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Phenolic constituents and biological properties of *Eryngium pristis* Cham. & Schltdl (Apiaceae)

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ABSTRACT

The present study evaluated the total phenolic and flavonoid contents and the antioxidant, photoprotective and cytotoxic properties of Eryngium pristis. Dried and powdered of E. pristis leaves were exhaustively extracted with ethanol by static maceration followed by partition to obtain the hexane, dichloromethane, ethyl acetate, and butanol fractions. Phytochemical screening and the total phenolic and flavonoid contents were determined. The antioxidant activity was evaluated by DPPH, reducing power of Fe^{+3}, ABTS and β-carotene/linoleic acid assays. The UV absorption spectra were recorded in the range of 290 to 450 nm and the sun protection factor (SPF) was calculated. The cytotoxic test was performed by brine shrimp lethality bioassay. Tannins, flavonoids, coumarins, terpenoids and steroids, saponins and alkaloids were detected in the ethanol extract and fractions. In these samples, the total phenolic and flavonoid contents ranged from 5.05 to 20.10 g/100 g and 4.00 to 9.37 g/100 g, respectively. The ethanol extract and fractions showed antioxidant effect as free radical scavengers and inhibitors of lipid peroxidation, while the photoprotective capacity was concentration dependent manner. In addition, the tested samples were cytotoxic against brine shrimp. These results suggest that E. pristis is an important and promising source of bioactive compounds with relevant biological properties and can be used as strategy to develop new products for the treatment of several pathological conditions.

Keywords: *Eryngium pristis*, phenolic compounds, antioxidant activity, photoprotective effect, cytotoxicity

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INTRODUCTION

In recent decades, a large number of studies have shown the significance of free radicals and oxidants as being responsible for the molecular mechanisms that trigger different pathologic processes. These products, such as superoxide (O_2•-), hydroperoxyl (HO_2•), hydroxyl (•OH), and peroxyl (ROO•) radicals, are reactive oxygen species (ROS) and may be generated from cellular metabolism or from environmental sources of ionizing radiation, UV light, pesticides, alcohol, cigarette smoke, and oxygen shortage [1]. ROS is able to damage DNA and can oxidize lipids and proteins, as well as have been associated with the development of various human diseases as neurodegenerative disorders, cardiovascular diseases, pulmonary disorders, diseases related to the premature infants, autoimmune diseases, renal disorders, gastrointestinal disturbances, tumors and cancers, ageing process, diabetes, skin lesions, immunodepression, liver diseases, pancreatitis, infertility, among others [1,2,3]. Besides, as result of ROS actions, the exposure to ultraviolet (UV) radiation is involved with a variety of harmful effects ranging from photoaging to skin cancer [4].

On the other hand, the natural antioxidants can have great importance as therapeutic agents in several pathological conditions related to oxidative stress since they are able to neutralize the actions of free radicals [3,5]. Typically, these agents produce beneficial effects on the human health, because they are clinically efficient with low toxicity

[3]. Among the antioxidants found in vegetables, phenolic compounds, such as flavonoids, have the ability to scavenge free radicals and chelate metals [6,7]. For triggering these effects, many flavonoids and polyphenols exhibit conjugated π-electron systems that allow ready donation of electrons, or hydrogen atoms, from the hydroxyl moieties to free radicals [6].

Considering the Brazilian biodiversity, the *Eryngium* gender, Apiaceae family, found in southeastern and southern Brazil, is represented by 230 to 250 species [8]. The medicinal value of this genus is related to the preventive and curative actions against gallstones and fatty deposits in the liver, as well as for reducing cholesterol levels [9]. Species of *Eryngium* have been known for its antimicrobial properties [10], as diuretic, bronchodilator and for the treatment of skin disorders [8], being also reported as anthelmintic, anti-convulsant, anti-inflammatory, analgesic, and antimalarial [11,12]. From a chemical point of view, flavonoids, tannins, saponins, triterpenoids, α-cholesterol, brassicasterol, campesterol, stigmasterol, clerosterol, β-sitosterol, δ-5-avenasterol, δ-(5)-24-stigmastadienol and δ-7-avenasterol have been identified in *Eryngium* [11-14].

