

Artículo original

Total phenolic, flavonoids content and antioxidant activity of ethanolic extracts of ecuadorian plants

Fenoles totales, contenido de flavonoides y actividad antioxidante de los extractos etanólicos de plantas ecuatorianas

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RESUMEN

Diecisiete especies de plantas de la Provincia de Santa Elena de las costas de Ecuador fueron sometidas a análisis cualitativo y cuantitativo, con la finalidad de proporcionar información preliminar sobre posibles nuevas fuentes de plantas con propiedades antioxidantes que puedan contribuir a futuras investigaciones. El contenido total de fenoles y flavonoides fueron medidos a partir del extracto crudo etanólico empleando los reactivos Folin-Ciocalteu y cloruro de aluminio, respectivamente. Los valores del contenido de fenoles oscilaron en el rango entre 21.79 ± 0.02 a 19.28 ± 0.1 EAG/g extracto seco; mientras que el contenido de flavonoides se observó entre 150.23 ± 3.46 a 18.47 ± 2.88 EQ/g extracto seco; siendo *Cynophalla ecuadorica* la especie con el más alto valor de compuestos fenólicos; mientras que *Hyptis mutabilis* presentó el contenido más alto de flavonoides. La actividad antioxidante se evaluó empleando el método de DPPH (1,1-difenil-2-picryl-hidrazil). El extracto con mayor poder antioxidante fue el correspondiente a la especie *Cynophalla ecuadorica* ($IC_{50} = 0.013 \pm 0.02$ mg/mL), seguido por *Vitex gigantea* ($IC_{50} = 0.059 \pm 0.001$ mg/mL); *Swinglea glutinosa* ($IC_{50} = 0.06 \pm 0.003$ mg/mL) y *Murraya paniculata* ($IC_{50} = 0.08 \pm 0.01$ mg/mL). Hasta el momento, este es el primer reporte sobre el contenido

total de flavonoides y fenoles, así como de la actividad antioxidante de estas especies colectadas en Ecuador.

PALABRAS CLAVE

Actividad antioxidante, flavonoides, fenoles totales, tamizaje fitoquímico, Ecuador.

ABSTRACT

Seventeen plant species from Santa Elena province at Ecuador coast have been subjected to qualitative and quantitative phytochemical screening, in order to provide preliminary information about possible new source of plants with antioxidant capacity that might contribute to future research. Total phenolic and flavonoids content were measured on the crude ethanol extracts using Folin-Ciocalteu and aluminum chloride reagents, respectively. Values for phenolic content ranged between 21.79 ± 0.02 to 19.28 ± 0.1 GAE/g dry extract and for flavonoids content 150.23 ± 3.46 to 18.47 ± 2.88 QE/g dry extract; being *Cynophalla ecuadorica* the specie that exhibited the highest value of phenolic compounds; while *Hyptis mutabilis* showed the highest value of flavonoids. Antioxidant activity was evaluated by 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging. The most effective extract was *Cynophalla ecuadorica* ($IC_{50} = 0.013 \pm 0.02$ mg/mL), followed by *Vitex gigantea* ($IC_{50} =$

0.059±0.001 mg/mL); *Swinglea glutinosa* (IC₅₀= 0.06±0.003 mg/mL) and *Murraya paniculata* (IC₅₀= 0.08±0.01 mg/mL). To the best of our knowledge, this is the first report on the total phenolic, flavonoids content and antioxidant activity of these Ecuadorian species.

KEY WORDS

Antioxidant activity, flavonoids, total phenols, phytochemical screening, Ecuador.

INTRODUCTION

Polyphenolic compounds, anthocyanins, carotenoids and vitamins present in fruits, vegetables and many plants species have shown significant antioxidant properties [1,2]. This is due to a type of compounds that may play important roles in free radicals inhibition and are responsible for reducing oxidative stress produced as a consequence of reactions that undergo the lipids during normal aerobic metabolism, which generates free radicals and active oxygen species such as singlet oxygen and hydroxyl radicals causing destructive and lethal cellular effects. Antioxidant property found in phenolic compounds has advantages for human health since aids to prevent cardiovascular diseases, cancer, and neural disorders as Alzheimer's disease, Parkinson's disease and atherosclerosis [3]. Several methods, *in vitro* and *in vivo*, are tested to measure antioxidant capacity of different fruit and herb extracts mediated by different mechanisms including hydrogen atom transfer (HAT), singlet electron transfer (ET) and reducing power and metal chelation [4,5,6]. In present investigation 1,1-diphenyl-2-picryl hydrazyl (α,α -diphenyl- β -picryl hydrazyl), DPPH, radical scavenging assay was used. This is an ET-based method with HAT mechanism and is widely used either in preliminary screening or in evaluation of novel antioxidant compounds or natural product extracts. This activity is frequently reported as IC₅₀, which is defined as the effective concentration of antioxidant necessary to decrease by 50% the initial DPPH concentration [3].

