decoction; **FD:** Fraction prepared by decoction.

Fig. 03. Scanning spectra (from 200 to 600 nm) of the *a.* aqueous extracts and *b.* fractions of *T. aculeata leaves*

According to the Agência Nacional de Vigilância Sanitária (ANVISA), the minimum SPF value proven for its use as a multifunctional product must be at least 2, and for its use as photoprotector, the specified value is 6, in addition to the minimum required critical wavelength being 370 nm (ANVISA 2012). The extracts showed SPF values that ranged from 7.45 to 8.14, while the fractions ranged from 17.07 to 17.27 (Fig 04). Thus, all extracts and fractions of *T. aculeata* showed promising values for their use as multifunctional and photoprotective.



Blank: Commercial cream, without incorporated extract; **EI:** Extract prepared by infusion; **FI:** Fraction prepared by infusion; **EM:** Extract prepared by maceration; **FM:** Fraction prepared by maceration; **ED:** Extract prepared by decoction; **FD:** Fraction prepared by decoction.

Fig 04. SPF value of extracts, fractions and extracts incorporated into the commercial cream.

Another important factor to consider for photoprotection is the calculation of the critical wavelength. The critical wavelength is the one corresponding to at least 90% of the spectrum area, and correlates how much the extract is able to protect against UVA radiation (Velasco et al. 2011). In this sense, all extracts and fractions showed higher values determined by ANVISA (>370), been indicated for this purpose (Table 02).

Considering the high toxicity of the fractions, in addition to the large amount of solvent needed to obtain them, only the extracts were selected for incorporation into the commercial non-ionic cream. The commercial cream used in the study showed a photoprotection value of 7.29 ± 0.24. The calculated wavelength values were equal to 375.67 ± 0.58 nm for e extract incorporated from the leaves in the form of infusion (EII); 380 ± 0.01 nm for extract incorporated in the form of maceration (EMI), and 376.01 for the extract incorporated in the form of decoction (EDI).

The extracts were incorporated to the commercial cream caused an increase in the SPF value of the extracts, with values ranging from 16.93 to 17.27, with the highest value attributed to EII (Fig. 04). Silva et al. (2016) found similar results when incorporating pomegranate extract into a synthetic organic filter and found a 20% increase in SPF and attributed this increase to the synergy of the compounds present in the plant extract and the organic filters used in the study.

After the incorporation of extracts, SPF analyzes were performed daily for 7 days. The FPS values showed an average deviation of 3-5%, showing that all extracts/fractions have good stability. As for the organoleptic analysis of the cream, it was noted that EI, EM and ED incorporated to the cream did not show changes in color and appearance over the days. All samples had a slightly sweet to pleasant smell, which did not change over time. The pH values were equal to 5 for all formulations, a result that can be attributed to the presence of phenolic compounds.

4. Conclusion

The extracts and fractions of the leaves of *T. aculeata* showed phenolic compounds and tannins in their composition, in addition to antioxidant, antimicrobial and photoprotective activities. The fractions showed the highest values of phenolic compounds compared to their respective aqueous extracts, in addition to presenting broad-spectrum antioxidant and antimicrobial activities, and high toxicity. The extracts showed promising results of antimicrobial and photoprotective activity, in addition to low toxicity, and were therefore selected for incorporation into commercial cream. The extracts incorporated to the commercial cream caused an increase in the SPF value, in addition to good stability results. Thus, the results found suggest the use of *T. aculeata* is a potential photoprotective/multifunctional product.

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