Eryngium pristis Cham. & Schltdl, known as "língua-de-tucano", is a shrubby species commonly used as emmenagogue, diuretic for diabetics, in the treatment of inflammation, thrush and throat and mouth ulcers [15]. Despite its importance in folk medicine, few reports have been published in scientific literature. In this sense, considering that oxidative damages are associated with different physiological changes in the body, this study evaluated the total phenolic, flavonoid contents and the antioxidant, photoprotective and cytotoxic properties of *Eryngium pristis* leaves.

EXPERIMENTAL SECTION

Plant material

Leaves of *Eryngium pristis* Cham. & Schltdl. (Apiaceae) were collected in the city of São João del Rei, Minas Gerais State, Southeast region of Brazil, in January 2010. The species was identified by Dr Glauciemar Del-Vechio-Vieira and a voucher specimen (number 207576) was deposited in the Herbarium of the National Museum of Brazil (R), Federal University of Rio de Janeiro, Brazil. The leaves were dried at room temperature with forced ventilation for a loss of 90–96% humidity. After drying, all material was triturated by an industrial blender and pulverized using a tamise nº 18 for the extract preparation.

Extract preparation

Dried and powdered mature leaves (690 g) were exhaustively extracted in 95% ethanol (4.0 l) by static maceration for 3 weeks at room temperature with renewal of solvent every 2 days. The ethanol extract was filtered and evaporated under a rotary vacuum evaporator (Rotavapor RII, Büchi, Flawil, Switzerland) at controlled temperature (50–55^{o}C). This material was placed into a desiccator with silica to yield 65.79 g. The ethanol extract (EE, 30 g) was suspended in water:ethanol (9:1) followed by liquid/liquid partition with increasing organic solvent polarity: hexane, dichloromethane, ethyl acetate, and butanol. After this procedure, hexane (HF), dichloromethane (DF), ethyl acetate (EF), and butanol (BF) fractions were obtained [16].

Phytochemical screening

The phytochemical tests to detect the presence of tannins, flavonoids, terpenes and phytosterols, saponins, coumarins, anthraquinones, and alkaloids were performed according to the method described by Tiwari et al. [17]. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents.

Chemicals and reagents

Chemicals and reagents used in this study (and their sources) were as follows: DPPH, ABTS, linoleic acid, β-carotene, tween® 40, BHT, gallic acid, and rutin (Sigma Chemical Co, St. Louis, MI, USA); aluminum chloride, calcium chloride, potassium chloride, magnesium chloride, potassium bromide, sodium sulfate, sodium bicarbonate, potassium ferrocyanide, ferric chloride, sodium chloride, dichloromethane, hexane, butanol, methanol, ethanol, pyridine, and sodium carbonate (Labsynth, Diadema, SP, Brazil) and Folin-Ciocalteu reagent, trichloroacetic acid, and ascorbic acid (Cromoline Química Fina, Diadema, SP, Brazil). All the chemicals used including the solvents were of analytical grade.

Total phenolic content determination

The total phenolic content was determined by Folin-Ciocalteu method [18] using gallic acid as reference standard (standard curve was prepared with concentrations from 10 to 50 µg/ml). The samples (EE, HF, DF, EF and BF) were oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance

of the resulting blue color was measured at 765 nm in a spectrophotometer (SHIMADZU®, UV-1800, Tokyo, Japan) after 60 min. The analyses were performed in triplicate and results were expressed as gram of gallic acid equivalent.

Total flavonoids content determination

Aluminum chloride colorimetric method described by Sobrinho et al. [19] was used for total flavonoid content determination using rutin as standard. The reaction was performed using samples (EE, HF, DF, EF and BF), acetic acid, pyridine:ethanol (2:8), 8% aluminum chloride, and distilled water at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm in a spectrophotometer (SHIMADZU®, UV-1800, Tokyo, Japan). The calibration curve was prepared with rutin solutions in ethanol (from 2 to 30 µg/ml) and results were expressed as gram of rutin equivalent.