Considering the importance of identifying new source of components or extracts with antioxidant capacity

and to prove scientific information on some Ecuadorian plant species, our research group collected seventeen plant species belonging to different families that grow spontaneously in the dry forests of the Ecuadorian coast (Table 1). Ecuador is a megadiverse country with more than 17,000 vascular plant species [7], however most of these are still unexplored regarding its chemical and biological potential.

Moreover, some studies have revealed that one third of all Ecuadorian plants species are traditionally used by people of this country [8]. Some species studied in this investigation such as *Gustavia serrata* S.A. Mori and *Capparidastrium bonifazianum* (Cornejo & Iltis) Cornejo & Iltis have restricted distribution in coastal Ecuador, while *Cynophalla ecuadorica* (Iltis) Iltis & Cornejo and *Erythrina smithiana* Krukoff both also extends the pattern of distribution to northwestern Peru. The aim of this study is to determine the antioxidant capacity, flavonoid and phenolic contents in ethanolic extracts of selected plants. To the best of our understanding, this is the first report on the antioxidant activity of these species.

MATERIAL AND METHODS

Plant material.

All the specimens were collected in February 2017 from Olón (Santa Elena Province; 1°41'52" S; 80°46'46" W) in the coast from Ecuador, about 195 kilometers northwest of Guayaquil and were identified by MsC Xavier Cornejo. Voucher specimens were deposited in the Herbarium GUAY of the Faculty of Natural Science of the University of Guayaquil, Ecuador. Table 1 shows the botanical species collected.

Chemicals.

All reagents are of analytical grade: Quercetin (Acros Organic™); ascorbic acid, gallic acid, potassium acetate, sodium carbonate, sodium hydroxide, potassium hydroxide, lead acetate, sulphuric acid, ferric chloride (Fisher); Folin-Ciocalteu reagent (MP Biomedicals LLC), Aluminium chloride (BDH Chemical) and 2,2-diphenyl-1-picrylhydrazyl (DPPH 90%) was provided by Sigma-Aldrich (Steinheim, Germany). Methanol and ethanol used were supplied by Merck.

TABLE 1:
Species collected from Olón (Ecuadorian coast)

Species	Vernacular name and uses	Family	Code
<i>Alternanthera</i> sp.		Amaranthaceae	s.n
<i>Xanthosoma dodsonii</i> Croat		Araceae	s.n.
<i>Liabum eggersii</i> Hieron.		Asteraceae	Rubio 1986
<i>Parthenium hysterophorus</i> L.		Asteraceae	Cornejo & Bonifaz 2154
<i>Hippobroma longiflora</i> (L.) G. Don		Campanulaceae	Cornejo & Bonifaz 5322
<i>Capparidastrium bonifazianum</i> (Cornejo & Iltis) Cornejo & Iltis	Maduro, food	Capparaceae	Cornejo & Bonifaz 1308
<i>Cynophalla ecuadorica</i> (Iltis) Iltis & Cornejo	Decoction for stimulation	Capparaceae	Cornejo 7344
<i>Dioclea virgata</i> (Rich.) Amshoff		Fabaceae	s.n.
<i>Erythrina smithiana</i> Krukoff	Pepito colorado	Fabaceae	Cornejo 654
<i>Hyptis mutabilis</i> (Rich) Briq		Lamiaceae	Cornejo & Bonifaz 5796
<i>Vitex gigantea</i> Kunth	Pechiche, food	Lamiaceae	Cornejo 1798
<i>Gustavia serrata</i> S.A. Mori	Membrillo	Lecythidaceae	Cornejo & Bonifaz 947
<i>Murraya paniculata</i> (L.) Jack	Ornamental	Rutaceae	Bonifaz & Cornejo 2538
<i>Swinglea glutinosa</i> (Blanco) Merr.	Ornamental	Rutaceae	s.n.
<i>Solanum adhaerens</i> Willd.	Cariño de suegra	Solanaceae	Cornejo & Bonifaz 1303
<i>Stachytarpheta cayennensis</i> (Rich.) Vahl		Verbenaceae	Cornejo 4161

Extraction.

