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ANTIOXIDANT POTENTIAL OF SOME SPECIES OF THE GENUS *Bomarea* (ALSTROEMERIACEAE)

POTENCIAL ANTIOXIDANTE DE ALGUNAS ESPECIES DEL GÉNERO *Bomarea* (ALSTROEMERIACEAE)

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ABSTRACT

This work evaluated the antioxidant activity of ethanol extracts from 11 species of the genus *Bomarea* (Alstroemeriaceae) by means of two *in vitro* methods. Values of CE_{50} between 51 and 333 μg/mL were obtained for DPPH the test, and the highest activity levels were found for *B. glaucescens, B. setacea, B. pardina* and *B. euryantha*, which presented a similar CE_{50} or lower than the reference used, silymarin (70.6 ug/mL). Likewise, the TBARS method showed that the maximum inhibition of lipid peroxidation of the linoleic acid was produced by *B. hirsuta* (malondialdehyde = 0.429 μM), followed by *B. bredemeyerana* (0.474 μM), *B. callejasiana* (0.479 μM), *B. euryantha* (0.489 μM), *B. glaberrima* (0.497 μM), and *B. setacea* (0.500 μM). Additionally, the concentration of phenol compounds was evaluated by the Folin-Ciocalteau method, finding that *B. setacea* presented the highest content of these (159.75 gallic acid equivalents/mg of extract). *Bomarea setacea* showed the highest antioxidant properties demonstrated by its free-radical scavenging and significant inhibition capacity of the oxidation of linoleic acid.

Key words: Antioxidant, *Bomarea,* DPPH, Folin-Ciocalteau, plant TBARS.

RESUMEN

En este trabajo se evaluó la actividad antioxidante de extractos etanólicos de 11 especies del género *Bomarea* (Alstroemeriaceae) por medio de métodos *in vitro*. Los valores de CE_{50} obtenidos con la prueba de DPPH se encuentran entre 51 y 333 μg/mL, siendo los valores más altos los obtenidos para *B. glaucescens, B. setacea, B. pardina* y *B. euryantha*, los cuales presentaron CE_{50} similares o inferiores al referente utilizado: silimarina (70,6 μg/mL). Con el método TBARS se encontró que la máxima inhibición de la lipoperoxidación del ácido linoléico, se produce por *B. hirsuta* (malondialdehido = 0,429 μM), seguida por *B. bredemeyerana* (0,474 μM), *B. callejasiana* (0,479 μM), *B. euryantha* (0,489 μM), *B. glaberrima* (0,497 μM) y *B. setacea* (0,500 μM). Se evaluó además el contenido de compuestos fenólicos por el método Folin-Ciocalteau, encontrando que *B. setacea* presenta el mayor contenido de éstos (159,75 equivalentes de ácido gálico /mg de extracto). La especie *B. setacea* presenta mayores propiedades antioxidantes, evidenciado esto en su actividad estabilizadora de radicales libres y significativa capacidad de inhibir la oxidación del ácido linoléico.

Palabras clave: antioxidante, *Bomarea,* DPPH, Folin-Ciocalteau, TBARS.

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INTRODUCTION

Living organisms constantly consume oxygen as a natural part of the process of cell energy production. As a consequence of this metabolic activity, highly reactive molecules known as free radicals are produced. These molecules are chemical species derived from oxidative metabolism which have one or more unpaired electrons in their last energy level (1). This reactive oxygen species (ROS), in which we can find superoxide ion, hydroxyl radical, alcoxyl, peroxyl, nitrogen oxide, oxygen peroxide, singlet oxygen and peroxynitrite (2), has shown multiple types of damage at the cellular level (3). The production of these substances can induce oxidative stress (4, 5), generated by an imbalance of free radicals as product of the increase of its production or the decrease in the ability to eliminate them (6). Increased production of free radicals could initiate and promote the progression of some chronic diseases such as cancer, cardiovascular problems, atherosclerosis, inflammation and other (7-9). The reactive oxygen species acts like molecular targets to search biologically active compounds that possess the ability to reduce or inhibit the effects caused by the action of free radicals.

