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THE ANTI-OXIDANT ACTIVITY OF EXTRACTS AND FRACTIONS OF *CHROMOLAENA BULLATA* (KLATT) KING & ROBINSON

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Abstract

The current trend in this field is the search for compounds derived from our vegetal species which have an anti-oxidant activity that is beneficial for human health. Colombia has a number of species known as field weeds (arvenses), like those of the Chromolaena genus, which are reported to have a high content of flavonoids. This study evaluated the anti-oxidant activity of extracts and fractions of the aerial parts of *Chromolaena bullata* plants found in the departments of Cundinamarca and Boyacá in Colombia, using the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$. For that purpose, extracts and fractions were obtained with the use of the Soxhlet method with solvents of different polarities of flowers and leaves. This study evaluated concentrations of 1, 10, 62.5, 100 and 250 mg of the extract or fraction per/ liter of MeOH, obtaining percentages for the capture of DPPH$^{\bullet}$ radicals of between 46.16 ± 0.7 and 92.21 ± 0.2, at 250 ppm, for the leaves, and between 46.19 ± 3.1 and 91.13 ± 0.3, at 250 mg/L of MeOH, for the flowers. With the ABTS$^{\bullet+}$ method, the range was between 90.14 ± 1.1 and 99.55 ± 0.2, at 250 mg/L of MeOH, for the leaves, and 64.65 ± 1.0 and 97.84 ± 0.6, at 250 mg/L, for the flowers. The study found that the methanolic fractions of flowers and leaves showed a high anti-oxidant activity when evaluated by the ABTS$^{\bullet+}$ y DPPH$^{\bullet}$ techniques and that the ABTS$^{\bullet+}$ showed a greater sensitivity.

Keywords: Anti-oxidant, *Chromolaena bullata*, ABTS$^{\bullet+}$, DPPH$^{\bullet}$, relative anti-oxidant activity (RAA).

Introduction

Chromolaena species were initially included in the genus Eupatorium, a large group composed of plants with large morphological differences belonging to the Asteraceae family. The Chromolaena genus is made up of 165 species, spread around South America, Central America and western India. The Eupatorium genus was made a separate one on the basis of the studies of King and Robinson, who divided the group into smaller and more homogeneous genii. Their findings were confirmed after several revisions were made of various species [1,2] using chromosomal and bio-geographical studies which provided additional data for the recognition of the taxa. Schmidt and Schilling used ribosomal DNA in regions of internal transcription of ribosomal genes to test the theories of King and Robinson in 36 species with a total of 40 Asteraceae specimens [3].

C. bullata (Klatt) King & Robinson is a herbaceous plant with bushes which are erect or scattered along the soil, found in the Colombian departments of Cundinamarca, Valle del Cauca, Antioquia and Boyacá. Investigations of this plant showed that it has the biological property of causing the activation of human dendritic cells and anti-microbial activity [4] but its anti-oxidant activity has not been explained.

The identification of anti-oxidants in natural products highlights the recent interest in promoting health and the conservation of food, pharmaceutical and cosmetic products, among others [5,6] which depend on the stability of their compounds. The relation which exists between the radicals and cellular aging is seen in illnesses like Alzheimer´s disease, arteriosclerosis and similar vascular diseases [6]. Given the importance of anti-oxidants, a series of techniques have been developed to find and measure them, and among the most frequently used there is the use of spectrophotometry, which is based on measuring their reaction with stable free radicals, like ABTS 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) or DPPH (2,2-diphenyl-1-picrylhydrazyl). The measure of the reduction of the absorption of the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical cations is one of the most widely used. There are also studies which report an anti-oxidant activity in *C. odorata* [9-12]. That is why the anti-oxidant activity of the *C. bullata* species was determined with methods for the discoloration of the $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals.

Anti-oxidant activity: Method for the discoloration of the $DPPH^{\bullet}$ radical.

$DPPH^{\bullet}$ is a stable organic nitrogenous radical, with an intense purple color, which does not require any previous preparation. The method was set forth by Brand-Williams [13] and serves to determine the anti-oxidant capacity on the basis of the reduction of color, measured at 517 nm, by the activity of an anti-oxidant compound. That activity can also be measured by electron spin resonances.

