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Determination of Radical Scavenging Activity of Hydroalcoholic and Aqueous Extracts from *Bauhinia divaricata* and *Bougainvillea spectabilis* Using the DPPH Assay

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ABSTRACT

Bauhinia divaricata and *Bougainvillea spectabilis* are medicinal plants widely distributed in Mexico and they are used because of its potential hypoglycemic action; however, no free radical scavenging activity (RSA) studies over these plants are known. Thus, aqueous and hydroalcoholic extracts from leaf and stem samples were evaluated for their RSA using 1,1-diphenylpicrylhydrazyl free radical ($DPPH^{\cdot}$). Total phenolics and flavonoids extracts were determined too. Statistical analyses were performed using the SPSS statistical program with the significance level set at $P<0.05$. *Bauhinia divaricata* stem aqueous extracts with total phenols content of 12.98 mg GAE/g DW had the highest amount between samples. The same behavior was shown in flavonoids determination. However, when RSA was estimated it was found that stem aqueous extracts from *Bougainvillea spectabilis* produced more DPPH absorbance reduction (95.66%), with an IC_{50} (the concentration to inhibit the oxidation of DPPH by 50%) and AP (reciprocal of IC_{50}) values of 0.03 µg/mL and 33.33, respectively. These results were superior to common synthetic antioxidants used in the food industry like butylated hydroxyl toluene (BHT, IC_{50}=62 µg/mL) and can be useful for further applications of these plants or its constituents in pharmaceutical and alimentary preparations.

Keywords: antioxidants, *Bauhinia*, *Bougainvillea*, flavonoids, phenolics

INTRODUCTION

Chemistry of natural products is a research field with endless potential, and is especially important in countries possessing great biodiversity (1), as México. In recent years there is an intensive increase in researches objecting the evaluation of the antioxidant activity of extracts and other materials from natural sources (2–5) since antioxidant compounds could be applied to treat and prevent cancer and cardiovascular diseases as well as to the aging process (6–8). Currently available synthetic antioxidants like BHT, butylated hydroxyl anisole and tertiary butylated hydroquinones have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants (9). Several studies revealed that phenols, mainly the type of flavonoids, from some medicinal plants, have antioxidant properties and exert anticarcinogenic, antimutagenic, antitumoral, antibacterial, antiviral and anti-inflammatory effects (10) due to their redox properties, acting as reducing agents, hydrogen donors, singlet oxygen

quenchers and chelating metals (11–13). Assays based on the scavenging of DPPH has been widely used to measure the antioxidant activity of different phenolic compounds and the results obtained are, in most cases, in agreement with those derived by lipid peroxidation assays in bulk oils (14–16). DPPH is one of a few stable available organic nitrogen radicals and has a UV-vis absorption maximum at 515 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of color (17–18).

Bougainvillea is a genus of flowering plants native to South America and are popular ornamental plants in all over the world (19). Narayanan (20), Senapati (21) and Menakshi (22) found that the alcoholic extract of *B. spectabilis* has significant hypoglycemic effect in alloxan-induced diabetic albino mice and that it is free from any acute toxicity. In another work, Edwin (23) reported the free radical scavenging activity of ethanol extracts from *B. glabra*. It is claimed that D-pinitol (3-*O*-methylchiroinositol) extracted from *B. spectabilis* exert insulin-like effects (24–25).

The *Bauhinia* genus comprises about 500 species of shrubs, small trees, and lianas in the tropics. It can be found in the rainforests and tropical regions of Africa, Asia and Latin America. Many plants of the genus are used in traditional medicine for their interesting biological activities such as analgesic, antidiabetic, antiinflamatory, antimicrobial, astringent and diuretic effects (26–27). There are several works dealing with the antioxidant capacity of the *Bauhinia* genus, mainly over *B. candicans* (28), *B. forticata* (29), *B. variegata* (30) and *B. purpurea* (31). In this project, *Bauhinia divaricata* L. was studied.

