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Research Article

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Antioxidant and Cytotoxic Properties of the Ethanol Extract and Fractions from *Eremanthus erythropappus* (DC) MacLeish Leaves

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ABSTRACT

The present study evaluated the total phenolic and flavonoid contents and the antioxidant and cytotoxic properties of Eremanthus erythropappus. Dried and powdered of E. erythropappus leaves were 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} and β-carotene/linoleic acid assays. The cytotoxic test was performed by brine shrimp lethality bioassay. Tannins, flavonoids, coumarins, terpenoids and steroids and saponins were detected in the ethanol extract and fractions. In these samples, the total phenolic and flavonoid contents ranged from 0.98 ± 0.15 to 16.27 ± 0.23 g/100 g and 0.84 ± 0.06 to 5.09 ± 0.22 g/100 g, respectively. The ethanol extract and fractions showed antioxidant effect as free radical scavengers and inhibitors of lipid peroxidation. In addition, the tested samples were cytotoxic against brine shrimp. These results suggest that E. erythropappus is an important and promising source of bioactive compounds with antioxidant and cytotoxic properties.

Keywords: *Eremanthus erythropappus*; Phytochemical screening; Phenolic content; Antioxidant activity; Cytotoxic property

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INTRODUCTION

Oxidative damages caused by the action of reactive oxygen species (ROS) have been related to the different pathological conditions, including degenerative diseases such as heart disease, atherosclerosis, and pulmonary disorders, as well as inflammation, fibrosis, and cancer and toxic effects [1]. In this sense, the existence of therapeutic options that could inhibit the oxidative mechanisms can represent a potentiality for the treatment of these pathologies [2]. Natural compounds or herbal medicines that are free radical scavengers or inhibitors of lipid peroxidation, for example, can be beneficial to people's health because they present antioxidant effects [1,2]. In addition, phenolic acids, phenolic diterpenes, flavonoids, volatile oils and others special metabolites are the major plant-derived antioxidants that can reduce the oxidative stress [3]. In this way, the search for natural antioxidant agents may be a strategy to prevent and/or treat oxidative stress and harmful effects on the body.

Eremanthus erythropappus (DC) McLeisch (Asteraceae) (*Vanillosmopsis erythropappa* Schultz-Bip), known as "candeia-da-serra", has its application in folk medicine related to the inflammatory and infectious diseases and as antibacterial, antimycotic, dermatological and spasmodic [4]. In addition, extracts, essential oil or isolated compounds of this species has presented antimicrobial [4-7], antioxidant [4], and antinociceptive and anti-inflammatory activities [8,9]. These properties have been associated with chemical components found in *E. erythropappus* [4-9]. Among these chemical constituents identified, β-caryophyllene, germacrene-D, α-copaene, β-pinene and α-bisabolol, components from essential oils [4,6-8,10,11], as well as sesquiterpene lactones have been highlighted [12-15].

Considering the medicinal applications implicated in diseases associated with the generation of free radicals and its toxic effects, this study evaluated the total phenolic and flavonoid contents and the antioxidant and cytotoxic properties of the ethanol extract and fractions obtained from *E. erythropappus* leaves.

EXPERIMENTAL SECTION

Oxidative damages caused by the action of reactive oxygen species (ROS) have been related to the different pathological conditions, including degenerative diseases such as heart disease, atherosclerosis, and pulmonary disorders, as well as inflammation, fibrosis, and cancer and toxic effects [1]. In this sense, the existence of therapeutic options that could inhibit the oxidative mechanisms can represent a potentiality for the treatment of these pathologies [2]. Natural compounds or herbal medicines that are free radical scavengers or inhibitors of lipid peroxidation, for example, can be beneficial to people's health because they present antioxidant effects [1,2]. In addition, phenolic acids, phenolic diterpenes, flavonoids, volatile oils and others special metabolites are the major plant-derived antioxidants that can reduce the oxidative stress [3]. In this way, the search for natural antioxidant agents may be a strategy to prevent and/or treat oxidative stress and harmful effects on the body.
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RESULTS AND DISCUSSION

Yield from the ethanol extract and fractions

500g of dried and powdered from *E. erythropappus* leaves yielded 58.62 g of EEEE equivalent to 11.72%. The partition procedure, using 30 g of EEEE, produced 8.92, 3.08, 4.26, and 5.82 g of hexane, dichloromethane, ethyl acetate and butanol fractions, respectively.

Phytochemical screening

Table 1 show that tannins, flavonoids, coumarins, terpenoids and steroids and saponins were detected in EEEE. However, the positive reactions for these classes of metabolites varied according to the polarity of the extracting solvent of each fraction.