Authors like Sánchez-Moreno [15) classified the kinetic behavior in terms of time, as follows: rapid (<5min), medium (5-30min), slow (>30min), and introduced a parameter for anti-oxidant capacity called anti-radical efficiency [16].

The sweep model reaction between the $DPPH^{\bullet}$ radical and the antioxidant (AH) is:

$$DPPH + AH \longrightarrow DPPH - H + A^{\bullet}$$

$$DPPH^{\bullet} + A^{\bullet} \longrightarrow DPPH - A$$

$$A^{\bullet} + A^{\bullet} \longrightarrow A - A$$

A new radical is formed during the interaction of the DDPH radical and the anti-oxidant and the secondary reactions lead to stable compounds [17].

Anti-oxidant activity: Method for the discoloration of the $ABTS^{\bullet+}$ radical.

This method was proposed in 1993 by Miller N.J. and collaborators [18], and is based on the antioxidant ability of $ABTS^{\bullet+}$. to sequester long-lived radical anions. In the test the ABTS is oxidized by radical peroxide, by potassium persulfate [19], by hydrogen peroxide [20], horseradish peroxidase [21] or another oxidant to form the radical cation $ABTS^{\bullet+}$, which has an intense green-blue color, and in the measurement the compounds with antioxidant capacity react, directly reducing the color of the radical cation $ABTS^{\bullet+}$.

The results obtained are expressed as inhibition and are brought to a trolox-related concentration, hence the method measures the Trolox Equivalent Antioxidant Capacity (TEAC). The radical has solubility in polar and nonpolar media and is not affected by ionic strength; Therefore, it evaluates hydrophilic and lipophilic anti-oxidants from plant extracts and biological fluids [6,12,14]. This method is based on the ability of antioxidant molecules (trolox) to satiate the long life $ABTS^{\bullet+}$, a blue-green chromophore with a maximum absorption at a wavelength of 754 nm.

$$ABTS + K_2S_2O_8 \longrightarrow ABTS^{\bullet+}$$

$$\lambda Max = 754 \text{ nm}$$

$$ABTS^{\bullet+} + ArOH\ (Antiox) \longrightarrow ABTS + ArO^{\bullet} + H^{+}$$

It is a screening method for the evaluation of the anti-oxidant capacity of different hydrophilic and lipophilic substances. The monocationic radical 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$) is

produced by the reaction between 50 mg of ABTS in H2O and 2.45 mg of potassium persulfate. That reaction yields a colored compound which, after 12-16 hours, is stable and is then diluted with methanol until it reaches an absorbance of 0.7 measured at 754 nm.

Methods

Gathering of the plant

The plant was gathered at the Hacienda El Rosal, rural district (vereda) El Rosa, at kilometer 1 on the road between Sibaté and San Miguel in the department of Cundinamarca. The plant was flowering when it was gathered, which allowed for a control sample to be taken, which was sent to the Herbarium of the Universidad Nacional (National University of Colombia), where it was found to be *C. bullata* (Klatt) R.M. King & H. Rob – Asteraceae, Code COL-539592.

Preparation of Trolox, quercetin, ascorbic acid, ABTS and DPPH

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 97%), quercetin and ascorbic acid were used as the reference anti-oxidants.

For the preparation of the $DPPH^{\bullet}$ radical, 2 mg of it were dissolved in 100 ml of methanol; the solution reacted at room temperature, in the dark, for 30 minutes. Then, working solutions were prepared to obtain an absorbency of 0.700 ± 0.050 for all cases [20], at a wavelength of 517 nm. For the preparation of the $ABTS^{\bullet+}$ radical, 50 mg were dissolved in 50 ml of deionized water and then 2.45 mg of potassium persulfate ($K_2S_2O_8$) were added. The solution reacted at room temperature, in the dark, during 16 hours. Later, working solutions were prepared to reach an absorbency of 0.750 ± 0.050 for all of the cases, at a wavelength of 754 nm.

In the preparation of ascorbic acid for the $DPPH^{\bullet}$ technique, a stock solution of 1000 mg/L MeOH was prepared by dissolving 10 mg of ascorbic acid in 10 ml of methanol, then dilutions were prepared at concentrations ranging from 400 to 1 mg/L MeOH. The preparation of the quercetin solution for the $DPPH^{\bullet}$ technique was done with a stock solution of 800 mg/L MeOH, dissolving 20 mg of ascorbic acid in 25 ml of methanol; later, dilutions were prepared at concentrations ranging from 20 to 1 mg/L MeOH.