In summary, *Bougainvillea spectabilis* and *Bauhnia divaricata* are species whose infusion extracts have been used in hypoglycemic treatments in México, but today no free radical scavenging activity reports on these natural sources are known. Thus, in this work it was carried out the determination of the phenols and flavonoids content and the evaluation of the antioxidant activity of aqueous and methanolic extracts from leaves and stem samples of these plants, in order to contribute to the knowledge for further applications in food and pharmaceutical industries.

MATERIALS AND METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl, gallic acid, cathequin, 2,6 di-tert-butyl-4-methylphenol (BHT), butylated hydroxyanysole (BHA), sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide and methanol were purchased from Sigma Co. (St. Louis, MO, USA) and Folin Ciocalteu reagent from Fluka Chemical Co. (Buchs, Switzerland).

Plant material

Leaf and stem of *Bauhinia divaricata* L. and *Bougainvillea spectabilis* were collected manually throughout 2008 in Alamo-Veracruz, México and they were ground and milled into flour. Voucher specimens are deposited at the Herbarium "IZTA" of FES-Iztacala, Universidad Nacional Autonoma de México under the number 42167.

Extract preparations

Portions of 200 mg of the leaf and stem samples were separately homogenized with 20 mL of 95% methanol for 50 min at room temperature. For the aqueous extraction, 200 mg of the samples were separately homogenized with 20 mL of deionized water and then boiled at 98°C for 3 h. After this procedure, all samples were filtered under vacuum using Whatman No. 1 paper and filtrates were frozen and lyophilized and were finally stored at -18°C until their use (32–33).

Total phenols determination

Total phenols were determined by Folin-Ciocalteu colorimetric method (34–35). To 0.5 mL of 50% hydroalcoholic solution of each extract was mixed 0.5 mL of the Folin-Ciocalteau reagent and 0.5 mL of 10% Na_2CO_3, and the absorbance was measured at 760 nm after 1 h incubation at room temperature, with an Aquarius Cecil CE7200 spectrophotometer. The results were given in mg GAE/100 g DW of gallic acid equivalent. The standard curve was prepared using 0, 50, 100, 150, 200, and 250 mg/L solutions of gallic acid in methanol:water (50:50, v/v).

Total flavonoids determination

Total flavonoids were determined by the Aluminum chloride colorimetric method (36), where 250 mL of each sample was mixed with 1.25 mL of deionized water and 0.075 mL of 5% sodium nitrite. After 6 min, 0.15 mL of 10% aluminum chloride was added and after another 6 min the product was mixed with 0.5 mL of 1M sodium hydroxide and 2.5 mL of deionized water. Total flavonoids were measured at 510 nm (37). The results were given in mg CE/100 g DW of cathequin equivalent. The standard curve was prepared using 5, 10, 20, 40, 60, 80 and 100 mg/L solutions of cathequin in methanol:water (50:50, v/v).

Estimation of RSA using the DPPH assay

The %RSA of phenols was determined using the DPPH assay (38–39). The decrease of the absorption at 516 nm of the DPPH solution after addition of the antioxidant was measured in a cuvette; concentration of the extracts was varied in the reaction mixtures adding 0, 200, 400, 600, 800 or 1000 µL of each of them to a 3.9 mL hydroalcoholic solution of DPPH (25 ppm), completing a final volume of 4.9 mL with methanol. The absorption was monitored at the start and continuously every 30 sec until a constant value (plateau) was reached. Then, DPPH was calculated through a calibration straight line obtained in a range of concentrations of this substance. The results are expressed as %RSA= [Abs_{516} nm (t = 0) - Abs_{516} nm($t = t'$)] × 100/Abs_{516} nm(t = 0). Measurements were performed in triplicate. Absorbance values were corrected for radical decay using blank solutions. The quality of the phenol antioxidants in the extracts was measured by determining the IC_{50} (the concentration to inhibit the oxidation by 50%) of the pooled phenol extracts for each sample. IC_{50} was determined graphically from the sigmoidal-shaped curve of antioxidant concentration (µg/mL) *vs.* %RSA. For comparison purposes the results were expressed as AP, defined as the reciprocal of IC_{50} ($AP=1/IC_{50}$). The higher number of AP the better quality antioxidants (40).