The continuous raise in public concern of the toxic effects that have been generated by some of the commonly synthetic antioxidants used in food preparation and other edible products, increased the need to look for other sources of antioxidant compounds (10). Some plant taxa, such as members of the families Asteraceae, Euphorbiaceae, Lamiaceae, Zingiberaceae, among others, have been frequently included in analyses of the antioxidant activity (11-15). Many studies have produced promising results due to their ability to generate free-radical scavenger substances such as phenolic compounds, carotenoids, vitamins and nitrogenated compounds, all of which are useful as potential sources of antioxidant compounds.

The Alstromeriaceae family, restricted to the Neotropics, includes the genera *Alstroemeria* and *Bomarea* (16), the latter being the most diverse with about 110-120 species distributed from Chile to Mexico (17). *Bomarea* includes lianescent and erect herbs with abundant inflorescences, growing especially in high and middle lands from the Andes region and Central America. Amazonian indigenous communities have used some species of this genus as food (e.g., *B. edulis*). Additionally, medicinal properties and toxicities are known for species of the genus (e.g. *B. salsilla*) (18).

Species of the genus *Bomarea* are common elements of the high Andean forest of Colombia where they grow as showy plants (17). Some species are considered to be endangered due to the human transformation of the Andean forest landscape. Although few uses have been reported for the genus, inflorescences are frequently harvested as ornamental flowers (16).

In this work, we evaluated the antioxidant capacity of ethanol extracts of 11 *Bomarea* species, constituting the first evaluation of this activity performed to this group of plants. The end goal is to produce more biological information for this genus and to increase the knowledge of its potentialities as antioxidants.

MATERIALS AND METHODS

Plant material of Bomarea species were collected from several localities in Colombia and one Ecuador (*B. glaucescens*). All specimens were determined by F. Alzate and vouchers were deposited at the Universidad de Antioquia's herbarium (HUA). Species were collected in four fieldworks carried out through Andean forest zones of Colombia and Ecuador. The plant material, consisting of stems and leaves, was dried in an oven at 45°C with circulating air for 48 h 400 g of powdered dry plant were extracted exhaustively in 3 L of 96% ethanol during 72 h. The obtained extracts were concentrated under vacuum pressure and stored at 4°C in dark glass.

PHENOLIC CONTENTS

The phenolic content was determined according to the Folin Ciocalteau method previously modified by Londoño *et al.,* 2006 (19). The reaction mix was composed of 750 μL ultrapure water (Milli-Q), 100 μL of extract to be evaluated, 100 μL of sodium carbonate solution (Na_2CO_3), and Folin Ciocalteu reagent 2N (Sigma® Chemical Co). The mixture was conserved in the dark for one hour after being agitated. The absorbance was determined at 760 nm, in a UV/Vis spectrophotomemeter Varian Cary®. A calibration curve was made with gallic acid (Sigma® Chemical Co) in a concentration range between 10-100 μg/mL. Results were expressed as gallic acid equivalents (μg/mL) per milligram of dried extract (GAE/mg).

FREE-RADICAL SCAVENGING ACTIVITY

A spectrophotometric assay was utilized based on the method proposed by Brand-Williams *et al.,* 1995 (20) and modified later by Jimenez *et al.,* 2005 (21). Extracts of each plant were concentrated (10.000 μg/mL) and diluted in a 1:2 (v/v) proportion with DPPH ethanol solution (5.07 x 10^{-5} M) (Sigma® Chemical Co.) The spectrophotometer was utilized to establish the absorbance at 517 nm five minutes after the reaction initiated. Discoloration was compared with the same ethanol and DPPH solution proportion (1:2).

DPPH discoloration percentage was calculated according to the following equation:

$$\% \text{ Discoloration} = \left(1 - \frac{As - Abs}{Ab_{DPPH}}\right) * 100 \quad \textbf{Equation 1.}$$

where: *As*: Sample absorbance, *Abs*: blank sample absorbance; Ab_{DPPH}: blanc DPPH absorbance.

The initial solution was diluted to 75, 50, 25, 10, 5 and 1%, to estimate EC_{50} for every extract (the necessary concentration to decrease the initial DPPH concentration in 50%). Three tests were made for every extract concentration, ensuring that the variation was not more than 10%. Additionally, Silymarin (Genfar®) 150 mg lot 0509061 was used as a comparison control. Silymarin is a mixture of flavolignanes obtained from *Silibus marianus*, which is used as a reference in this study because it is a natural free radical scavenger with high antioxidant activity (22).