The preparation of the quercetin solution for the $ABTS^{\bullet+}$ technique was done in a stock solution of 2.6 mM of Trolox, dissolving 0.005 g of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Acros Organics in 10 mL of methanol, after which dilutions were prepared with concentrations ranging from 3 to 15 µM. The preparation of the quercetin for the $ABTS^{\bullet+}$ technique was done in a stock solution of 2.6 mM, dissolving 0.02 g of quercetin in 25 mL of methanol, after which dilutions were made at concentrations ranging from 3 to 12 µM. Finally, the preparation of the ascorbic acid for the $ABTS^{\bullet+}$ was done in a stock solution of 5.7 mM, dissolving 0.01 g of ascorbic acid (vitamin C) in 10 ml of methanol, after which dilutions were prepared with concentrations ranging from 3 to 12 µM.

Statistical analysis

A completely univariate randomized design with two independent responses, IC50 DPPH and IC50 ABTS, was applied to a single-way analysis of variances - ANOVA - to determine if there were significant differences between the extracts and fractions of the study. With this we made post-hoc HSD statistical analyses (following Tukey and Scheffé), which, together with the graphs of arithmetic means, allowed us to elucidate the most significant treatments for each one of the answers. All of that was done by taking into account compliance with the premises of the Shapiro–Wilk test of normality in frequentist statistics ($p>0.05$; $\alpha=0.05$) and the Levene test for equality of variances ($p>0.05$; $\alpha=0.05$) and independence of the variables.

Results

Extraction

From the material which was previously dried and ground, an extraction with ethanol was made in in a Soxhlet extractor to obtain the total ethanolic extract concentrated in the rotary evaporator (Table 1).

Solid-liquid fractionation

A solid-liquid fractionation (percolation) was made of the total ethanolic extracts of the leaves and flowers, using silica gel 60 (MN Kieselgel 60 0.063 0.2 mm/70 –230 mest ASTM) as the stationary phase and petroleum ether as the mobile phase. This was continued with dichloromethane (CH_2Cl_2), ethyl acetate (AcOEt) and methanol (MeOH), obtaining the fractions of petroleum ether, CH_2Cl_2, AcOEt and MeOH, which were concentrated in the rotary evaporator.

Method for the discoloration of the $DPPH^{\bullet}$ radical

The patterns used for $DPPH^{\bullet}$ were ascorbic acid and quercetin at concentrations of 1, 3, 5 and 12 mg/L MeOH and 1, 2, 3, 5 mg/L MeOH respectively, with inhibition coefficients 50 (IC_{50}), for ascorbic acid: IC_{50} = 3.09, and for quercetin : IC_{50} = 0,95, at concentrations necessary to obtain percentages of inhibition of 10% to 95%.

The method for the discoloration of the $DPPH^{\bullet}$ radical on leaves revealed that the total extract and the methanolic fraction showed a similar behavior in surpassing 50% of the capture of $DPPH^{\bullet}$ at 10 mg/L MeOH, in contrast with

the fraction of dichloromethane, which did not reach 50% of capture at 250 mg/L MeOH.

Method for the discoloration of the ABTS$^{\bullet+}$ radical

The patterns used for ABTS$^{\bullet+}$ were ascorbic acid, quercetin and Trolox in concentrations of 3, 6, 9 and 12 µM for ascorbic acid, equivalent to 0.53, 1.05, 1.58, 2.11 mg/L MeOH; 3, 6, 9 and 12 µM for quercetin, equivalent to 0.92, 1.85, 2.77, 3.75 mg/L MeOH; and for Trolox, 3, 6, 9, 12, 15 µM, equivalent to 0.75, 1.50, 2.25, 3.00, 3.75 mg/L MeOH; con IC_{50} coefficients for the ascorbic acid of = 0.89; IC_{50} = 0.87 for quercetin; and IC_{50} = 1.00 for Trolox at concentrations necessary to obtain percentages of inhibition of 10% to 95%.

