August 30, 2020

PharmacologyOnLine

Archives • 2020 • vol.2 • 83-91

ANTI-OXIDANT ACTIVITY OF LEAVES AND FLOWERS EXTRACTS in *Chromolaena scabra* PLANT SPECIES (L. f.) R.M. King & H. Rob

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Abstract

Chromolaena is a genus of *phanerogamic* plants belonging to the *Asteraceae* family. They are native to America, from the southern United States to South America (especially Brazil). The species, *Chromolaena scabra* (L.f.) R.M. King and H. Rob, is registered in Colombia and Ecuador. *Objective*: The objective of this research was to determine the antioxidant activity of the leaf and flower extracts of *Chromolaena scabra*. *Methods*: The antioxidant activity was evaluated by the $ABTS^{*+}$ cationic radical scavenging method (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and by the $DPPH^{*}$ radical scavenging test (radical method 1,1-diphenyl -2-picrylhydrazyl); Antioxidant activity tests were performed using Trolox's antioxidant capacity in the $ABTS^{*+}$ free radical generator and its long-lasting radical scavenging capacity, which is based on the discoloration of the nitrogenous compound $DPPH^{*}$. *Results*: The dichloromethane extract has a higher% of free radical uptake evaluated by the $DPPH^{*}$ method, likewise the ethanolic extract has the highest% of free radical uptake by $ABTS^{*+}$ method; The evaluation of the flower and leaf extracts determined that the extract with the highest IC_{50} by both methods was ethanol. *Conclusion*: The antioxidant activity obtained by the $DPPH^{*}$ test was higher than that obtained by the $ABTS^{*+}$ test, these values are significantly different, however, a good correlation was found between both methods, the results obtained in this study show that *Chromolaena scabra* has significant potential for use as a natural antioxidant agent.

Keywords: *Chromolaena scabra, antioxidant activity, soxhlet extraction,* $DPPH^{*}$, $ABTS^{*+}$

Introduction

Natural products have been shown to be an important source of compounds for the development of new drugs [1]. Natural products have provided many important medicines that are used in the arsenal of modern medicine. However, among the 250,000–400,000 estimated plant species, only 6% have been studied for biological activity and 15% have been phytochemically examined [2]. Active ingredients obtained from plants can protect humans from a variety of diseases. Phytochemicals are non-nutritive plant compounds, which have protective, curative or disease-preventive properties [3]. From a pharmacological perspective, plants are a treasure. In fact, the plant itself or its secondary metabolites are the source of useful drugs. They remain the main source of bioactive compounds that can be used directly in remedies or can inspire the synthesis of more active derivatives [4]. Throughout the years, research has been carried out to develop new drugs from medicinal plants, these plants produce different metabolites that act as therapeutic agents, presenting pharmacological activity against a disease. Species of the Asterácea family and among them the Chromolaena genus, to date present chemical and biological studies that have demonstrated the presence of secondary metabolites such as flavonoids with cytotoxic activity and that could carry out their pharmacological activity in different uses in the industry. the antioxidant activity of plants is the basis for counteracting many diseases; This is the case of the Chomolaena genus such as *C. odorata* [5,6], where they have evaluated the extracts of different plant polarity for their antioxidant effects and have evaluated purified fractions on cultured fibroblasts and keratinocytes. [7] Previous phytochemical investigations of this plant described the presence of flavonoids, phenolics, alkaloids, terpenoids and essential oil. [8-15].

The antioxidant activity of any substrate can be measured by various methods, such as 1,1-diphenyl 2-pichilhidazil (DPPH) [16], 2,2-azinobis -3-ethylbenzathiazoline 6-sulfonic acid [17-18], CUPRAC (CUPric reducing antioxidant capacity) [18] and ferric reducing antioxidant power [19-20]. However, the DPPH technique is the common method chosen by the investigator. This method is characterized by a color change from purple to yellow [21]. Besides antioxidant, the flavonoid component can also be used for antibacterial application. [22]

Methods

To determine the antioxidant activity of *Chromolaena Scabra* (L. f.) R.M. King & H. Rob cationic radical decolorization methods ABTS^{*+} (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) and the radical fading test DPPH* (radical method 1, 1-diphenyl-2-picrylhydrazyl); The antioxidant activity tests were performed using the antioxidant capacity of Trolox in the ABTS^{*+} free radical generator and its ability to scavenge long-lasting radicals, which is based on the discoloration of the nitrogen compound DPPH*.

