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ANTIOXIDANT CAPACITY OF *LOURTEIGIA STOECHADIFOLIA* (L. F.) R.M. KING & H. ROB. ASTERACEAE

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Abstract

This study aimed to evaluate Antioxidant Capacity of extracts and fractions obtained from *Lourteigia stoechadifolia* (L. f.) R.M. King & H. Rob. Asteraceae, leaves using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. Extracts were obtained in dichloromethane and ethanol, using Soxhlet extraction, subsequent, a solid-liquid fractionation with solvents of increasing polarity has been carried out: Hexane, Chloroform, and Methanol for each of the extracts; antioxidant capacity were evaluated for each fraction and extract. Using the DPPH method, the ethanolic extract showed a relative antioxidant capacity equivalent to ascorbic acid of 28.6 and it's methanolic fraction of 32.0, and using the ABTS method, the ethanolic extract showed a relative antioxidant capacity equivalent to ascorbic acid of 58.5 and its methanolic fraction of 49.8. The ethanolic extract and its methanolic fraction showed the highest antioxidant capacity, these data show a potential of free radical scavengers, property that can be exploited in the cosmetic and food industry.

Keywords: *Lourteigia stoechadifolia*, *Antioxidant Capacity, DPPH*. ABTS*+*

Introduction

Lourteigia is a genus belong to Asteraceae family, [1-4], includes eleven species restricted to the high areas of the extreme north of the Andes. These have been reported above 2500 meters of altitude; three are endemic to Venezuela, seven thrive in the paramos and subparamos of Colombia, one grows in areas of both countries [5,6], *L. aroensis, L. ballotaefolia, L. dichroa, L. fimbriata, L. humilis, L. lanulata, L. microphylla, L. morenoi, L. ornatiloba, L. scandens* and *L. stoechadifolia*.

Lourteigia stoechadifolia (L. f.) R.M. King & H. Rob., has as synonyms. *Eupatorium stoechadifolium* L. f. and *Eupatorium stoechadifolium* var. pamplonense B.L. Rob. A shrub or sub-shrub is a small to medium sized, sometimes procumbent, cylindrical stem, striated, puberulous or densely tomentose, white hairs, leaves opposite, petiolate, sometimes very short petioles; leaf blade ovate to narrowly elliptic, margin serrated to crenulate, underside often provided with white tomentum.

Terminal inflorescence, densely corymbose, short pedicels; filarias 20-30, weakly to moderately subimbrincadas, arranged in 3-4 unequal series [6].

In 2013, Mondolis and colleagues reported two diterpenes identified as jhanidiol acetate (18-acetoxy-1β-hydroxymanoyl oxide), jhanidiol (1β, 18-dihydroxymanoyl oxide), purified from *Lourteigia stoechadifolia*, and its vasodilator effect [7]. Likewise in 2017, Moran et al., purified and identified two flavonoids from this specie with vasodilatory activity, the flavonoids were 5.3'-dihydroxy-6,7,4'-trimethoxyflavone, 5-hydroxy-6,7,3',4'-tetrametoxyflavone) [8]. The aim of this study was to evaluate Antioxidant Capacity of extracts and fractions obtained from *Lourteigia stoechadifolia* leaves (DPPH and ABTS methods).

Methods

Chemicals. The solvents were purchased from Riedel-de Haen, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS); trolox, ascorbic acid and rutin were purchased from Sigma-Aldrich.

Plant material. Samples of *Lourteigia stoechadifolia* were collected at Tenjo/La Punta (Cundinamarca-Colombia; 4°52′11″N 74°08′38″O), a region characterized by a warm climate. The plant was at flowering stage. *Lourteigia stoechadifolia* was identified at the Herbarium of the National University, voucher COL539592.

Extraction procedures. Dried and milled material of *L. stoechadifolia*, was extracted with dichloromethane and ethanol in a Soxhlet equipment, subsequent, a solid-liquid fractionation with solvents of increasing polarity was carried out (petroleum ether, dichloromethane and methanol). Stock solutions of the extracts and fractions were adjusted at 1000 mg/L.

Antioxidant capacity. Antioxidant capacity was evaluated by DPPH (1,1-Diphenyl-2-picryl-hydrazyl) and ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) methods.