DPPH radical scavenging activity

DPPH was used for determination of free radical-scavenging activity as recommended by Mensor et al. [20]. Different concentrations of each sample [EE (from 20 to 70 µg/ml), HF (from 35 to 140 µg/ml), DF (from 5 to 80 µg/ml), EF (from 1 to 30 µg/ml), and BF (from 30 to 60 µg/ml)] were added, at an equal volume, to methanol solution of DPPH (0.03 mM). After 60 min at room temperature, the absorbance was recorded at 518 nm in a spectrophotometer (SHIMADZU®, UV-1800, Tokyo, Japan). The experiment was performed in triplicate and rutin was used as standard. EC_{50} values denote the concentration (µg/ml) of sample, which is required to scavenge 50% of DPPH free radicals.

Test of Fe^{+3} reducing power

The reducing power of Fe^{+3} was determined using a serial dilution of EE, HF, DF, EF and BF (from 53.48 to 6.68 µg/ml) with 2.5 ml of 0.2 mM phosphate buffer pH 6.6, and 2.5 ml of 1% potassium ferrocyanide [$K_3Fe(CN)_6$] [21]. The mixture was incubated at 50ºC for 20 min. Five milliliters of this mixture received 2.5 ml of 10% trichloroacetic acid and was centrifuged at 3,000 g for 10 minutes. The supernatant was separated and mixed with 2.5 ml distilled water containing 0.5 ml of 1% ferric chloride. The absorbance of this reaction, in triplicate, was measured at 700 nm in a spectrophotometer (SHIMADZU®, UV-1800, Tokyo, Japan). Ascorbic acid was used as reference substance. The measurement was considered the possible antioxidant activity.

ABTS radical scavenging capacity

The free radical ABTS (2,2'-azinobis-3-etilbenzotiazoline-6-sulfonic acid) method was used to evaluate the antioxidant activity of ethanol extract and fractions from *E. pristis* leaves [22]. A standard solution of $ABTS^{\bullet+}$ (7 mM, 5 ml) was mixed with 88 µl of potassium persulfate (140 mM). The mixture was stirred and kept in a dark room temperature for 16 h. After this period, 1 ml was removed and the volume completed to 100 ml with ethanol (P.A.). The absorbance was read at 734 nm (yield 0.70 nm) in a spectrophotometer (SHIMADZU®, UV-1800, Tokyo, Japan). Different dilutions of the ethanol extract and fractions of *E. pristis* (from 500 to 5 µg/ml) were prepared and 30 µl removed and added to 3.0 ml of $ABTS^{\bullet+}$ solution. After 6 minutes, the measurement was performed at 734 nm and the EC_{50} values denote the concentration (µg/ml) of sample, which is required to scavenge 50% of $ABTS^{\bullet+}$ free radicals.

β-carotene/linoleic acid assay

Antioxidant activity (AA) was determined by *in vitro* method modified by Miller [23], using linoleic acid, Tween 40 and β-carotene. This system was maintained at approximately 40°C and the spectrophotometric absorbance measurements were made in a microplate reader (THERMOPLATE®, TP-Reader) at 492 nm every 15 minutes during 105 minutes (t_0, t_{15}, t_{30}, t_{45}, t_{60}, t_{75}, t_{90} e t_{105}). From these data, it performed a kinetic study of the ethanol extract and fractions compared to the antioxidant agents BHT and rutin. In addition, the percentage inhibition of lipid peroxidation (%I) was determined.

***In vitro* determination of sun protection factor (SPF)**

All samples (1.0 g) were weighed, transferred to a 100 ml volumetric flask, diluted to volume with ethanol, followed by ultrasonication for 5 min. From this stock solution (10 mg/ml), the ethanol extract and fractions at concentrations of 0.05, 0.10 and 0.20 mg/ml were prepared. The UV absorption spectra of these solutions were obtained in the range of 290 to 450 nm, with 3 determinations at each point, every 5 nm, using 1 cm quartz cell, and ethanol as a blank. SPF was calculated using the application of Mansur´s equation [24].