Statistical analyses of results

To verify the statistical significance of the studied parameters, means and standard deviation of three measurements were determined. Where it was appropriate, differences between groups were tested by 2-way ANOVA using SPSS v11.0 software (SPSS Inc. Chicago, IL). Significant differences (*P* values < *0.05*) were assessed using Tukey`s test (41).

RESULTS

Total phenols determination

Comparison of total phenols content (Table 1) between *Bauhinia divaricata* leaf and stem extracts show slightly significant difference in each solvent used though not different enough to be statistically significant (*P<0.05*). However, significant differences were found between solvents used, being total phenols content in aqueous extraction higher than that with hydroalcoholic extraction procedure. The same behavior was found in *Bougainvillea spectabilis* samples although there are significant differences between samples and solvents used. *Bahuinia divaricata* stem aqueous extracts with total phenol contents of 12.98 ± 0.29 mg GAE/g DW had the highest amount among the plants analyzed in this study.

Table 1: Total phenols determination results in *Bauhinia divaricata* L. and *Bougainvillea spectabilis.**

Sample	Total phenols (mg GAE/g DW)	
	Aqueous extraction	Methanol extraction
Bauhinia divaricata		
Leaf	11.99 ± 0.82[a]	4.34 ± 0.21[d]
Stem	12.98 ± 0.29[b]	4.14 ± 0.78[d]
Bougainvillea spectabilis		
Leaf	11.10 ± 0.08[a]	2.6 ± 0.09[e]
Stem	9.64 ± 0.02[c]	6.0 ± 0.07[f]

* Average values of three measurements (For n = 3 ± s. d.). The letters indicate statistically significant differences (Tukey, P<0.05) between all samples.

Table 2: Total flavonoids determination results in *Bauhinia divaricata* L. and *Bougainvillea spectabilis.**

Sample	Total flavonoids (mg CE/g DW)	
	Aqueous extraction	Methanol extraction
Bauhinia divaricata		
Leaf	7.11 ± 0.07[a]	4.49 ± 0.32[d]
Stem	7.02 ± 0.14[a]	0.36 ± 0.03[e]
Bougainvillea spectabilis		
Leaf	5.33 ± 0.08[b]	3.62 ± 0.09[c]
Stem	3.95 ± 0.02[c]	2.86 ± 0.07[f]

* Average values of three measurements (For n = 3 ± s. d.). The letters indicate statistically significant differences (Tukey, P<0.05) between all samples.

Total flavonoids determination

Studying the main group of phenolic compounds, the total flavonoid content was analyzed. Results are exhibited in Table 2 and they indicate that total flavonoids contents in leaf and stem extracts from *Bauhinia divaricata* shown no statistical significant difference for the aqueous extraction procedure, whereas for hydroalcoholic extracts there is more flavonoids in leaf than in stem. Aqueous extraction method allows obtaining higher total flavonoids content in comparison with hydroalcoholic extraction procedure. In case of flavonoid concentration in *Bougainvillea spectabilis* it was found that leaf aqueous extracts presented the highest values (5.33 ± 0.08 mg CE/g DW); but, *Bauhinia divaricata* leaf aqueous extracts had the highest among all the samples.