Percentage of captation of radicals

The percentage of captation was calculated by the following formula [22]:

% of captation = (initial A – final A/initial A) x 100

The anti-oxidant activity of the different compounds is measured as a function of the degree of discoloration they cause in the solution of the mono-cationic radical ABTS$^{\bullet+}$ or the radical DPPH$^{\bullet}$. The bleaching test is based on the ability of the test substance to capture the missing electron from the radical or to release a proton.

Relative antioxidant activity (RAA)

The anti-oxidant activity of a substance when compared with that of a pattern in the same experimental conditions and units of concentration is calculated with the inhibition coefficient (IC_{50}) of the analyzed sample, divided by the IC_{50} of the patterns used (Trolox, quercetin or ascorbic acid).

The IC_{50} is calculated by linear regression or the straight-line equation of each sample or reference pattern being analyzed. The RAA is calculated with the following formula:

RAA = (IC_{50} (m) / IC_{50} (R))

Where IC_{50}(R) is the inhibition coefficient of the reference pattern being used and IC_{50}(M) is the coefficient of the analyzed sample.

The method for the discoloration of the ABTS$^{\bullet+}$ radical used on leaves showed a similar behavior in all the extracts and fractions, surpassing 50% of capture at 1 mg/L MeOH (Table 3). An evaluation of the capacity to inhibit the ABTS$^{\bullet+}$ radical in the fractions (dichloromethane and methanol) and the total extract of leaves showed that the total extract had a greater activity, with 79.2% at 1 mg/MeOH, reaching 95.91% at 10 mg/L MeOH, which remained stable. It was followed, in turn, by the methanolic fraction, with a 95.12% inhibition of the ABTS$^{\bullet+}$ radical, at 10 mg/L MeOH, in contrast with the fraction of dichloromethane, which showed a scaled growth, depending on the concentration, and came to surpass 90% at 250 mg/L MeOH. In general, both the extract and fractions surpassed 50% of inhibition at a concentration of 1 mg/L MeOH.

The methanolic and dichloromethane fractions of the flowers registered a capture of 50% with the method for the discoloration of the DPPH$^{\bullet}$ radical, at 10 ppm; the petroleum ether fraction did not reach 50% of capture at 250 mg/L MeOH, and thus had the lowest yield (Table 3).

The inhibitory concentration 50 (IC_{50}) of fractions and leaf and flower extract were compared with the patterns used by the DPPH$^{\bullet}$ radical discoloration method and the ability to inhibit the ABTS$^{\bullet+}$ radical.

Percentage of relative anti-oxidant activity (%RAA) shown by the method for the discoloration of the DPPH$^{\bullet}$ radical

An evaluation of the capture capacity of the DPPH$^{\bullet}$ radical showed an IC_{50} of 1.86 for the methanolic fraction of flowers, 3.33 for the total ethanolic extract of leaves and a 4.63 for the dichloromethane fraction of flowers.

The percentage of RAA for the DPPH$^{\bullet}$ method was calculated with the IC_{50} of the patterns used, which showed a greater anti-oxidant activity in the methanolic fraction of flowers with respect to the ascorbic acid (60.16%) and quercetin (195.55%). The total ethanolic extract of leaves registered (107.78%) for the RAA with regard to the Vitamin C and (350.33%) for the RAA with regard to quercetin. The fraction of dichloromethane of the leaves showed less activity compared to the patterns and other samples analyzed (Table 4).

Percentage of relative anti-oxidant activity (%RAA) shown by the method for the discoloration of the ABTS$^{\bullet+}$ radical

The percentage of relative antioxidant activity was calculated with the three standards, trolox, quercetin and ascorbic acid, and the IC_{50} of each of the samples was used with respect to the IC_{50} of each standard (Table 4).

The total extract of leaves showed a greater relative anti-oxidant activity under the ABTS$^{\bullet}$ method with respect to the ascorbic acid (0.01%), quercetin (0.01%) and Trolox (0.01%). The second biggest activity was shown by the methanolic fraction of the leaves, with (5.26%) of RAA with respect to the ascorbic acid, quercetin (5.39%) and Trolox (4.67%). The lowest activity was shown by the petroleum ether fraction. When the two methods were compared, the discoloration assay of the ABTS$^{\bullet+}$ radical had the greatest effectiveness.