Brine shrimp lethality bioassay

The artificial seawater used in the experiments presented the following composition: NaCl 24 g/l, $CaCl_2 \cdot 2H_2O$ 1.5 g/l, KBr 0.1 g/l, KCl 0.7 g/l, Na_2SO_4 4.0 g/l, $NaHCO_3$ 0.3 g/l, and $MgCl_2 \cdot 6H_2O$ 11 g/l. Ethanol extract and fractions were dissolved in tween 80 and DMSO (1:1) followed by artificial seawater. Ten shrimps (*Artemia salina* Leach) were transferred into test tubes in quadruplicate, containing the following concentrations: 10, 50, 100, 500 and 1,000

μg/ml. The tubes were maintained under illumination. Survivors were counted 24 h after exhibition to the ethanol extract and fractions. Thymol was used as standard [25]. LC_{50}'s and 95% confidence intervals were from the 24-hour counts using the probit analysis method [26].

Statistical analysis

Data were expressed as mean ± S.E.M. Statistical significance was analysed by the one-way analysis of variance followed by the Tukey or Student Newman-Keuls test. *P* values below 0.05 were considered significant.

RESULTS AND DISCUSSION

Yield from the ethanol extract and fractions

690 g of dried and powdered leaves from *E. pristis* yielded 65.79 g of ethanol extract equivalent to 9.53%. After partition, 30 g of this extract produced 10.81, 2.64, 4.96, and 4.79 g of hexane, dichloromethane, ethyl acetate and butanol fractions, respectively.

Phytochemical screening

Table 1 shows positive reactions to tannins, flavonoids, coumarins, terpenoids and steroids, saponins and alkaloids in ethanol extract. These chemical classes were also detected in the ethyl acetate and butanol fractions. Reactions to flavonoids and alkaloids were well characterized in the samples.

Table 1. Phytochemical screening of the ethanol extract and fractions from *Eryngium pristis* leaves

Chemical classes	Reactions	EE	HF	DF	EF	BF
Tannins	Iron salts	+	-	-	+	+
	Lead salts	+	-	+	+	+
	Copper acetate	+	+	+	+	-
	Alkaloids	-	-	-	-	-
	Gelatine	+	+	+	+	+
Flavonoids	Aluminum chloride	+	-	-	+	+
	Sodium hydroxide	+	-	-	+	+
	Shinoda	+	-	-	+	+
Coumarins	Potassium hydroxide	+	-	-	+	+
Terpenes and steroids	Libermann-Burchard	+	+	+	+	+
	Kedde	+	-	+	+	+
	Baljet	+	-	+	+	+
Saponins	Foam Index	+	-	-	-	+
Alkaloids	Dragendorff	+	+	+	+	+
	Mayer	+	+	+	+	+
	Bouchardat	+	+	+	+	+
	Bertrand	+	+	+	+	+
Anthraquinones	Borntraeger	-	-	-	-	-

EE: Ethanol extract; HF: Hexane fraction; DF: Dichloromethane fraction; EF: Ethyl acetate fraction; BF: Butanol fraction; (+) positive reaction; (-) negative reaction.

Total phenolic and flavonoid contents

Total phenolic content was estimated by using Folin-Ciocalteu reagent, expressed as gram of gallic acid equivalent, while flavonoid was quantified by aluminum chloride method and expressed as gram of rutin equivalent. In *E. pristis*, the total phenolic varied from 5.05 ± 0.01 to 20.10 ± 0.05 g/100 g and flavonoid ranged from 4.00 ± 0.02 to 9.37 ± 0.12 g/100 g (Table 2). The hexane and dichloromethane fractions showed no reactions to determine the total flavonoid content. In addition, Table 2 also showed that the ethyl acetate fraction exhibited the highest total phenolic (20.10 ± 0.05 g/100 g) and the highest amount of flavonoid contents (9.37 ± 0.12 g/100 g).

Table 2. Total phenolic and flavonoid contents obtained with ethanol extract and fractions from *Eryngium pristis* leaves

Plant extract	Total phenolic (g/100g)	Total flavonoid (g/100 g)
Ethanol extract	10.62 ± 0.04	4.28 ± 0.05[a]
Hexane fraction	6.46 ± 0.02	-
Dichloromethane fraction	9.05 ± 0.11	-
Ethyl acetate fraction	20.10 ± 0.05	9.37 ± 0.12
Butanol fraction	5.05 ± 0.01	4.00 ± 0.02[a]

Each value in the table is represented as mean ±S.E.M. (n = 3). Same letters in the same column indicate that there was no significant difference between the means considering p < 0.05 after ANOVA followed of Tukey's test.