Estimation of RSA Using the DPPH Assay

These results shown the DPPH scavenging kinetics measured at 515 nm for 20 min (Fig. 1–2). Stem aqueous extracts from *Bougainvillea spectabilis* produced more DPPH

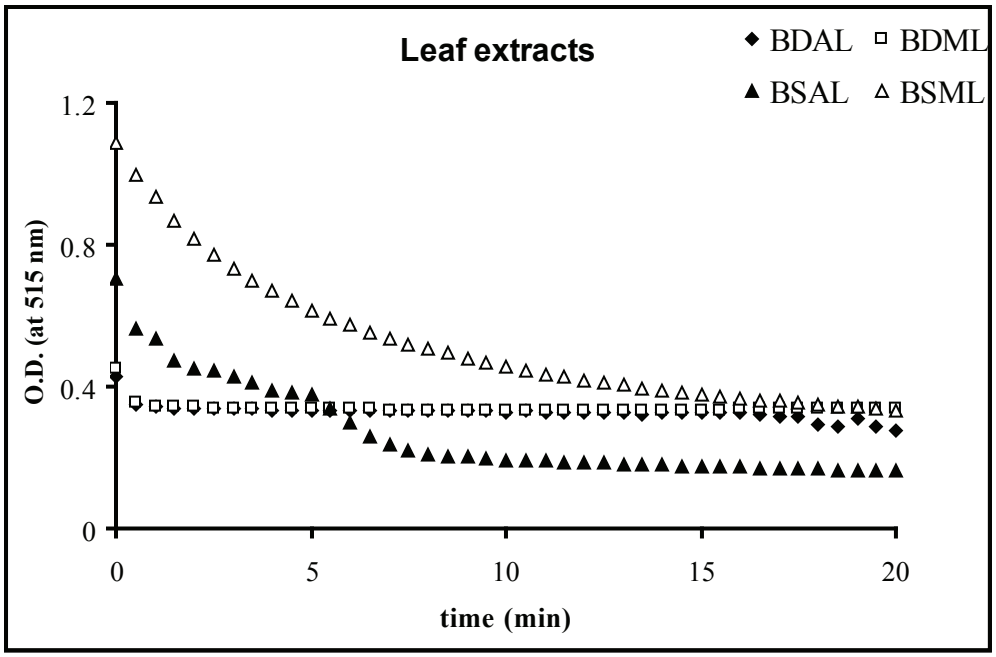

Figure 1: DPPH scavenging kinetics in the presence of aqueous and methanolic extracts from *Bauhinia divaricata* and *Bougainvillea spectabilis* leaf samples.
O.D.) Optical deviation, BDAL) *Bauhinia* aqueous extracts, BDML) *Bauhinia* methanolic extracts, BSAL) *Bougainvillea* aqueous extracts and BSML) *Bougainvillea* methanolic extracts