Dried and ground aerial parts (50 g) were vigorously stirred and separately with 250 mL of ethanol at room temperature for 24 hours. The mixture was filtered and concentrated under reduced pressure at 60 °C. The obtained extracts were stored in a refrigerator at -4°C until the performance of analysis.

Phytochemical screening.

Crude extracts were phytochemically evaluated to determine the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, tannins, anthraquinones according to standard methods [9]. Any change of colours or precipitate formation was indicative of positive response to these tests.

Total Phenolic Contents.

Total phenolic contents of each extract were determined using a Folin-Ciocalteu colorimetric method [10]. 1 mL properly diluted of each extract solution was mixed with 0.5 mL of Folin-Ciocalteu reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 8 min at room temperature, 2 mL of (7.5% w/v) sodium carbonate solution were added. The solutions were mixed and allowed to stand for 30 min at room temperature. Then, the absorbance was measured with a spectrophotometer UV-visible Genesys 10 BIO at 765 nm. A standard calibration curve was prepared, using a gallic acid solution of (5, 10, 20, 25, 30, 40 and 50 µg/mL). Results are expressed as mg of

gallic acid equivalents (GAE)/100 g dry weight (dw) extract. Data are reported by means of at least three replications.

Total Flavonoids Contents.

Total flavonoid content was estimated according to the method described by Zhishen [11]. 0.5 mL of properly diluted extract was mixed with 0.1 mL NaNO₂ (5% w/v). Additionally, 0.1 mL AlCl₃ (10 % w/v) was also added, 5 min later and leaved it to react for another 6 min and after reaction, 0.1 mL of potassium acetate 1 M solution was added; the mixture was completed up to 2.8 mL with distilled water. The solution was mixed carefully and the absorbance was measured with a spectrophotometer UV-visible Genesys 10 BIO at 415 nm. A calibration curve was plotted using quercetin as standard flavonoid reference (10, 20, 30, 40, 50, 60, 70, 80, 100 µg/mL). The results are expressed as mg of quercetin equivalents (QE)/100 g dry weight (dw) extract. Data are reported by means of at least three replication.

DPPH radical method.

The antioxidant capacity of each extract was assessed using a method described by Lai [12]. A solution of DPPH• (6 x 10⁻² mM) in methanol was prepared, and 2.8 mL of this solution was mixed with 200 µL of each extract previously dissolved in methanol at concentration of 15, 30, 60, 125, 250, and 500 µg/mL (starting with a solution of 4 mg/

mL). The mixture were kept in the dark at room temperature for 30 min. The absorbance was measured on a spectrophotometer UV-visible Genesys 10 BIO at 517 nm. A solution of 2.8 mL of DPPH and 0.2 mL of methanol was used as negative control while ascorbic acid at the concentration of 176 µg/mL was used as standard antioxidant reference. Results are expressed as inhibition percentage (% IP) and are calculated following this equation [13,14]:

$$\%IP = [\text{Abs DPPH} - \text{Abs sample} / \text{Abs DPPH}] \times 100$$

The concentration required to obtain 50% of the maximum capacity of free radicals scavenging (IC₅₀) were calculated by linear regression. Data are reported by means of at least three replications.

Statistical analysis.

Data was expressed by means of ± standard deviation (SD) of three replications in all tests. Data from the antioxidant activity assay was analyzed statistically by one-way variance analysis (ANOVA), followed by Tukey test using the statistical software SPSS 23.0 for Windows. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

Ethanollic extracts were phytochemically evaluated through simple qualitative tests to observe the presence of secondary metabolites in the extracts. Results are shown in Table 2.