INHIBITION OF LIPID PEROXIDATION

Previous studies conducted with fully marked antioxidant activity extracts confirmed the lipoperoxidation inhibition of methyl linoleate to 500 μg/mL (ppm) concentration (23). For this reason, we decided to utilize extracts in a final concentration of 300 μg/mL, defining this concentration limit value as a control parameter to select the most potential antioxidant extracts, for including them in a posterior exhaustive investigation using TBARS methodology. The procedure proposed by Guillensans R, Guzmanchozas, 1998 (24) was modified for this assay. 80 μL of linoleic acid (Sigma® Chemical Co) 20mM, 10μL of each extract (3000 μg/mL) was added by triplicate in the plates. The mixture was pre-incubated and after this stage, 10 μL of $CuSO_4.5H_2O$ were added. Then, the solution was incubated at 37°C to allow the oxidation of the linoleic acid. After this stage, the oxidation was stopped by means of EDTA addition, and TBA solution was added to the final solution (thiobarbituric acid 0.67% –Sigma Chemical Co, trichloroacetic acid 15% –Merck®, HCl 0.1M – Merck®). The plate was heated at 90°C to allow chromophore formation. After cooling, the content of each plate was filtered and it was read in a Universal Microplate Reader ELx 800Ns at 532 nm.

Results were compared with the positive control, which represents 100% of the lipid matrix oxidation, and finally expressed as μM MDA concentration.

STATISTICAL ANALYSIS

The GraphPad Prism® (Graph- Pad software, Inc, San Diego, CA 2003) statistical package was employed to estimate EC_{50} and its statistical parameters (goodness of fit and confidence intervals) in Free radical scavenging activity. An analysis of variance (ANOVA), followed by a Newman–Keuls multiple comparison test, was used to perform a TBARS and Folin Ciocalteau assay. $p < 0.05$ values were considered significant (*), $p < 0.01$ very significant (**), and $p < 0.001$ extremely significant (***).

RESULTS AND DISCUSSION

The free radical scavenging activity, the phenolic contents, and the inhibitory effects on linoleic acid peroxidation of ethanolic extracts of 11 *Bomarea* species are presented in table 1. In general, extracts exhibited a DPPH free radical scavenging activity, which becomes a stable violet radical in ethanolic solution. This radical was absorbed at 517 nm and contains a unpaired electron that can be stabilized, producing a decrease of color, which can be measured by means of an espectrophotometry (25). CE_{50} varied between 333.3 and 39.0 μg/mL as it is shown in table 1. The extracts of *B. glaucescens, B. setacea, B. pardina* and *B. euryantha* showed high free radical scavenger activity, represented by their CE_{50} value (39.0, 50.99, 54.39 and 75.10 μg/mL, respectively); which is similar to the value found for the reference substance, silymarin (CE_{50} = 70.66 μg/mL).

Table 1. Free radical scavenging estimated by the DPPH method, oxidation parameters of the linoleic acid, and the phenolic content by means of the Folin-Ciocalteau test for ethanolic extracts of 11 *Bomarea* species.

		DPPH	TBARS	Folin-Ciocalteau
Species	**Voucher**	**CE_{50} μg/mL [a]**	**Concentration μM de MDA [b]**	**Phenolic content GAE/mg [c]**
B. bredemeyerana	Alzate 2897	168.20 (158.20; 178.90)	0.474 ± 0.0147**	27.42 ± 0.68
B. callejasiana	Alzate 3010	333.30 (311.00; 357.30)	0.479 ± 0.0138**	57.56 ± 1.96
B. diffracta	Alzate 2714	293.20 (285.60; 301.00)	0.511 ± 0.0130*	27.44 ± 4.56
B. euryantha	Alzate 2909	75.10 (57.85; 97.49)	0.489 ± 0.0079**	37.08 ± 3.61
B. glaberrima	Alzate 2923	277.60 (263.20; 292.70)	0.497 ± 0.0055*	26.85 ± 1.87
B. glaucescens	Alzate 2930	39.00 (38.52; 39.48)	0.532 ± 0.0244*	97.89 ± 2.92
B. hirsuta	Alzate 2927	212.40 (187.20; 240.90)	0.429 ± 0.0038***	28.74 ± 1.90
B. linifolia	Alzate 2931	159.10 (141.90; 178.40)	0.587 ± 0.0722	31.89 ± 1.04
B. pardina	Alzate 2922	54.39 (45.10; 65.58)	0.545 ± 0.0038	42.84 ± 4.54
B. setacea	Alzate 2713	50.99 (47.70; 54.52)	0.500 ± 0.0075*	166.00 ± 17.99
B. vestita	Alzate 2801	119.80 (115.90; 123.70)	0.551 ± 0.0113	50.69 ± 5.38
Silymarin	-	70.66 (66.92; 74.60)	-	-
Positive control	-	-	0.646 ± 0.0121	-