Methodology developed for the test of discoloration of radical 1-1- Diphenyl-2-Picrilhidrazil (DPPH*)

For the DPPH* radical: 2 mg of Sigma-Aldrich DPPH was dissolved in 100 ml of analytical reagent grade methanol, the solution was allowed to react at room temperature for 24 hours in the dark. Subsequently, working solutions were prepared until obtaining an absorbance of 0.750 ± 0.050 for all cases, at a wavelength of 517 nanometers. [23]

For ascorbic acid, a stock solution of 100 milligrams per liter of methanol was prepared by dissolving 10 mg of ascorbic acid in 100 ml of methanol, then dilutions of concentrations of 2, 3, 3.5, 7 and 8 milligrams per liter were prepared of methanol. . to make the reference curve.

Routine preparation A stock solution of 100 milligrams per liter of methanol was prepared by dissolving 10 mg of rutin in 100 mL of methanol, then dilutions with concentration ranges of 3, 5, 10, 20 and 30 mg/L of methanol were prepared. to make the reference curve.

Preparation of the reference curve

A final volume of 1 mL of the used standards (ascorbic acid and rutin) was added to a glass cell with reduced volume plus the radical DPPH* necessary to reach a concentration of 100 ppm, the measurement was carried out at 517 nm, and the

percentage of absorption was calculated based on the following equation:

% absorption = ((A1-An) / A1) x 100

The absorption percentage represents the change from purple to yellow of the DPPH* radical, when an antioxidant compound is added, thus reducing the absorbance of the solution, which is measured at 517 nm; the initial absorbance is taken at minute zero without the addition of the standard antioxidant, the absorbance data is taken every 30 seconds for 10 minutes after adding the standard antioxidant.

Measurement of the antioxidant activity DPPH* of extracts of leaves and flowers *Chromolaena scabra* (L. f.) R.M. King and H. Rob. A final volume of 1 ml of each of the *C. scabra* plant extracts was added to a 1.5 µl glass cell. King & H. Rob plus DPPH* equivalent to a radical concentration at 100 ppm, the measurement was carried out at 517 nm. To evaluate the antioxidant activity of each of the foliar extracts of *C. scabra*, stock solutions were prepared for each extract, at a concentration of 1000 milligrams per liter of methanol. Dilutions of 10, 25, 50, 100, 150 and 200 milligrams per liter of methanol were made from each sample to determine the concentrations necessary to obtain absorption percentages from 10% to 95%. The absorbance of the DPPH***** (alone) is measured, which is known as the initial absorbance, then the mixture is made between the DPPH* and the different extracts, the absorbance is measured every 30 seconds for 10 minutes of the previous mixture to determine antioxidant capacity.

Methodology developed for discoloration test with the cationic radical 2,2-Azino-bis-3-ethylbenzothiazolin-6-sulfonic (ABTS+).*

For ABTS*+: radical, 50 mg of (ABTS) the diamonic salt of (2,2-Azino-bis-3-etIlbenzothiazoline-6-sulfonic) from Sigma-Aldrich, were dissolved in 50 mL of deionized water, then 2.45 mg of potassium persulfate ($K_2S_2O_8$) were added, the solution was allowed to react at a temperature of 3 ° C for 48 hours in the dark, then working solutions were prepared until obtaining an absorbance of 0.750 ± 0.050 for all the cases, at a wavelength of 754 nanometers. [24-26]

For Trolox: A stock solution of 100 milligrams per liter of methanol was prepared by dissolving 10 mg of (6-hydroxy-2,5,7,8-tetramethylchrome-2-carboxylic acid) 97% (Trolox) from across organic, in 100 mL of methanol, then dilutions were prepared with concentration ranges between 1, 2, 2.5 and 3 milligrams per liter of methanol, in order to perform the reference curve. [27]

Routine Preparation, a stock solution of 100 milligrams per liter of Methanol was prepared by dissolving 10 mg of routine, in 100 mL of methanol, then dilutions were prepared with concentration ranges between 3, 5, 10 and 20 milligrams per liter of methanol, with in order to make the reference curve.