Alkaloids, tannins, flavonoids and triterpenes were the most abundant metabolites in all tested species. Particularly, *Hippobroma longiflora* (Campanulaceae); *Capparidastrum bonifaziarum*, *Cynophalla ecuadorica* (Capparaceae); *Dioclea virgata* (Fabaceae); *Gustavia serrata* (Lecythidaceae); *Hyptis mutabilis* (Lamiaceae) and *Solanum adhaerens* (Solanaceae) extracts showed abundant to moderate presence of alkaloids in all the assays carried out with Dragendorff, Mayer, Wagner and Bouchardat reagents. Although these species belonging to families that have reported the presence of alkaloids, the isolation and identification of alkaloids from these species is very limited in the scientific literature consulted and very few reports

have been found. (-)-lobeline, a type piperidine alkaloid, has been isolated from *Hippobroma longiflora*, with atherosclerosis and antihypertensive properties [15]. Additionally, other alkaloids such as tetrahydropyridine have been reported from *H. longiflora* [16]. Some species of *Gustavia* [17], *Dioclea* [18] *Erythina* [19] and *Solanum* [20] genus have shown presence of alkaloids with important biological activities.

Tannins and flavonoids were present between abundant to moderate, in most of the extracts assayed using FeCl₃ 5%, AcOPb 10 % solutions and NaOH 10% and Shinoda reagent, respectively, as detection methods. Previous investigations have reported the presence of this class of metabolites and its pharmacological activities. From *Dioclea* species, flavonoids have been isolated with vasodilation effect [21]. Novel flavonoids and its anti-inflammatory effects were reported from *Erythina livingstoniana* [20]. *Vitex gigantea*, known in Ecuador as “Pechiche” showed presence of polyphenols, although *Vitex* genus is known mainly by presence of iridoids, furthermore lignans and diterpenes have also been identified [22,23]. Additionally, from extracts of *M. paniculata* oxygenated flavones with antioxidant activity has been found recently [24].

Triterpenes and quinones were observed in all extracts, while saponins were poorly detected in all samples analyzed. Triterpens such as β-sistosterol, stigmasterol and spinasterol have been isolated from some species of *Alternanthera* [25], while triterpenoid lactones structurally related to oleanonic, betulinic and lantanilic acid with nematocidal properties were isolated from *Lantana camara* [26].

Quinones and anthraquinones were observed by using Borntrager reagent. *H. longiflora* and *G. serrata* exhibited abundant presence of these metabolites, while extracts of *V. gigantea*, *C. ecuadorica*, *L. eggersii* and *Althernanthera sp.* showed moderate presence of these metabolites. Emodin, an anthraquinone derivative was reported of *Murraya tetramera* and other *Murraya* species [27].

Table 2: Phytochemical screening of ethanolic extracts of aerial parts

Botanical species	DR	MR	WR	BR	FeCl ₃ 5%	AcOPb 10%	NaOH 10%	SR	SR*	L- BR	H ₂ SO ₄	Foam- I	BR*
<i>Alternanthera sp</i>	+	+	-	++	+++	++	+++	-	+++	+++	+++	-	++
<i>Xanthosoma dodsonii</i>	++	-	-	-	++	++	-	-	+++	-	-	-	-
<i>Liabum aggersii</i>	++	-	-	-	++	+++	-	-	++	-	+	-	++
<i>Parthenium hysterothorus</i>	++	-	-	-	+++	-	-	-	+++	-	+++	-	-
<i>Hippobroma longiflora</i>	++	++	++	+++	+++	++	+++	-	+++	+++	+++	-	+++
<i>Cappariadstrum bonifazianum</i>	+++	+++	++	+++	+++	+++	+++	-	-	++	-	-	-
<i>Cynophalla ecuadorica</i>	+++	+++	+++	++	++	+++	+	-	+	-	-	-	++
<i>Dioclea virgata</i>	+++	+++	+++	+++	+++	+++	+++	-	-	+++	-	-	-
<i>Erythrina smithiana</i>	+++	-	-	-	+	-	-	-	+++	-	+++	+	-
<i>Hyptis mutabilis</i>	++	++	++	++	+++	+++	+++	-	+++	+++	+++	-	+
<i>Vitex gigantea</i>	++	-	-	-	++	++	++	++	+++	-	+++	+	++
<i>Gustavia serrata</i>	++	++	++	++	+++	++	+++	-	+++	+++	+++	-	+++
<i>Murraya paniculata</i>	++	++	-	-	+++	-	+++	-	+++	-	+++	-	-
<i>Swinglea glutinosa</i>	+++	++	++	-	-	+++	+++	+++	+++	+++	-	-	-
<i>Solanum adhaerens</i>	++	++	++	+++	-	+	-	-	+	+++	+	+	-
<i>Lantana sp</i>	-	-	-	-	+++	+++	-	-	+++	-	+++		
<i>Stachytarpheta cayennensis</i>	++	-	-	-	++	++	-	-	+++	+++	+	-	++