DPPH radical scavenging, Fe^{+3} reducing power and ABTS radical scavenging activities

Table 3 shows the scavenging effects obtained with samples on DPPH radical in the following order: EF > DF > EE > BF > HF. The EC_{50} values were statistically different ($p < 0.05$) that ranged from 27.82 ± 0.08 to 203.17 ± 1.99

μg/ml (Table 3). EF and DF were more active to inhibit the DPPH radical with EC_{50} equal to 21.47 ± 0.25 and 43.76 ± 0.53 μg/mL, respectively (Table 3). In addition, the ranking order for Fe^{+3} reducing power was EF > EE > DF = BF > HF. These samples produced EC_{50} values between 18.79 ± 0.10 and 213.48 ± 0.76 μg/ml. As noted in the DPPH test, EF was more potent in convert Fe (+3) to Fe (+2) with EC_{50} of 18.79 ± 0.10 μg/ml. Using the ABTS assay, EC_{50} values of the samples were in the following order: EF > EE > BF > HF > DF. These values ranged from 18.72 to 66.86 μg/ml, and the ethyl acetate fraction (EC_{50} = 18.72 ± 0.27 μg/ml) was more active in inhibiting free radical.

Table 3. Antioxidant activity of the ethanol extract and fractions obtained from *Eryngium pristis* leaves by DPPH, Fe^{+3} reducing power and ABTS methods

Plant extract/Chemical	EC_{50} (μg/ml)		
	DPPH	Fe^{+3} Reducing Power	ABTS
Ethanol extract	72.36 ± 0.73	42.05 ± 0.14	29.33 ± 0.22
Hexane fraction	397.85 ± 1.61	213.48 ± 0.76	49.30 ± 0.09
Dichloromethane fraction	43.76 ± 0.53	59.29 ± 0.31[a]	66.86 ± 0.74
Ethyl acetate fraction	21.47 ± 0.25	18.79 ± 0.10	18.72 ± 0.27
Butanol fraction	78.15 ± 0.50	60.14 ± 0.09[a]	35.60 ± 0.32
Rutin	8.58 ± 0.15	-	-
Ascorbic acid	-	3.65 ± 0.10	-
BHT	-	-	13.82 ± 0.34

Each value in the table is represented as mean ± S.E.M. (n = 3). Same letters in the same column indicate that there was no significant difference between the means considering p < 0.05 after ANOVA followed of Tukey´s test.

Beta-carotene bleaching antioxidant activity

Besides showing good antioxidant activity and significant reducing power, the ethanol extract and fractions were also able to inhibit the oxidation of the β-carotene/linoleic acid system. In this study, the β-carotene decolouring mechanism was evaluated in a system mediated by free radicals formed from linoleic acid. The presence of extract and fractions with antioxidant activity can partially inhibit the loss of β-carotene colour through the neutralisation of free radicals formed in the system, the % of oxidation inhibition being concentration-dependent. The order of oxidation inhibition power observed with the ethanol extract and fractions analysed was as follows: Ethyl acetate fraction > hexane fraction > ethanol extract > dichloromethane fraction > butanol fraction (Table 4). The results obtained in this study indicate that the potential of *E. pristis* to inhibit oxidative processes in emulsified systems should be exploited. The ethyl acetate fraction, with the highest total phenolic and flavonoid contents, also had the greatest oxidation inhibition power in this system, indicating the high capacity of these compounds to scavenge free radicals liberated during linoleic acid oxidation.

Table 4. Inhibition of lipid peroxidation of the ethanol extract and fractions from *Eryngium pristis* leaves in β-carotene/linoleic acid system

Plant extract/Chemical	% Inibição da peroxidação lipídica
Ethanol extract	58.12 ± 1.07
Hexane fraction	62.49 ± 1.68
Dichloromethane fraction	47.60 ± 1.02
Ethyl acetate fraction	67.47 ± 1.57[a]
Butanol fraction	42.38 ± 0.71
BHT	65.08 ± 0.72[a]
Rutin	32.30 ± 0.90

Each value in the table is represented as mean ± S.E.M. (n = 3). Same letters in the same column indicate that there was no significant difference between the means considering p < 0.05 after ANOVA followed of Tukey's test.