Preparation of ascorbic acid, a stock solution of 100 milligrams per liter of methanol was prepared by dissolving 10 mg of ascorbic acid (Vitamin C) in 100 mL of methanol, then dilutions of 1, 2, 2.5 and 3 milligrams per liter were prepared of methanol, in order to make the reference curve.

Preparation of the reference curve.

To a 1.5 µL glass cell was added a final volume of 1 mL of the standards used (ascorbic acid, rutin and trolox) plus radical ABTS*+ necessary to obtain a concentration of 100 ppm, the measurement was made at 754 nm, and the percentage of uptake was calculated, Uptake percentage represents the blue-green color change of the ABTS*+ radical, when an antioxidant compound is added to it, thus decreasing the absorbance of the solution, which is measured at 754 nm; the initial absorbance is taken at minute zero without adding any standard, the absorbance data is taken every 30 seconds for 10 minutes after adding the standard antioxidant.

Measurement of the antioxidant activity ABTS*+ of the leaf and flower extracts of *Chromolaena scabra* (L. f.) R.M. King & H. Rob, A final volume of 1 mL of each of the plant extracts was added thanks to a 1.5 µL glass cell. plus the radical ABTS*+ concentration equivalent to 100 ppm, the measurement was carried out at 754 nm. To evaluate the antioxidant activity of each of the foliar extracts of *C. scabra*, stock solutions were prepared for each extract, at a concentration of 1000 milligrams per liter of methanol. Dilutions of 50, 100, 150 and 200 milligrams per liter of methanol were

made of each extract, in order to determine the concentrations necessary to obtain uptake percentages from 10% to 95%. The $ABTS^{*+}$ absorbance is measured, known as the initial absorbance. Then, the mixture between $ABTS^{*+}$ and the extract was carried out, on which the evaluation of the antioxidant capacity was carried out. The absorbances of the $ABTS^{*+}$ mixture and the *C. scabra* extract were measured every 30 seconds for 10 minutes.

Results and Discussion

The antioxidant activity was evaluated for the extracts of leaves and flowers obtained from solvents of different polarities (petroleum ether, dichloromethane and ethanol), at different concentrations 10, 25, 50, 100, 150 and 200 mg/L MeOH) , with the $DPPH^{*}$ radical method, we observe that there is a trend of greater antioxidant activity as the concentration of the extracts increases. Comparing the results of the test carried out for the leaf extract by the $DPPH^{*}$ method (Figure 1) we can see that at concentrations of 50 and 100 mg/L of MeOH, the petroleum ether extract has a lower uptake of free radicals with 17.8 and 28 mg/L MeOH mg/L MeOH respectively, it is observed that the dichloromethane and ethanolic extracts in concentrations of 50 mg/L MeOH, presented a superior behavior with 65.2 % and 66.1 % of free radical uptake respectively and at 100 mg/L MeOH 75.7% and 83.9%, this being the extract with the highest activity against the $DPPH^{*}$ radical.

Comparing the results for the flower extracts by the $DPPH^{*}$ method (Figure 1) we can determine that at concentrations of 50 and 100 mg/L of MeOH, the petroleum ether extract has a lower antioxidant activity with 12.9 % and 23.7 % of free radical uptake respectively. As in leaves, it is observed that the dichloromethane extract and the ethanolic extract show a superior behavior at concentrations between 50 and 100 mg/L MeOH, with a percentage of free radical uptake for dichloromethane between 32.1 % and 45, 4 % for the ethanolic extract between 40.9 % and 61.8%, this being the one with the highest activity against $DPPH^{*}$.