[a] CE_{50} with superior and inferior limits at a confidence interval of 95%.

[b, c] Media ± Standard error.

Statistical differences between the extracts and the positive control of oxidation for TBARS test: *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$).

The polyphenol content related to the free radical scavenger capacity could indicate that a high content of these compounds constitutes a possible explanation for the behavior observed in the ethanolic extracts of *B. glaucescens* and *B. setacea*.

A contrary case is evident for *B. pardina* and *B. euryantha*, in which the quantity of phenolic content was relatively low (42.84 ± 4.54 and 37.08 ± 3.61 GAE/mg, respectively), although these had strong free radical scavenger (CE_{50} = 54.39 and 75.10 μg/mL, respectively).

The free radical scavenger activity can be attributed to diverse mechanisms that not necessarily involve the plant phenolic content (26). From this point of view, can be re-evaluated as the content of phenolic compounds is a parameter directly correlated with the antioxidant activity, as it was proposed Londoño *et al*., 2006 (19). The last idea could explain the behavior of the 11 evaluated species, as they show an inverse correlation between polyphenol content and free radical scavenger activity (illustrated in figure 1). This fact was demonstrated by the Pearson's correlation coefficient (r = -0.4991), with r^2 = 0.2491, indicating that only 24.91% of the free radical scavenging activity exhibited for these *Bomarea* species would be explained by the phenolic content in the extracts.

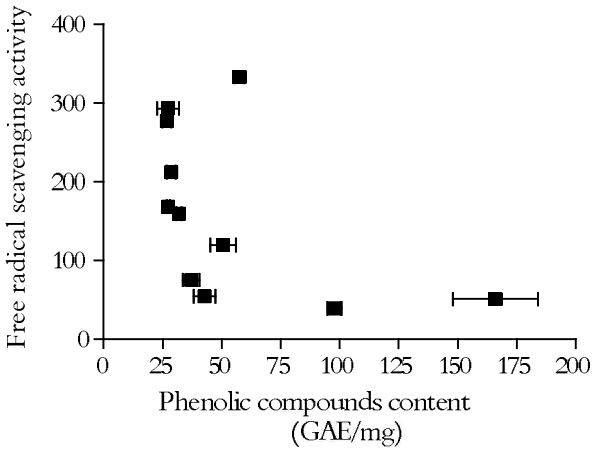

Figure 1. Pearson's correlation coefficient between the free radical scavenging activity and the plant phenolic compound content (r = -0.4991) obtained for ethanolic extracts of 11 species of *Bomarea*.

The TBARS test is based on the determination of malondialdehyde (MDA), which is one of the final products of the lipid peroxidation. When this reacts with thiobarbituric acid, it forms a pink chromophore (thiobarbituric acid reactivating substance), which allows to spectrophotometrically follow the reaction to 532 nm (27, 28).

Results (shown in table 1) demonstrated that 50% of extracts present low capacity to inhibit the linoleic acid oxidation, comparatively with the

results between the concentrations of MDA (μM) found for the samples, and the positive control of oxidation (100% of the oxidation of the oily matrix). The highest inhibition values of the linoleic acid oxidation were obtained for the *B. hirsuta*'s extract, which had the lowest MDA average concentration (0.429 ± 0.0038 μM) (***p < 0.001).

Extracts of *B. setacea* (*p < 0.05), *B. glaberrima* (*p < 0.05), *B. euryantha* (**p < 0.01), *B. callejasiana* (**p < 0.01), and *B. bredemeyerana* (**p < 0.01), presented interesting results to continue the chromatographic isolations studies, because they diminished significantly MDA's formation to a concentration of 300 μg/mL.