Table 5. Sun protection factor (SPF) of the ethanol extract and fractions from *Eryngium pristis* leaves in three different concentrations

Plant extract/Chemical	SPF			
	0.05 mg/ml	0.10 mg/ml	0.20 mg/ml	50 mg/ml
Ethanol extract	1.22 ± 0.001	2.58 ± 0.002	5.00 ± 0.003	-
Hexane fraction	1.68 ± 0.002	3.28 ± 0.003	6.44 ± 0.002	-
Dichloromethane fraction	2.48 ± 0.003	5.15 ± 0.001	9.91 ± 0.003	-
Ethyl acetate fraction	15.10 ± 0.006	31.22 ± 0.040	39.70 ± 0.200	-
Butanol fraction	6.70 ± 0.002	13.72 ± 0.002	27.21 ± 0.030	-
PBISA	-	-	-	13.84 ± 0.11

Each value in the table is represented as mean ± S.E.M. (n = 3). In the same column, the means are different considering p < 0.05 after ANOVA followed of Tukey's test. PBISA = 2-Phenylbenzimidazole-5-sulfonic acid.

Photoprotective effect

Considering the ethanol extract and fractions from *E. pristis* leaves, the SPF value increased concentration dependent manner (Table 5). When compared with the standard (2-phenylbenzimidazole-5-sulfonic acid, SPF =

13.84), the SPF at 50 mg/ml was equivalent to the concentration of the butanol fraction at 0.10 mg/ml. As observed in Table 5, at 0.20 mg/ml, EF was more potent (SPF = 39.70) than 2-phenylbenzimidazole-5-sulfonic acid (50 mg/ml). This result can be explained by the higher total phenolic content and the antioxidant activity detection reported in the present study. In addition, the UV absorption spectra profiles of the ethanol extract and fractions revealed the difference among them which it can is related to the chemical composition (Figure 1).