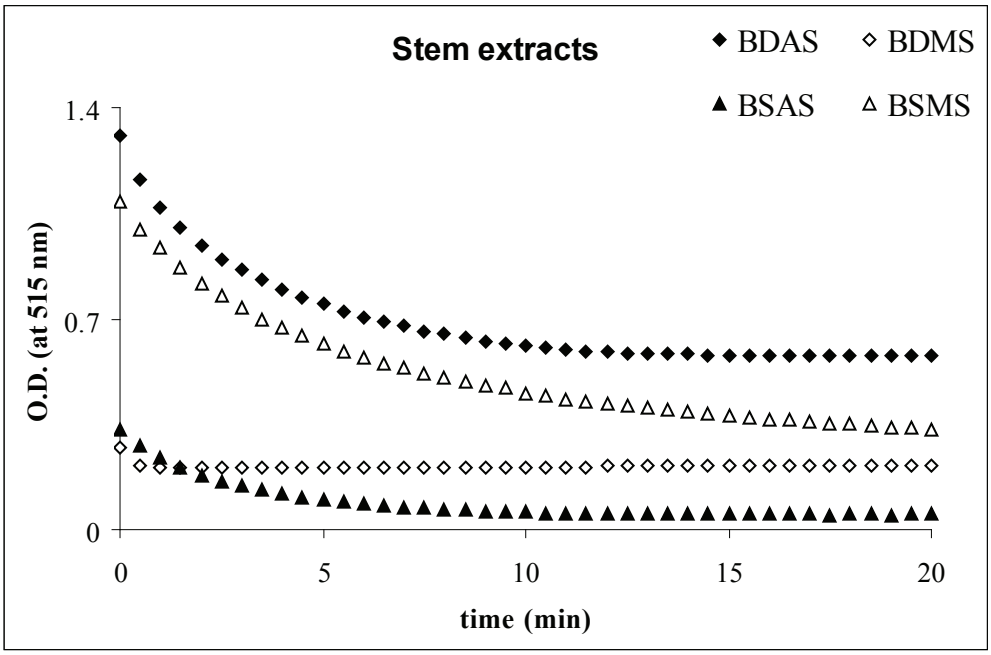

Figure 2: DPPH scavenging kinetics in the presence of aqueous and methanolic extracts from *Bauhinia divaricata* and *Bougainvillea spectabilis* stem samples.
O.D.) Optical deviation, BDAS) *Bauhinia* aqueous extracts, BDMS) *Bauhinia* methanolic extracts, BSAS) *Bougainvillea* aqueous extracts and BSMS) *Bougainvillea* methanolic extracts

Table 3: Radical scavenging activity, IC_{50} and AP determination results in *Bauhinia divaricata* L. and *Bougainvillea spectabilis.**

	% RSA		IC_{50} µg/mL		AP	
Parameter Solvent	**A**	**MetOH**	**A**	**MetOH**	**A**	**MetOH**
Bauhinia divaricata						
Leaf	75.0 ± 0.79[a]	74.62 ± 0.56[a]	0.145[a]	0.108[e]	6.89[a]	9.25[e]
Stem	53.7 ± 0.38[b]	84.11 ± 0.81[c]	0.165[b]	0.035[d]	6.06[b]	28.57[f]
Bougainvillea spectabilis						
Leaf	85.63 ± 0.01[c]	66.57 ± 0.11[e]	0.134[c]	0.06[f]	7.46[c]	16.66[g]
Stem	95.66 ± 0.02[d]	88.60 ± 0.01[f]	0.03[d]	0.061[f]	33.33[d]	16.39[g]

* Average values of three measurements (For n = 3 ± s. d.). The letters indicate statistically significant differences (Tukey, P<0.05) between samples in each parameter analyzed. A) Aqueous extraction, MetOH) Methanolic extraction

Table 4: Comparison of phenol content and %RSA results of *Bauhinia divaricata* L. and *Bougainvillea spectabilis* with other medicinal plants.

Plant material	Taxonomical Family	Phenol content (mg GAE/g DW)	%RSA	Reference
Bougainvillea spectabilis	Nyctaginaceae	9.64	95.66	In this work
Bauhinia divaricata	Leguminosae	12.98	84.11	In this work
Gynura procumbens	Compositae	21.74	58.21	
Camellia sinensis	Theaceae	288.5	94.2	
Sesbania grandiflora	Leguminosae	54.5	89.3	Kumar (42)
Thespesia populnea	Malvaceae	31.2	44.1	
Cassia auriculata	Fabaceae	24	70.9	
Mellilotus officinalis	Fabaceae	289.5	94.3	
Equisetum maximum	Equisetaceae	54.5	89.9	
Plantago major	Plantaginaceae	31.4	89.3	Pourmorad (43)
Adiantum capillus	Adiantaceae	22.3	44	
Urtica dioica	Urticaceae	24.1	70.8	

absorbance reduction (95.66 %) than hydroalcoholic extracts and this value was higher than *Bauhinia divaricata* results in samples analyzed (table 3). *Bauhinia divaricata* aqueous extracts exhibited a %RSA values in the range of 53–75% in stem and leafs versus 74-84% in hydroalcoholic extracts. The IC_{50} and AP values showed that stem aqueous extracts from *Bougainvillea spectabilis* has the better quality of antioxidants, because at lower concentrations produce high radical scavenging activity (0.03 µg/mL and 33.33, respectively). IC_{50} values of the standard compounds were 28.83 for gallic acid, 3.3 for cathequin, 62 for BHT and 363 µg/mL for BHA.