Key: (-) Absence; (+) Poor; (++) Moderate; (+++) Abundant. DR: Dragendorff reactive; MR: Mayer reactive; WR: Wagner reactive; BR: SR: Shinoda reactive; SR* Salkowski reactive; L-BR: Lieberman-Burchard reactive; Foam-I: Foam (out sodium bicarbonate); BR* Borntrager reactive.

Phenols and Flavonoids contents

Phenolic contents, was expressed as gallic acid equivalents using the linear regression equation ($y=0.0618x-0.4436$) $r^2=0.9984$. All samples analyzed showed at range between 21.79 ± 0.02 GAE/g dry extract to 19.28 ± 0.1 GAE/g dry extract (Table 3), where *C. ecuadorica* revealed the highest value while *H. longiflora* showed the lowest. No significant differences in phenolic content were observed in all the extracts analyzed.

When comparing the results obtained with previous reports for these species, it was only found the analysis of phenolic content for *M. paniculata* carried out by Gautam et al., [28] which reported a

value of 15.40 ± 0.38 GAE for ethanolic extract while in the present study a higher value for the same species (20.56 ± 0.12 GAE/g dry extract) was observed. Similarly, ethanolic extract of *S. cayennensis* reported by Onofre et al., [29], exhibited a significantly lowest range value (6.99 ± 0.02 GAE/g dry extract and 8.23 ± 0.08 GAE/g dry extract) comparing to the reported in this investigation (20.04 ± 0.03 GAE/g dry extract).

Gallic acid is a phenolic compound commonly present in plant; it shows a variety of pharmacological activities such as antioxidant, anticancer, anti-inflammatory, neuroprotective and has also been used in phytomedicine [30].

On the other hand, the amount of total flavonoids found in the extracts were determined using the regression equation standard ($y=0.00117x+0.0483$)

$r^2 = 0.9906$. Results demonstrated a wide range between 150.23 ± 3.46 QE/ g dry extract to 18.47 ± 2.88 QE/ g dry extract, being the highest value observed in *H. mutabilis* and the lowest value in *E. smithniana*.

Furthermore, *H. longiflora* expressed the second highest value (148.27 ± 0.55 QE/ g dry extract). Moderate levels were found in *Lantana sp.*, *C. bonifaziarum* and *G. serrata* with the following values 94.94 ± 1.66 ; 91.41 ± 6.72 and 91.01 ± 2.41 QE/ g dry extract, respectively. Results on phenolic and flavonoids content such as DPPH inhibition percentage are shown in the Table 3, Figure 1 and 2. Results observed in present investigation regarding total phenols in ethanolic extracts are in accordance to phytochemical screening described in table 2.

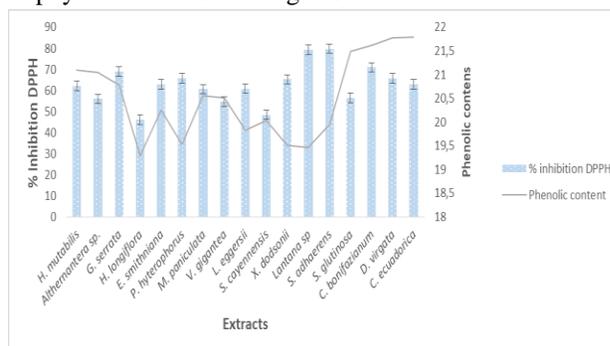


Figure 1: Phenolic content and DPPH inhibition percentage

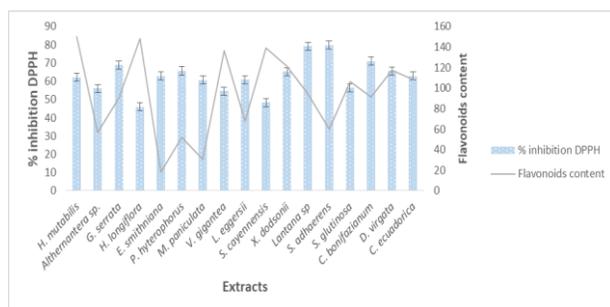


Figure 2: Flavonoids content and DPPH inhibition percentage.