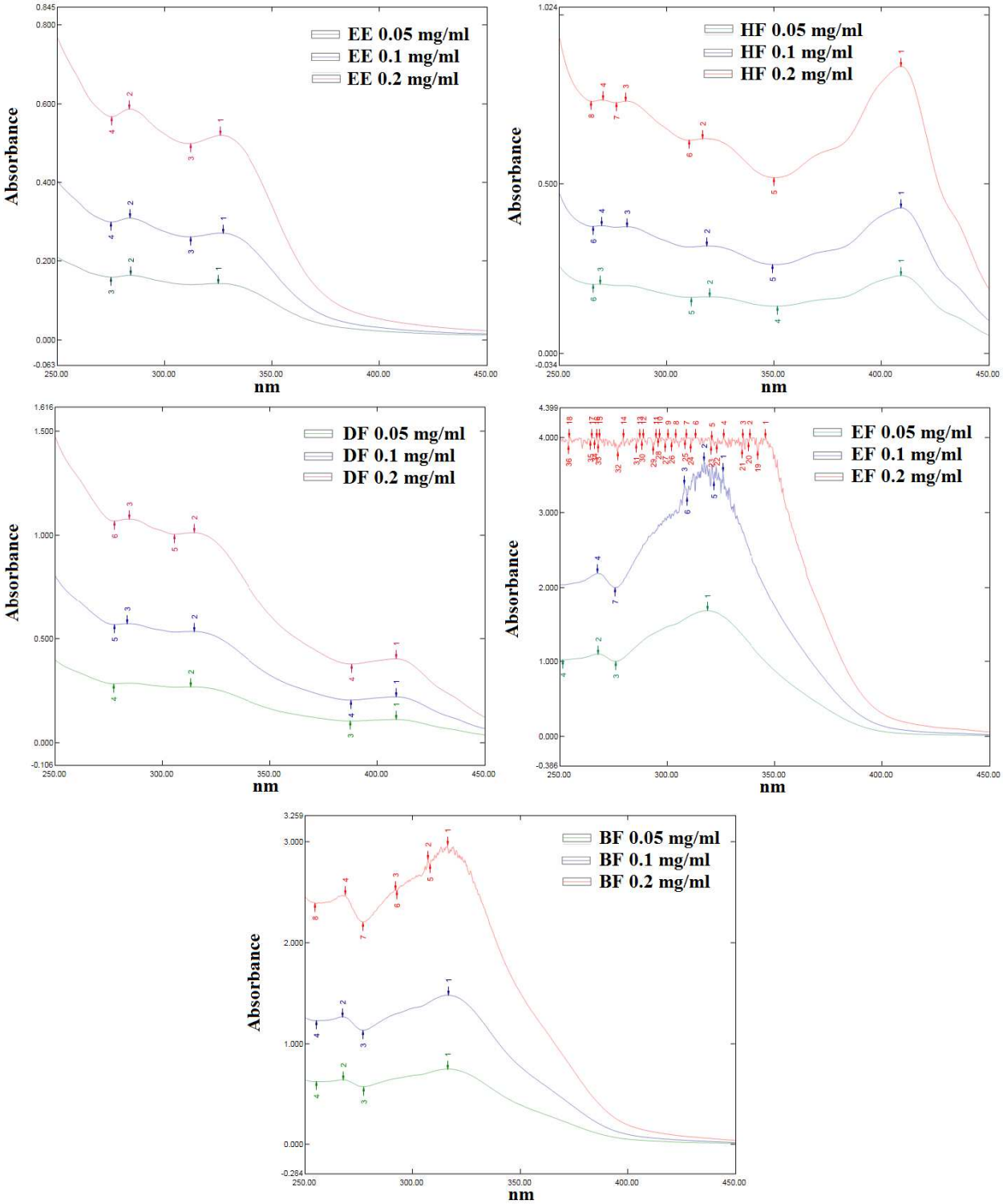

Figure 1. UV absorption spectra profiles of the ethanol extract and fractions from *Eryngium pristis* leaves in three different concentrations. EE: Ethanol extract; HF: Hexane fraction; DF: Dichloromethane fraction; EF: Ethyl acetate fraction; BF: Butanol fraction

Cytotoxicity in the brine shrimp assay

The ethanol extract and fractions from *E. pristis* leaves were poisonous against *Artemia salina* producing LC_{50} values lower than 1,000 μg/ml (Table 6). According to the data presented in Table 6, the results showed that the dichloromethane and hexane fractions were three times more active than thymol (LC_{50} = 438.13 μg/ml), the reference substance.

Table 6. Toxicity of the ethanol extract and fractions from *Eryngium pristis* leaves on *Artemia salina*

Tested product	LC_{50} (μg/ml)	Confidence interval (95%)
Ethanol extract	518.17	313.44 – 856.64
Hexane fraction	113.42	67.76 – 189.85
Dichloromethane fraction	110.92	64.10 – 192.00
Ethyl acetate fraction	470.93	252.24 – 879.21
Butanol fraction	763.31	414.43 – 1405.91
Thymol[a]	438.13	243.97 – 786.80

[a] *Reference drug.*

Plant species have significantly contributed for the supply of useful substances to treat diseases that affect humans and animals [27]. The phytochemical screening of *E. pristis* detected the presence of tannins, flavonoids, alkaloids, terpenes and steroids, and saponins (Table 1). This finding corroborates the chemical profile described for the genus species [12,28]. Among the special metabolites detected and quantified, phenols, and especially flavonoids, have been extensively studied for its antioxidant and photoprotective actions [29]. Our results (Table 2) confirmed the positive reactions of phenolic compounds (Table 1) and, in part, these constituents can justify the medicinal properties of *E. pristis.* Accordingly, the variation of the total phenolic and flavonoid contents in the fractions was influenced by the polarity of solvent, since hexane is able to remove terpenes and steroids and dichloromethane promotes the extraction of lignans, methoxylated flavonoids, sesquiterpenes, lactones, coumarins and triterpenes [16]. In addition, free flavonoids, tannins, xanthones, triterpenic acids, saponins and phenolic compounds are extracted with ethyl acetate, while glycosylated flavonoids, tannins, saponins and carbohydrates are separated by the butanol action [16].