In flowers by the $DPPH^{*}$ method at a concentration of 150 mg/L of MeOH, the ethanol extract had a cativity of 56.6 %, the dichloromethane extract 46.6% and the petroleum ether extract of 17.0%, we can infer that the higher the polarity, the higher the activity.

Applying the $ABTS^{*+}$ method, we observe, as with the $DPPH^{*}$ method, that there is a trend towards greater antioxidant activity as the concentration in the working solutions of the extracts increases.

Comparing the results of the test carried out for the leaf extract by the $ABTS^{*+}$ method (Figure 2) we can see that the dichloromethane and ethanol extracts presented a greater uptake of free radicals unlike the petroleum ether extract, which presented to the same concentrations; The ethanol extract of leaves at a concentration of 200 mg/L MeOH presented a free radical uptake of 94.0 % $ABTS^{*+}$, comparing the results of the test carried out for the flower extract by the $ABTS^{*+}$ method (Figure 2), to 200 mg/L of MeOH, presented 70.6 %, lower activity in flowers than in leaves.

Analyzing the IC_{50} results obtained by the $DPPH^{*}$ method, it was observed that the leaf extracts presented a higher inhibitory concentration 50, resulting in 28.66 mg/L MeOH for the dichloromethane extract and 25.60 mg/L MeOH for ethanol being This is the one with the best result for this test (Table 1). Analyzing the results obtained from IC_{50}, it was observed that the leaf extracts presented a higher inhibitory concentration 50, resulting in 88.67 mg/L MeOH for the dichloromethane extract and 75 for ethanol 58 mg/L MeOH, this being the best result for this test.

The relative antioxidant activity (AAR) of *C. scabra* leaf and flower extracts compared to the $DPPH^{*}$ radical decolorization method (Table 2) shows that for leaf extracts the best relative antioxidant activity is ethanol leaves with a value 9.6 AAR compared to ascorbic acid, and compared to routine the value is 3.6 AAR; for flower extracts, the best relative antioxidant activity is flower ethanol, presenting 24.5 AAR compared to ascorbic acid and 9.1 AAR compared to rutin. We can observe that by the $DPPH^{*}$ decolorization method for the ascorbic acid pattern, the extracts that presented the highest relative antioxidant activity are: ethanol leaves with 9.6 AAR and dichloromethane leaves with 10.8 AAR, the extract that presented the lowest relative antioxidant activity was dichloromethane flowers

with 40.1 AAR. For the Rutin pattern, the extracts that presented the highest relative antioxidant activity are: ethanol leaves with 3.6 AAR and dichloromethane leaves with 4.0 AAR, the extract that presented the lowest relative antioxidant activity was dichloromethane flowers with 14.9 AAR. Comparing the two standards used, it is observed that the results obtained by routine are lower with respect to ascorbic acid. By the $DPPH^{*}$ radical decolorization method, higher percentages of antioxidant activity were presented with respect to the $ABTS^{*+}$ cationic radical decolorization method with respect to ascorbic acid. The AAR of a substance with a lower value has a greater antioxidant capacity.

When comparing the antioxidant activity at 100 ppm of *C. bullata* with respect to *C. scabra* verified that *C. bullata* shows greater activity in each of the extracts for the $DPPH^{*}$ and $ABTS^{*+}$ tests see Figure 3 [28].

The results obtained in this study indicate that *Chromolaena scabra*, has significant potential to use as a natural antioxidant agent. The ethanol extracts of leaves and flowers were determined to have a higher relative antioxidant activity than that obtained by the other solvents used. Petroleum ether extracts that have low polarity compounds do not have a significant antioxidant activity, on the contrary with those of high polarity, the best AAR was presented by ethanol and dichloromethane extracts. The antioxidant activity obtained by the $DPPH^{*}$ test was greater than that obtained by the $ABTS^{*+}$ test, these values are significantly different, however, a good correlation was found between both methods.