Antioxidant Activity

In this study, DPPH assay was used for the *in vitro* evaluation of ethanolic extracts and demonstrated the presence of antioxidant compounds therein. Results are shown in Figures 1, 2 and Table 3. The

inhibition values of the extracts were expressed at the concentration of 4 mg/mL. Most of the extracts showed from high to weak percentage of inhibition (79.77 ± 0.055 % to 46.04 ± 0.007 %).

The highest DPPH value was observed for *Solanum adhaerens* 79.77 ± 0.005 %; followed by *Lantana sp* 79.29 ± 0.05 %. An analysis by Anova revealed that there is no significant difference ($p > 0.05$) on percentage of inhibition between the samples and ascorbic acid (86.73 ± 0.01 %). Lowest percentage of inhibition was observed in *Stachytarpheta cayennensis* and *Hippobroma longiflora*, showing a very weak scavenging capacity ≤ 50 % (48.36 ± 0.01 % and 46.04 ± 0.007 %, respectively) therefore it was not possible to calculate the IC_{50} values by linear regression (the minimum amount of plant extracts necessary to decrease the concentration of initial DPPH absorbance is 50%).

On the other side, *C. ecuadorica* (0.013 ± 0.02 mg/mL) showed the best antioxidant capacity even higher than the ascorbic acid used as control (0.039 mg/mL) followed by *V. gigantea* (0.059 ± 0.001 mg/mL); *S. glutinosa* (0.06 ± 0.003 mg/mL) and *M. paniculata* (0.08 ± 0.01 mg/mL). No significant difference was observed between the sample assay and ascorbic acid used as control ($p > 0.05$). The lowest antioxidant capacity (IC_{50} 1.90 ± 0.03 mg/mL) was observed for *C. bonifaziarum* and *Alternanthera sp.* (IC_{50} 1.44 ± 0.24 mg/mL).

Numerous investigations indicate that there are a strong relationship between the phenolic and flavonoids content and the antioxidant activity, this may be due to the redox property and metal chelation potential of these secondary metabolites [1, 2, 31,32]. In the present study, no significant correlation could be observed between the total phenolic and flavonoids content and antioxidant capacity of the plant extracts analyzed. Ethanolic extract of *C. ecuadorica* exhibited 108.66 ± 4.83 QE/ g dry extract and 21.79 ± 0.02 GAE/g dry extract while *H. mutabilis* showed 150.23 ± 3.46 QE/ g dry extract and 21.09 ± 0.11 GAE/g dry extract with a IC_{50} 0.49 ± 0.04 mg/mL. Furthermore, *S. adhaerens* showed 60.03 ± 3.88 QE/ g dry extract and 19.95 ± 0.14 GAE/g dry extract, however the IC_{50} was 0.34 ± 0.08 mg/mL.

Table 3: Total phenolic and flavonoids content, percentage inhibition measured by the DPPH method and IC₅₀ values of examined extracts plants.