Antioxidant activity data showed that the action of phytochemicals from *E. pristis* against free radicals can be related to the redox properties. Many natural constituents, especially phenolic compounds, have the ability to scavenge a variety of oxidants (superoxide, hydroxyl and peroxyl radicals and hypochlorous acid, singlet oxygen quenching and metal ion chelation) that are generated in our body [1]. These oxidants can cause cellular damages through the peroxidation of unsaturated fatty acids, denaturation of proteins and reaction with carbohydrates and nucleic acids triggering pathophysiological processes associated with various diseases [1-3]. In this sense, our results can have great importance in the therapeutic approaches of different disorders, because the ethanol extract and fractions of *E. pristis* leaves were effective as radical scavengers and inhibitors of lipid peroxidation. Probably, the neutralization of free radicals is associated with the action of phenolic compounds mainly found in the ethyl acetate and butanol fractions, while the inhibition of lipid peroxidation may be due to the presence of less polar components, as terpenes, since the hexane fraction was more active in this assay. Phytochemicals identified from species of *Eryngium*, such as terpenoids, triterpenoid saponins, flavonoids, coumarins, polyacetylenes, and steroids, can corroborate these observations [12].

Whereas that the UV radiation causes oxidative damage with generation of free radicals, the photoprotective power of plant extracts may reduce the risk of sun-induced skin cancer [30]. In cosmetic practice, the photoprotective power has been determined by SPF as the UV energy necessary to produce a minimal erythemic response after 16 to 24 h of exposure on protected skin divided by the UV energy requested to cause this erythema on unprotected skin in this same time [31]. In the last decades, due to the increase of cases of early photoaging, actinic keratosis, cancer and basal cell carcinoma and melanoma, many natural products have been investigated as UV protective agents [30,32,33]. In this sense, plant extracts, particularly those with antioxidant activity, have been potential targets for the development of new sunscreens, since contain compounds as phenolic acids, flavonoids, and polyphenols, which cover the full range of UV wavelengths [32,34]. Constituents of this kind were detected and quantified in our study and may be responsible for the SPF and prevention of the UV-induced oxygen free radical generation and lipid peroxidation. Furthermore, at 0.20 mg/ml, the ethyl acetate fraction was more active (SPF = 39.70 ± 0.200) than the standard. This result can be explained by the higher total phenolic and flavonoid contents and the antioxidant activity detection observed with the EF. In addition, the UV absorption spectra profiles revealed the difference among the ethanol extract and fractions (Figure 1). *In vivo* investigations have shown that the use of plant extracts with antioxidant activity associated with synthetic sunscreen is more effective to reduce skin damage caused by the exposure to solar radiation [35].

The *A. salina* bioassay is a simple model applied to the toxicity investigation [25] and can be a valuable tool as a screening effort in the search for compounds with protective action against damage by superoxide or other active oxygen species [36]. In addition, brine shrimp have been utilized in various bioassay systems (analysis of pesticide residues, mycotoxins, stream pollutants, anesthetics, dinoflagellate toxins, morphine-like compounds, toxicity of oil dispersants, cocarcinogenicity of phorbol esters, and toxicants in environments) and may be possible to monitor fractionation of natural products with cytotoxic activity on tumor cell lines [25]. Based on this test, our results showed that the ethanol extract and fractions from *E. pristis* leaves, particularly dichloromethane and hexane fractions, were toxic on the microcrustacean and this finding has not been previously described. The presence of compounds extracted with less polar solvents such as those cited by Cechinel Filho and Yunes [16] can explain the toxicity of these fractions, since the cytotoxicity of compounds from species of the genus *Eryngium* have been evaluated against different tumor cell lines [12]. *Eryngium* saponin, for example, exhibited moderate cytotoxicity against A549, PC-3, HL-60, and MRC-5 cell lines. Especial, Eryngioside H and I revealed potent and highly selective inhibition against four human tumor cells but almost no cytotoxicity against normal human cells [12]. Ethanol extracts from fruits of *E. planum* showed highly significant in inducing apoptosis in two human leukemic cell lines C8166 and J45 [37]. Therefore, our results can contribute to the research of antitumor agents from *E. pristis*.

CONCLUSION

The results obtained in the present study suggest that the ethanol extract and fractions from *E. pristis* leaves are an important and promising source of bioactive compounds with antioxidant, photoprotective and cytotoxic properties, since they were able to neutralize the action of free radical, inhibit the lipid peroxidation, increase the SPF and were noxious against brine shrimp.

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