Table 1: Phytochemical screening of EEEE and fractions from *Eremanthus erythropappus* leaves

Chemical classes	Reactions	EEEE	HFEE	DFEE	EFEE	BFEE
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	-	-	-	-	-

EEEE: Ethanol extract; HFEE: Hexane fraction; DFEE: Dichloromethane fraction; EFEE: Ethyl acetate fraction; BFEE: Butanol fraction; (+) positive reaction; (-) negative reaction

Total phenolic and flavonoids

In *E. erythropappus*, the total phenolic (gram of gallic acid equivalent) varied from 0.98 ± 0.15 to 16.27 ± 0.23 g/100 g, while total flavonoids (gram of rutin equivalent) ranged from 0.84 ± 0.06 to 5.09 ± 0.22 g/100 g (Table 2). In addition, this Table also showed that EFEE exhibited the highest total phenolic (16.27 ± 0.23 g/100 g) and the highest amount of flavonoid contents (5.09 ± 0.22 g/100 g).

Table 2: Total phenolic and flavonoids contents obtained with ethanol extract and fractions from *Eremanthus erythropappus* leaves

Plant Extract	Total phenolic (g/100g)	Total flavonoids (g/100 g)
Ethanol extract	8.09 ± 0.25	3.24 ± 0.06
Hexane fraction	0.98 ± 0.15	-
Dichloromethane fraction	2.03 ± 0.19	0.84 ± 0.06
Ethyl acetate fraction	16.27 ± 0.23	5.09 ± 0.22
Butanol fraction	4.57 ± 0.18	1.95 ± 0.21

Each value in the Table is represented as mean ± S.E.M. (n = 3). The values are significantly different ($P < 0.05$) after ANOVA followed of Tukey's test

DPPH Radical scavenging and reducing power activities

The antioxidant activity of *E. erythropappus* showed that EFEE (27.82 ± 0.08 μg/ml) and BFEE (41.76 ± 0.32 μg/ml) were more effective in inhibit the DPPH radical (Table 3). The EC_{50} values were statistically different ($p < 0.05$) and ranged from 27.82 ± 0.08 to 203.17 ± 1.99 μg/ml (Table 2). Using FRAP method, the EC_{50} values varied between 40.08 ± 0.19 and 239.80 ± 2.02 μg/ml. EFEE and BFEE were also more potent in convert Fe (+3) to Fe (+2) with EC_{50} of 40.08 ± 0.19 and 55.99 ± 0.44 μg/ml, respectively.

Table 3: Antioxidant activity of the ethanol extract and fractions obtained from *Eremanthus erythropappus* leaves by DPPH and FRAP methods

Plant extract/Chemical	EC_{50} (μg/ml)	
	DPPH	FRAP
Ethanol extract	59.30 ± 0.60	98.00 ± 0.45
Hexane fraction	203.17 ± 1.99	239.80 ± 2.02
Dichloromethane fraction	166.05 ± 1.15	197.18 ± 0.33
Ethyl acetate fraction	27.82 ± 0.08	40.08 ± 0.19
Butanol fraction	41.76 ± 0.32	55.99 ± 0.44
Rutin	8.73 ± 0.04	-
Ascorbic acid	-	5.10 ± 0.02

Each value in the Table is represented as mean ± S.E.M. (5 = 3). The values are significantly different (P < 0.05) after ANOVA followed of Tukey's test

Beta-carotene bleaching antioxidant activity

To verify another mechanism of the antioxidant action, EEEE and fractions were tested through the oxidation of the β-carotene/linoleic acid system. In this system, the generation of free radicals occurs from linoleic acid to promote the discoloration of the reaction with beta-carotene. Thus, our results showed that HFEE was more active to neutralize free radicals producing a decay curve similar to the positive control (BHT) and inhibition of lipid peroxidation of 68.44 ± 0.51% (Figure 1 and Table 4). EFEE and BFEE were less effective to scavenge free radicals liberated during linoleic acid oxidation (Figure 1 and Table 4) with inhibition of lipid peroxidation equal to 46.40 ± 0.86 and 34.63 ± 0.95%, respectively.

Table 4: Inhibition of lipid peroxidation of the ethanol extract and fractions from Eremanthus Erythropappus leaves in β-carotene/linoleic acid system

Plant extract/Chemical	% Inhibition of lipid peroxidation
Ethanol extract	48.84 ± 1.06
Hexane fraction	68.44 ± 0.63
Dichloromethane fraction	56.46 ± 1.40
Ethyl acetate fraction	30.08 ± 1.18
Butanol fraction	26.48 ± 1.16
BHT	70.80 ± 0.60

Each value in the Table is represented as mean ± S.E.M. (n = 3). The values are significantly different ($P < 0.05$) after ANOVA followed of Tukey's test

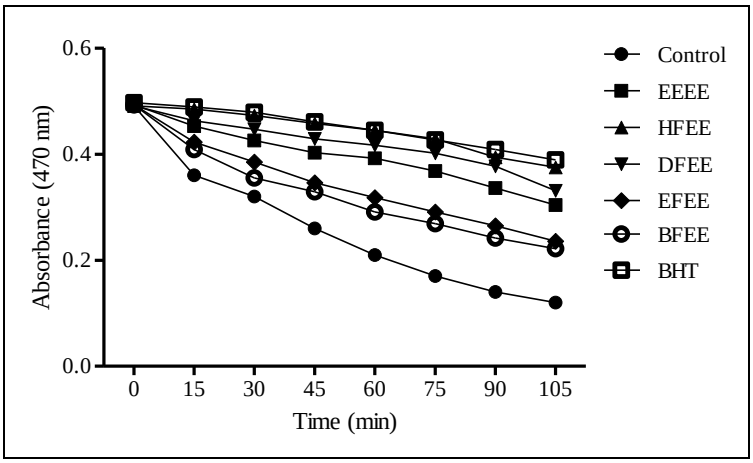

Figure 1: Decayment of coloring of the beta-carotene/linoleic acid system in the presence of EEEE and fractions. EEEE: Ethanol Extract; HFEE: Hexane Fraction; DFEE: Dichloromethane Fraction; EFEE: Ethyl Acetate Fraction; BFEE: Butanol Fraction; BHT: Butylated hydroxytoluene