Sample	^a Total phenolic	^b Total flavonoids	^c %I	^d IC ₅₀
<i>Alternanthera sp.</i>	21.05±0.1	56.50±2.21	56.00±0.02	1.44±0.24
<i>Capparidastrium bonifazianum</i>	21.62±0.06	91.41±6.72	70.89±0.01	1.90±0.03
<i>Cynophalla ecuadorica</i>	21.79±0.02	108.66±4.83	62.93±0.01	0.013±0.002
<i>Dioclea virgata</i>	21.77±0.08	117.29±4.40	65.82±0.02	0.21±0.04
<i>Erythrina smithiana</i>	20.25±0.21	18.47±2.88	62.83±0.009	0.13±0.001
<i>Gustavia serrata</i>	20.78±0.09	91.01±2.41	68.85±0.002	0.82±0.005
<i>Hippobroma longiflora</i>	19.28±0.10	148.27±0.55	46.04±0.007	*
<i>Hyptis mutabilis</i>	21.09±0.11	150.23±3.46	62.07±0.002	0.49±0.04
<i>Lantana sp.</i>	19.46±0.23	94.94±1.66	79.29±0.05	0.19±0.004
<i>Liabum eggersii</i>	19.82±0.09	67.88±3.32	60.89±0.01	0.86±0.01
<i>Murraya paniculata</i>	20.56±0.12	30.62±3.88	60.62±0.01	0.08±0.01
<i>Parthenium hysterophorus</i>	19.52±0.01	52.19±1.10	65.73±0.0004	0.14±0.01
<i>Solanum adhaerens</i>	19.95±0.14	60.03±3.88	79.77±0.005	0.34±0.08
<i>Stachytarpheta cayennensis</i>	20.04±0.03	138.86±4.43	48.36±0.01	*
<i>Swinglea glutinosa</i>	21.49±0.01	106.31±2.77	56.43±0.01	0.06±0.003
<i>Vitex gigantea</i>	20.51±0.05	136.11±1.92	54.65±0.01	0.05±0.001
<i>Xanthosoma dodsonii</i>	19.51±0.12	121.60±2.93	65.25±0.09	0.67±0.06
Ascorbic acid			86.76±0.01	0.039±0.08

Results are the mean of three data ^a As gallic acid equivalent (mg/100 g dry weight); ^b As quercetin equivalent (mg/100 g dry weight); ^c Percentage of Inhibition of DPPH (at 4 mg/mL); ^d IC₅₀ (mg/mL) using ascorbic acid as reference; *Not calculated.

Additionally, *Stachytarpheta cayennensis* and *Hippobroma longiflora* showed the lowest DPPH inhibition percentage (< 50%), even though phenolic levels were 138.86±4.43 and 148.27±0.55 QE/ g dry extract, respectively.

It is known that molecular antioxidant response of polyphenols compounds varies remarkably, depending on their chemical structure. The number of phenolic hydroxyl groups may play an important role in their scavenging activity [33]. Thus, the low scavenging capacity in both cases may be attributed to the presence of flavonoids whose chemical structure

possesses glycosylated or substituted hydroxyl groups, which might the antioxidant capacity of flavonoids. To date few reports on isolated and identification of flavonoids and phenolic compounds from these species has been found, this presumption has permitted to explain the results observed and is widely documented in the scientific literature [34].

Some highly oxygenated flavonoids have been isolated from *M. paniculata* [35], which might explain the excellent antioxidant activity observed to this species. *Vitex* genus (Lamiaceae) is frequently related to the flavonoids isolated with potent antioxidant activity and particularly in China are widely used in traditional medicine [22]. Other compounds such as lignans, diterpenoids, triterpenoids and phenolic glycosides isolated of *Vitex* genus have exhibited satisfactory antioxidant and anti-inflammatory properties [36].

On the other hand, some researchers have demonstrated a considerable antioxidant activity for *S. cayenensis* from Brazil with IC₅₀ values in the range between 38.60±5.42 to 288.44±22.12 µg/mL [29]; while *S. cayenensis*, included in this study, showed a percentage of inhibition ≤ 50%, thus, it was not possible to calculate the IC₅₀. Similarly, hydroxyl radical scavenging activity was observed between *P. hysterothorus* from India, (75-77%) [37] and *P. hysterothorus* from Ecuador, which showed percentage of inhibition of 65.73%. These marked differences in polyphenolic content values and the ability to scavenge free radicals between species of the same genus may be due to several factors such as genetic and environmental conditions in where these species grow [38].

CONCLUSION

In present study, seventeen plant extracts from Ecuador were tested with respect to their total phenolic and flavonoids content, and antioxidant capacity using DPPH method. This research showed that ethanolic extract of *C. ecuadorica* has excellent antioxidant activity followed by *V. gigantea*, *S. glutinosa* and *M. paniculata*, exhibiting a range of IC₅₀ between 0.013±0.02 mg/mL to 0.08±0.01 mg/mL. However, the highest flavonoids content was observed in *Hyptis mutabilis* and *Hippobroma longiflora*.

The antioxidant activity of the extracts may not be predicted based on its total phenolic content, therefore, it could be attributed to other class of compounds such as coumarins, anthraquinones, among others. Taking into account the results obtained in this research, these species could be promising source of natural antioxidants. However, more phytochemical analysis and *in vivo* studies are required to establish the safety of this species.

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