Acknowledgments

The authors owe our sincere thanks to, Universidad de Ciencias Aplicadas y Ambientales U.D.C.A., Fundación Universidad Juan N. Corpas Pontificia Universidad Javeriana and Universidad El Bosque

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Figure 1. % Radical Uptake DPPH* vs. *Chromolaena scabra* extracts

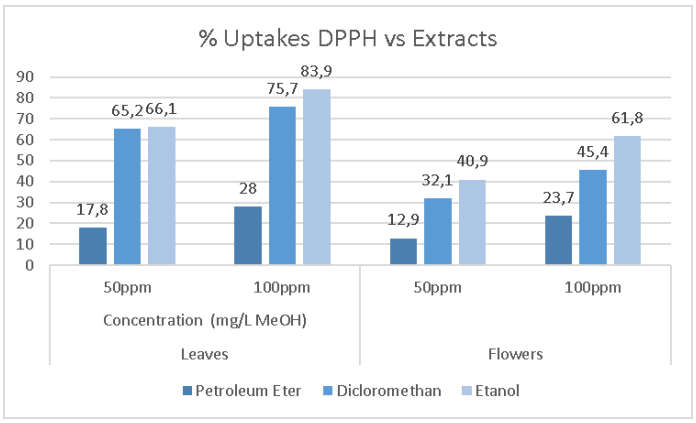

Figure 2. % Radical Uptake ABTS*+ vs. *Chromolaena scabra* extracts

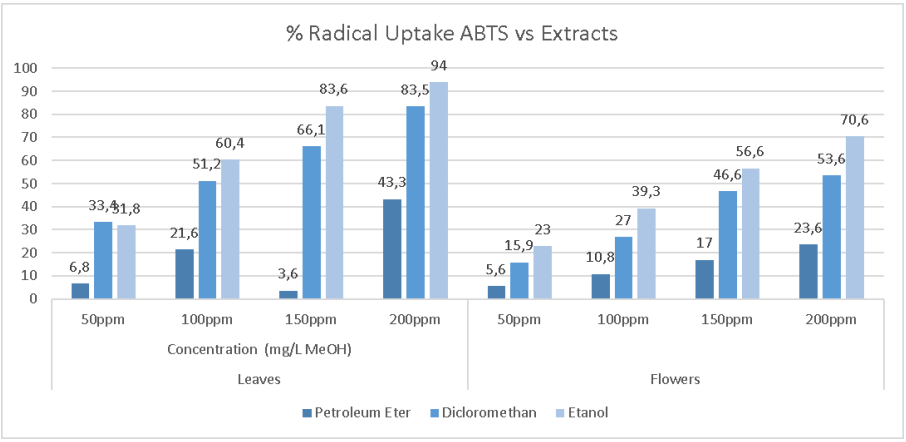

Table 1. Comparison of IC_{50} extracts of leaves and flowers of *Chromolaena scabra* Calculated by the DPPH* and ABTS*+ methods

EXTRACT	IC50 DPPH*	IC50 ABTS*+
Dichloromethane leaves	28,66	88.67
Ethanol leaves	25,60	75.58
Petroleum ether flowers	509,09	1927.86
Dichloromethane flowers	106,85	183.41
Ethanol flowers	65,42	119.59
Ascorbic acid	2,67	1.82
Routine	7,18	4.26
Trolox		2.26

Table 2. Comparison of relative antioxidant activity AAR of leaf and flower extracts of *Chromolaena scabra* Calculated by the DPPH* and ABTS*+ methods versus ascorbic acid and routine

Extract	DPPH*		ABTS*+		
	AAR Ascorbic Acid	AAR Routine	AAR Ascorbic Acid	AAR Routine	AAR Trolox
Petroleum ether leaves	111,4	41,4	144,5	61,7	116,3
Dichloromethane leaves	10,8	4,0	48,8	20,8	39,3
Ethanol leaves	9,6	3,6	41,6	17,8	33,5
Petroleum ether flowers	191,0	70,9	1.061,6	452,9	854,0
Dichloromethane flowers	40,1	14,9	101,0	43,1	81,2
Ethanol flowers	24,5	9,1	65,9	28,1	53,0

Figure 3. Comparative antioxidant activity at 100 ppm between *Chromolaena. bullata* with *Chromolaena. scabra*