A comparison of the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods for the extracts showed significant differences ($p<0.01$) in all of the treatments, depending on the method of analysis (DPPH$^{\bullet}$ or ABTS$^{\bullet+}$). In addition, the Tukey test showed that the most sensitive method was the ABTS ($p<0.05$)..

Discussion

It is evident that there are significant differences between the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods´ findings for the extracts and fractions under study (ANOVA p=0,000) In

turn, the post-hoc proofs showed that for the DPPH$^{\bullet}$ method, the leaves in the CH_2Cl_2 fraction and the flowers in the petroleum ether fraction (HSD Tukey p=1,000; Scheffe p=1,000) obtained the highest IC50, that is, they had a lower anti-oxidant capacity.
Furthermore, the ABTS$^{\bullet+}$ method yielded highly significant differences, showing that the total extract of leaves has the lowest IC_{50}, and thus shows a greater anti-oxidant activity (HSD test: Tukey p=1,000; Scheffe p=1,000).
Higher capture values were obtained in *C. bullata* than in the studies of Rao [10] in 2010, who evaluated the anti-oxidant activity of the methanolic fraction of the dichloromethane extract of C. odorata, finding a capture percentage under the DPPH$^{\bullet}$ method, at 100 ppm, of 23.48 ± 1.86 and 18.92 ± 0.61 under the ABTS$^{\bullet+}$ method at the same concentration. Kusuma [11] evaluated the anti-oxidant capacity of *C. odorata* and other species, finding a capture percentage under the DPPH$^{\bullet}$ method, at 50 ppm, of 86% for the ethanolic fraction, 79% for the ethyl acetate fraction and 18% for the petroleum ether fraction. Similarly, in 2010 Krishanti [12] evaluated the anti-oxidant capacity of C. odorata and other species, finding a capture percentage of 48%, under the DPPH$^{\bullet}$ method, at 1000 ppm.
Similar values were obtained by Varon [23] in 2011, who evaluated the anti-oxidant activity of extracts and fractions of the flowers and leaves of *C. leivensis*, using the methods for the discoloration of radicals (ABTS$^{\bullet+}$ and DPPH$^{\bullet}$); and obtained percentages for the inhibition of radical uptake by the DPPH$^{\bullet}$ method in a range between 21.44 ± 0.94 and 88.95 ± 0.00, and by the ABTS$^{\bullet+}$ method, between 37.64 ± 1.89 and 95.44 ± 0.20.

The fractions of leaves and flowers and the total extract of leaves in methanol of *C. bullata* showed an anti-oxidant capacity with regard to the ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radicals, because this activity is generated by substances capable of producing protons such as those of phenolic type, as well as the flavonoids contained in this plant. The results obtained in this study show that *C. bullata* may be a natural source of anti-oxidants with a potential application in the pharmaceutical industry, given the high activity found in the different extracts under the two in vitro models (DPPH$^{\bullet}$, ABTS$^{\bullet+}$).

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References

1. King RM, Robinson H. Studies in the Eupatorieae (compositae) XXIX the genus Chromolaena. Phytologia, 1970; 20(3): 196-209.

2. King RM, Robinson H. Studies in the Eupatorieae (Compositae). XXV. A new genus Eupatoriadelphus. Phytologia, 1970; 19: 431–2.

3. Schmidt GJ, Schilling EE. Phylogeny and biogeography of Eupatorium (Asteraceae, Eupatorieae) based on nuclear ITS sequence data. American Journal of Botany. 2000; 87(5): 716-26.

4. Santander SP, Urueña C, Rodríguez OE, Torrenegra RD, Fiorentino S. El flavonoide CB2 obtenido de *Chromolaena bullata* induce activación de células dendríticas humanas. Scientia et Technica. 2007; 33: 313.

5. Cárdenas E, Packer L. Handbook of antioxidants, second edition revised and expanded. Food-Derived Antioxidants: How to Evaluate Their Importance in Food and In Vivo. Measurement of Total Antioxidant Capacity in Nutritional and Clinical Studies; University of Southern California School of Pharmacy. California. Marcel Dekker INC. 2002; 18-70.