In order to evaluate the antioxidant capacity by DPPH method, 1 mL of DPPH solution (20 mg/L) was added to 0.25 mL of each extract and fraction in a concentration range from 50 to 500 mg/L. After 30 min of reaction, the absorbance was measured at 517 nm in a Spectronic 21D UV-Visible spectrophotometer. Inhibition percentage was calculated as follows: $\%I=[(A_o\text{-}Ae)/A0]x100$, where A_o is the absorbance value of the DPPH blank sample and Ae is the absorbance value of the test solution.

ABTS assay was performed, mixing 1450 µL of standardized solution of $ABTS^{*+}$ and 50 µL of each extract and fraction (50 to 500 mg/L). The absorbance was measured at 754 nm in a Spectronic 21D UV-Visible spectrophotometer. ABTS radical was prepared by dissolving 10 mg of the compound and 2.4 mg of potassium persulfate in 10 mL of water.

The calculation of inhibitory concentration 50 (IC50) was performed using the Probit function of the Minitab program (V17).

Antioxidant capacity was expressed in equivalents of standard solutions of ascorbic acid, rutin and trolox (100 mg/L).

Results and Discussion

The evaluation of the antioxidant capacity was performed according to the following tests: DPPH* radical discoloration test and ABTS*+ radical discoloration test.

*Discoloration method of DPPH**. The antioxidant capacity of the extracts and fractions from *L. stoechadifolia*, were evaluated using ascending concentrations between 50 to 500 mg/L. Table 1 shows the results of antioxidant.

The percentages of radical absorption using DPPH* method for ethanolic extract and it`s methanolic fraction are within the range from 25.30 ± 0.096 to 37.70±0.149. This last value indicated a maximum of total extract absorption in a concentration of 50 mg/L; at 500 mg/L the absorption percentage is in a range between 79.90±0.299 and 87.70±0.329 showing a maximum of absorption for the ethanolic extract (Table 1). With these percentages of free radical absorption, IC50 was calculated (table 2).

Ethanolic extract, the methanolic fraction from the ethanolic extract and the methanolic fraction from the dichloromethane extract, are the ones that have lower IC50 values (higher antioxidant capacity), comparing with the standards.

The relative antioxidant activity (RAA) of ethanolic extract with respect to ascorbic acid was 28.6 and 10.6 with respect to the rutin. Being this extract that presented the highest antioxidant capacity.

Discoloration method of radical ABTS+.* The results of the percentages of free radical's absorption of the extracts and fractions from *L. stoechadifolia*, evaluated between 50 to 500 mg/L, by ABTS*+ method, are reported in Table 1. For ethanolic extract and it`s methanolic fraction, It was found percentages of free radicals absorption in the range from 19.30±0.082 to 37.00±0.175, at a concentration of 50 mg/L; at 500 mg/L the absorption percentage is in a range from 91.50±0.372 to 94.20±0.425, showing a maximum absorption value with ethanolic extract (Table 1).

Taking into account the rate of free radical absorption IC_{50} of the extracts and fractions was calculated (Table 2). The ethanolic extract and its methanolic fraction, showed the lower values for IC_{50} comparing with the standards, 106.3 and 90.40 mg/L, respectively.

The relative antioxidant activity (RAA) of the ethanol extract with respect to standard solutions was 58.5 (ascorbic acid), 58.5 (trolox) and of 25.0 (rutin). The RAA of methanolic fraction from dichloromethane extract, was 49.8 (ascorbic acid), 40.1 (trolox) and 21.2 (rutin).

Comparing with previous studies performed by the same methods for other species of Asteraceae (*Chromolaena bullata* and *Diplostephium phylicoides*) [25-26], it can be observed that *Lourteigia stoechadifolia* has a lower antioxidant capacity (Table 3).

Conclusions

The results of antioxidant activity suggest that of all of *Loutergia stoechadifolia* extracts capture the DPPH and ABTS radicals in a concentration dependent manner. Using the DPPH and ABTS methods, the ethanolic extract and it´s metanolic fraction, showed the highest antioxidant capacity.

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Table 1. Percentage of free radical absorption by DPPH* and ABTS*+ methods.