DISCUSSION

Taking into account the use of *Bauhinia divaricata* and *Bougainvillea spectabilis* to design and formulate disease-preventing products in a future, it was observed that the phenol content range observed in the composition of the plant extracts may be completely acceptable due to several points: they were obtained from two sources (leaf and stem) and they were extracted by different methods (aqueous and hydroalcoholic procedures), rending different content of dry weight. In comparison with other vegetables resources, the phenol content values determined in this work for *Bauhinia divaricata* ranged between 11.99 to 12.98 mg/g, and for *Bougainvillea spectabilis* ranged 9.64 to 11.1, being values lower than the ones reported for other medicinal plants (table 4), like *Camellia sinensis* (288.5 mg/g), *Sesbania grandiflora* (54.5 mg/g) and *Mellilotus officinalis* (289.5 mg/g) (42–43). However, when %RSA is evaluated, *Bougainvillea spectabilis* has the highest activity (95.66%). It is seen that although *Bougainvillea spectabilis* had lowest contents of phenolics the %RSA is the highest between several medicinal plants. These results were complemented with the IC_{50} evaluation, where lower values are indicative of a higher free radical scavenging activity of a sample. Aqueous and hydroalcoholic stem extracts from *Bougainvillea spectabilis* shown strong activities, with IC_{50} values of 0.03 and 0.061 µg/mL. Their activities were 110 and 54-fold more potent than cathequin and 2066, and 1016-fold more potent than the known synthetic antioxidant BHT (62 µg/mL) reported here and elsewhere (44). In the case of *Bauhinia divaricata* stem hydroalcoholic extracts showed IC_{50} values of 0.035 µg/mL. In other reports, *B. variegata*, *B. purpurea*, *B. candicans*, *B. monandra*, *B. forticata* and *B. angulosa* exhibited IC_{50} values ranged between 60 to 2000 µg/mL and the authors indicate that these values represent a good potential as free radical scavengers (45–47).

Therefore, results from *Bauhinia divaricata* and *Bougainvillea spectabilis* shown that they could represent good options for antioxidants sources.

The mechanism of reduction of the DPPH molecule (48) is based on a scavenging activity. In this system, the structure (both planar and spatial) of the antioxidant compound, present in the extract, is important for its capacity of donating hydrogen ions. Compounds able to donate hydrogen are derived from the shikimate pathway, as for example, flavonoids (49), which were determined in *Bougainvillea spectabilis* and *Bauhinia divaricata*. These molecules are not produced by plants whose extracts display a very high IC_{50} in the DPPH test, opposite to the results found in this work. Plants with high IC_{50} are, in fact, very rich in compounds of the acetate pathway, like terpenoids and fatty acids, which are unable of scavenging the DPPH free radical, but are able to avoid oxidative damage of cell membranes (50).

CONCLUSION

The properties of the solvents with different polarities used in this work significantly affect the yield of total phenolics and flavonoids and the antioxidant activity, and correspond with the reviewed results of other medicinal plants. The results shown that water is the best solvent to extract total polyphenols compounds from leaves and stem of *Bauhinia divaricata* L. and *Bougainvillea spectabilis*, and that they have a very potent antioxidant activity, compared with the pure catechins and gallic acid used as positive controls. These results can be useful for further applications of *Bauhinia divaricata* and *Bougainvillea spectabilis* or its constituents in pharmaceutical preparations after performing clinical *in vivo* researches. With this kind of investigations it would be easier the establishment of natural extracts supposed to functionalize formulations to treat and prevent the human damages occurring due to free radicals and also to replace synthetic antioxidants in industry.

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