6. Roginsky V, Lissi EA. Review of methods to determine chainbreaking antioxidant activity in food. Food Chem. 2005; 92: 235-54.

7. Thaipong K, Boonprakob U, Crosby K, CisnerosZevallos L, Hawkins D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts Journal of Food Composition and Analysis 2006; 19: 669-75.

8. Floegel A, Dae-Ok K, Sang-Jin C, Sung K and Ock C. Comparison of ABTS/DPPH Assays to Measure Antioxidant Capacity in Popular Antioxidant-Rich US Foods. Journal of Food Composition and Analysis 2011; 24(7): 1043-48.

9. Thang PT, Patrick S, Teik LS, Yung CS. Anti-oxidant effects of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts and epidermal keratinocytes against hydrogen peroxide and hypoxanthine–xanthine oxidase induced damage, Burns 2001;27(4): 319-27.

10. Rao S, Chaudhury PK Pradhan A. Evaluation of anti-oxidant activities and total phenolic content of Chromolaena Food and Chemical Toxicology, 2010;48(2): 729-32.

11. Kusuma, IW., Murdiyanto, ET., Arung, Syafrizal, Y., Kim. Antimicrobial and Antioxidant Properties of

Medicinal Plants Used by The Bentian Tribe from Indonesia Food Science and Human Wellness, 2014; 3: 191–6

12.Krishanti M, Xavier R, Kasi M, Ayyalu D, Surash R, Sadasivam K, Sreeramanan S. A comparative study on the antioxidant activity of methanolic leaf extracts of Ficus religiosa L, *Chromolaena odorata* (L.) King & Rabinson, Cynodon dactylon (L.) Pers. and Tridax procumbens L. Asian Pacific Journal of Tropical Medicine, 2010;3(5): 348-50

13. Brand-Williams W, Cuvelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebens. Wiss. u.Technol, 1995; 28: 25-30.

14. Prior RL, Wu X, Schaich K. Standardized Methods for Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. J. Agric. Food Chem, 2005; 53: 4290-302.

15. Sánchez-Moreno C, Jiménez-Escrig A, Jiménez-Jiménez I, Saura-Calixto F. Evaluation of Free Radical Scavening of Dietary Carotenoids by the Stable Radical 2,2-diphenyl-1-picrylhydrazyl. J. Sci. Food Agric. 2000; 80: 1686-90.

16. Huang D, Ou B, Prior RL. The Chemistry behind Antioxidant Capacity Assays. J. Agric. Food Chem., 2005; 53: 1841-56.

17. Suja KP, Jayalekshmy A, Arumughuan C. Free Radical Scavenging Behaviour of Antioxidant Compounds of Sesame (Sesame indicum L.) in DPPH• System. J. Agric. Food Chem, 2004; 52: 912-15.

18. Miller NJ, Diplock AT, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A Novel Method for Measuring Antioxidant Capacity and its Application to Monitoring the Antioxidant Status in Premature Neonates. Clin. Sci. 1993; 84: 407-12.

19. Re R, Pellegrini N, Protegente A, Pannala A, Yang M, Rice-Evans C. Antioxidant Activity Applying and Improved $ABTS^{+\bullet}$ Radical Cation Decolorization Assay. Free Rad. Biol. Med, 1999; 26: 1231-37.

20. Villano D, Fernández-Pachón MS, Troncoso AM, García-Parilla MC. The Antioxidant Activity of Wines Determinated $ABTS^{\bullet+}$ Method: Influence of Simple dilution and Time. Talanta, 2004; 64: 501-9.

21. Labrinea EP, Georgiu CA. Stopped-Flow Method for Assessment of pH and Timing Effect on the ABTS Total Antioxidant Capacity Assay. Anal. Chim. Acta 2004; 526: 63-8.

22. Kulisic T, Radonic A, Katalini V, Milos M. Analytical, Nutritional and Clinical Methods Use of different methods for testing antioxidative activity of oregano essential oil, Food Chemistry, 2004; 85: 633-40.