	DPPH*					ABTS*+			
mg/L MeOH	**Fr. Petrol EX ETOH**	**Fr. CHCl3 EX. ETOH**	**Fr. MeOH EX. ETOH**	**Ex.ETOH**	**mg/L MeOH**	**Fr. Petrol EX. ETOH**	**Fr. CHCl3 EX. ETOH**	**Fr. MeOH EX. ETOH**	**Ex.ETOH**
50	28.2±0,122	12.9±0,056	25.3±0.096	37.3±0,149	50	7.0±0,028	5.5±0,022	19.3±0,082	34.2±0,175
100	36.7±0,161	23.3±0,102	56.0±0.229	55.7±0,214	100	16.5±0,072	10.3±0,041	42.2±0,185	38.3±0,171
200	41.2±0,158	31.6±0,121	76.3±0.302	66.7±0,260	200	38.7±0,194	18.5±0,077	75.8±0,328	44.6±0,242
500	53.1±0,207	65.9±0,262	79.9±0.299	87.7±0,329	500	91.8±0,379	60.5±0,257	91.5±0,372	94.2±0,425
mg/L MeOH	**Fr. Petrol Ex. CH2Cl2**	**Fr. CHCl3 Ex. CH2Cl2**	**Fr. MeOH Ex. CH2Cl2**	**Ex.CH2Cl2**	**mg/L MeOH**	**Fr. Petrol Ex. CH2Cl2**	**Fr. CHCl3 Ex. CH2Cl2**	**Fr. MeOH Ex. CH2Cl2**	**Ex.CH2Cl2**
50	13.3±0,051	6.9±0,022	21.8±0,070	10.7±0,039	50	22.6±0,114	4.9±0,022	19.2±0,086	6.9±0,030
100	31.0±0,114	15.3±0,055	38.0±0,115	29.0±0,087	100	25.8±0,113	6.8±0,030	24.7±0,106	15.5±0,066
200	32.7±0,103	34.8±0,110	49.7±0,173	46.3±0,156	200	30.3±0,152	19.3±0,095	28.5±0,120	43.7±0,173
500	67.2±0,238	49.5±0,168	82.9±0,244	71.9±0,216	500	48.1±0,213	42.3±0,189	67.1±0,280	90.3±0,359

Table 2. IC_{50} and relative antioxidant activity by $DPPH^*$ and $ABTS^{*+}$ methods.

	ABTS*+				DPPH*		
	IC_{50}	*RAA ASCORBIC*	*RAA TROLOX*	*RAA RUTIN*	IC_{50}	RAA ASCORBIC	RAA RUTIN
Ex.CH_2Cl_2	189.5	207.8	167.2	88.6	235.1	88.2	32.8
Fr Petrol Ex CH_2Cl_2	918.4	505.7	406.8	215.8	414.8	155.6	57.8
Fr $CHCl_3$ Ex CH_2Cl_2	704.3	387.8	312.0	165.5	350.9	131.7	48.9
Fr MeOH Ex CH_2Cl_2	377.3	207.8	167.2	88.6	179.5	67.3	25.0
Ex.ETOH	106.3	58.5	47.1	25.0	76.1	28.6	10.6
Fr Petrol EX ETOH	455.2	250.7	201.7	107.0	326.9	122.7	45.6
Fr $CHCl_3$ EX ETOH	190.7	105.0	84.5	44.8	372.8	139.9	51.9
Fr MeOH EX ETOH	90.4	49.8	40.1	21.2	85.2	32.0	11.9
ASCORBIC	1.8	1.0	0.8	0.4	2.7	1.0	0.4
RUTIN	4.3	2.3	1.9	1.0	7.2	2.7	1.0
TROLOX	2.3	1.2	1.0	0.5			

Table 3. Analysis of antioxidant activity by $DPPH^*$ and $ABTS^{*+}$ methods

SPECIES	IC_{50}				RAA/ ASCORBIC			
	DPPH*		ABTS*+		DPPH*		ABTS*+	
	Fr, MeOH - Ex, EtOH	Ex, ETOH	Fr, MeOH - Ex, EtOH	Ex, ETOH	Fr, MeOH - Ex, EtOH	Ex, ETOH	Fr, MeOH - Ex, EtOH	Ex, ETOH
Chromolaena bullata	4,9	3,3	0,8	0,5	1,8	1,2	0,4	0,3
Diplostephium phylicoides	2,3	13,8	8,6	10,1	0,9	5,1	4,8	5,6
Lourteigia stoechadifolia	85.2	76.1	90.4	106.3	32.0	28.6	49.8	58.5