Cytotoxicity in the brine shrimp assay

Table 5 shows that EEEE, HFEE, DFEE and EFEE were toxic against brine shrimp with LC_{50} values lower than 1,000 μg/ml. Considering the tested samples, DFEE and EFEE were more active than thymol (LC_{50} = 486.16 μg/ml), the reference compound, while BFEE produced LC_{50} greater than 1000 μg/ml, which was considered not poisonous.

Table 5: Citotoxicity of EEEE and fractions from Eremanthus erythropappus leaves against brine shrimp

Tested product	LC_{50} (μg/ml)	Confidence interval (95%)
Ethanol extract	646.68	395.94 – 1056.20
Hexane fraction	241.61	147.46 – 395.89
Dichloromethane fraction	182.07	114.30 – 290.03
Ethyl acetate fraction	969.02	533.74 – 1759.29
Butanol fraction	>1000.00	–
Thymol[a]	486.16	286.52 – 824.93

[a] Reference compound

For many generations, medicinal plants and herbal medicines have been used for the treatment of diseases and health problems, mainly by the poorest population. These products contain special metabolites that re-establish the people's health, since their actions inhibit the toxic effects produced by free radicals [2]. Phenolic compounds, for example, are able to eliminate free radicals and prevent the development of chronic diseases by its ability to donate electrons [25]. Our results showed that *E. erythropappus* is rich in special metabolites (Table 1), as tannins and flavonoids, which can justify the antioxidant action against free radicals associated with medicinal applications [4]. Considering the total phenolic and flavonoid contents, ethyl acetate fraction (EFEE) showed the highest concentrations of these constituents (Table 2). Probably, the polarity of ethyl acetate allowed a greater extraction of flavonoids from the ethanol extract as described by Cechinel Filho and Yunes [16]. However, it is important to mention that total phenolic and flavonoid contents from *E. erythroppapus* leaves have not previously been described in literature.

Using DPPH method, the EC_{50} value of the tested samples varied according to the polarity of solvent. As observed in Table 2, EFEE was more active to inhibit DPPH followed by BFEE. This antioxidant effect can be related to the presence of phenolic compounds (total phenolic and flavonoids), since these constituents have the ability to neutralize the DPPH radical. However, other special metabolites have also been associated with this antioxidant action, as components of essential oils found in *E. erythropappus* [4].

Ferric reducing antioxidant power (FRAP) assay determines the antioxidant action of compounds that convert of Fe^{+3} to Fe^{+2} [25]. All samples of *E. erythropappus* were active in transforming Fe^{+3} to Fe^{+2}, which corroborated the results observed with DPPH method. Phenolic compounds found in EFEE and BFEE may be responsible for this conversion to donate hydrogen atom to break the free radical chain [25]. In addition, the number and position of hydroxyl group in the phenolic compounds also regulate this antioxidant activity [25].

Our results can have great importance in the therapeutic approaches of different disorders, because EEEE and fractions from *E. erythropappus* leaves were effective as inhibitors of lipid peroxidation. Probably, this action may be due to the presence of less polar components, as terpenes, since HFEE was more active in this assay. Phytochemicals identified in *E. erythropappus*, such as terpenoids, triterpenoid saponins, flavonoids, coumarins, polyacetylenes, and steroids, can corroborate these observations (Table 1).

The *A. salina* bioassay has used to determine the toxicity of natural products [23] and investigate new compounds with action against free radical as superoxide or other active oxygen species [26]. This bioassay has also been used to evaluate the toxicity of pesticide residues, mycotoxins, stream pollutants, anesthetics, dinoflagellate toxins, morphine-like compounds, oil dispersants, cocarcinogenicity of phorbol esters, toxicants in environments and monitors the cytotoxic activity of natural products on tumor cell lines [26]. Except BFEE, our results showed that EEEE and fractions, mainly DFEE and EFEE, were toxic on brine shrimp. Apolar compounds, such as essential oils [4], have been involved in toxicity on *A. salina*, as well in antibacterial activities [6]. These results have not been previously described and can contribute to the research of antitumor agents from *E. erythropappus*.

CONCLUSION

The present study showed that EEEE and fractions from *E. erythropappus* leaves possess antioxidant and cytotoxic properties and could be related to the synergistic actions of bioactive compounds, which were able to neutralize the action of free radicals, inhibit the lipid peroxidation and be harmful against brine shrimp. However, new studies are needed for a better understanding of their medicinal uses.

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