23. Varón M., Torrenegra R.D., Rodriguez O.E., Antioxidante de la *Chromolaena leivensis* (Hieron) R.M. King & H.Rob. VITAE 2011; 18(2): 82

Table 1. Percentages of the yield from the extraction with ethanol from the leaves and flowers of *Chromolaena bullata.*

Chromalaena bullata	Dry vegetal material (g)	Total EtOH extract (g)	Percentage of yield (%)
Leaves	499	79	16
Flowers	525	63	12

Table 2. Yield of the fractions of leaves and flowers of *Chromolaena bullata.*

Chromolaena bullata	Leaves		Leaves	
Fraction	Mass (g)	Yield (%)	Mass (g)	Yield (%)
Petroleum ether	4.1	9.10	14.31	31.80
Dichloromethane	8.9	19.77	7.81	17.35
Methanol	24.4	54.22	15.48	34.40
Residue	7.6	16.88	7.40	16.40

Table 3. Percentage of the capture of the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals of extracts and fractions of leaves of *C. bullata.*

$DPPH^{\bullet}$ Leaves				$ABTS^{\bullet+}$ Leaves		
mg/L MeOH	Fraction CH_2Cl_2	Total extract	Fraction MeOH	Fraction CH_2Cl_2	Total extract	Fraction MeOH
1	25.93 ± 0.9	32.63 ± 1.1	28.89 ± 0.9	61.13 ± 2.3	79.20 ± 2.3	62.77 ± 1.0
10	28.36 ± 0.4	66.37 ± 3.1	57.07 ± 0.4	66.65 ± 1.3	95.91 ± 0.1	95.12 ± 0.1
62,5	32.45 ± 2.6	87.75 ± 1.5	89.80 ± 0.1	76.35 ± 1.0	96.79 ± 0.1	96.92 ± 0.6
100	42.89 ± 0.6	88.9 ± 0.7	90.21 ± 0.3	78.83 ± 2.4	97.03 ± 0.7	97.82 ± 0.1
250	46.16 ± 0.7	90.14 ± 0.8	92.21 ± 0.2	90.14 ±1.1	99.07 ± 0.2	99.55 ± 0.2
$DPPH^{\bullet}$ Leaves				$ABTS^{\bullet+}$ Leaves		
mg/L MeOH	Fraction CH_2Cl_2	Total extract	Fraction MeOH	Fraction CH_2Cl_2	Total extract	Fraction MeOH
1	26.17 ± 28.0	29.45 ± 0.8	42.76 ± 2.9	34.36 ± 1.3	50.96 ± 2.7	48.37 ± 1.8
10	28.72 ± 1.1	60.02 ±2.0	65.36 ± 2.4	43.81 ± 2.4	91.42 ± 2.9	83.01 ± 0.2
62,5	38.39 ± 0.8	86.83 ± 0.8	89.17 ± 0.3	61.01 ± 1.5	93.86 ± 0.6	89.89 ± 1.1
100	40.83 ± 1.4	88.5 ± 0.7	89.18 ± 0.8	62.58 ± 1.8	93.51 ± 0.3	94.01 ± 0.8
250	46.19 ± 3.1	90.53 ± 0.3	91.13 ± 0.3	64.65 ± 1.0	95.55 ± 0.5	97.84 ± 0.6

Table 4. IC_{50} of extract and fractions of leaves and fractions of flowers, calculated by the method for the discoloration of the $DPPH^{\bullet}$ radical and the inhibition capacity of the $ABTS^{\bullet+}$ radical. RAA: relative anti-oxidant capacity , Vit C; and RAA of ascorbic acid.

	DPPH•			ABTS•+			
Extract or Fraction	**IC 50**	**RAA Vit C**	**RAA quercetin**	**IC 50**	**RAA Vit C**	**RAA quercetin**	**RAA Trolox**
CH_2Cl_2 Fraction, Leaves	1.664,30	538,57	1.750,65	0,18	0,20	0,20	0,18
Total extract of leaves	3,33	1,08	3,50	0,00	0,00	0,00	0,00
MeOH Fraction, Leaves	4,88	1,58	5,13	0,05	0,05	0,05	0,05
Petroleum ether fraction, Leaves	1.219,35	394,58	1.282,61	15,92	17,88	18,33	15,90
CH_2Cl_2 Fraction, Flowers	4,63	1,50	4,87	0,29	0,32	0,33	0,29
MeOH fraction, Flowers	1,86	0,60	1,96	0,65	0,73	0,75	0,65