The antioxidant activity is often defined by the capacity to delay the beginning of the autoxidation of a substratum for ROS captation, or by the capacity to act like an antioxidant, breaking the chain reaction to disable the phase of the spread of the autoxidation of the mentioned substratum (29). The antioxidant capacity of the extracts of *B. setacea, B. glaberrima, B. euryantha, B. callejasiana,* and *B. bredemeyerana* was confirmed by means of the TBARS method, according to their capacity to avoid the formation of malondialdehyde (MDA), which is a product derived from the linoleic acid peroxidation, MDA (26-27).

The response and efficiency to the tests among species, changed according to the method used, reflecting the high complexity of the mechanisms involved in the antioxidant activity, which has been previously postulated by Matkowski *et al*., 2006 (30). The variability obtained in the response is evident for *B. callejasiana*, even though representing the extract with low free radical scavenging activity according to the DPPH method (CE_{50} = 333.3 μg/mL), and having a mean content of phenolic contents (57.56 ± 1.96 GAE/mg). Therefore, it was very effective to disable the linoleic acid peroxidation (MDA μM to 300 μg/mL = 0.479 ± 0.0138**). Similar behaviors appear in the *B. glaucescens* extract, which presents the highest free radical scavenging activity (CE_{50} = 39.0 μg/mL), and a great content of phenolic contents (97.89 ± 2.92 GAE/mg). However, it did not show a high capacity to avoid the oxidation of the lipid matrix (MDA μM = 0.532 ± 0.0244*).

The extracts of *B. glaucescens* and *B. setacea* presented better stabilizing capacity of free radicals than the reference ones used as control (Silymarin). This fact can be explained by its highest phenol content. The functional groups around the hidroxil aromatic have diverse chemical effects, providing molecules with antioxidant capacities (31).

The results obtained (phenolic content, free radical scavenging and inhibition of lipid peroxidation) strongly support the need for using different methods to establish the antioxidant activity on a complex matrix, such as crude extracts. The antioxidant activity should not possibly be attributed to the presence of specific molecules with phenol content, but it is possibly caused by mutual interactions between the diverse components of the matrix (32). In other studies conducted in parallel to this research, it was possible to determine the presence of phytosterol in *Bomarea* by means of GC/MS, represented mainly by campesterol, stigmasterol and a great content of β-sitosterol in all the species studied.

The presence of phytosterols might explain the antioxidant potential of several analyzed species, due to the fact that β-sitosterol has shown interesting results regarding its capacity to act as an antioxidant. Nevertheless, results depend on the method used, as it presents a great capacity to stabilize membrane models on which a lipid oxidative process has been induced (33). The assessed extracts showed to have, in addition, a moderate ability to stabilize free radicals (34), which allows considering that the antioxidant capacity is not necessarily involving such mechanism. It is then more likely to propose that this capacity is not due to the mere occurrence of any single metabolite, but rather to the interactions between different components of the analyzed *Bomarea*'s extracts.

Bomarea setacea was the most active extract in this study and a promising taxon with antioxidant activity, which was also evident for three parameters evaluated (CE_{50} = 50.99 μg/mL, 166.00 ± 17.99 GAE/mg and MDA μM to 300 μg/mL = 0.500 ± 0.0075* for the DPPH, Folin-Ciocalteau and TBARS tests respectively). Future chromatographic isolation is necessary for the extracts of the species with antioxidant activity, such as *B. euryantha, B. glaucescens, B. pardina* and *B. hirsuta*, aiming to establish the potential metabolites responsible of the antioxidant response.

CONCLUSIONS

This study constitutes the first evaluation of the antioxidant potential for species of the *Bomarea*

genus, demonstrating that some of the extracts present marked antioxidant activity represented by their capacity to stabilize DPPH free radical, and by their high content of phenolic compound and inhibition of lipid peroxidation. These species can constitute prospective sources of metabolites, useful in the treatment of problems derived from the oxidative metabolism (35-37). The antioxidant activity represents an additional biological activity reported for the genus, which has already been successfully tested in the control of protozoa (38).

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