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Original article

Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador

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ABSTRACT

Phytochemical screening of ethanolic extracts of *Acnistus arborescens* (L.) Schltdl. (Solanaceae); *Albizia multiflora* (Kunth) Barneby & J.W. Grimes (Fabaceae); *Capparidastrum petiolare* (Kunth) Hutch. (Capparaceae); *Colicodendron scabridum* (Kunth) Seem. (Capparaceae); *Gronovia scandens* L. (Loasaceae); *Gustavia angustifolia* Benth. (Lecythidaceae); *Piscidia carthagenensis* Jacq. (Fabaceae); *Psidium rostratum* Mc Vaugh (Myrtaceae); *Psidium guayaquilense* Landrum & Cornejo (Myrtaceae); *Psidium* cf. *rostratum* Mc Vaugh (Myrtaceae); *Salicornia fruticosa* L. (Amaranthaceae); *Simira ecuadorensis* (Standl.) Steyerm. (Rubiaceae); *Ruellia floribunda* Hook. (Acanthaceae) were assayed. Alkaloids, tannins and terpenoids were the secondary metabolites most frequently found, while flavonoids, quinones, anthraquinones, steroids and saponins were present in less proportion. Folin-Ciocalteu method was used to quantify the total phenolic content in the ethanolic extracts using a calibration curve of gallic acid. The range between 941.97 ± 30.69 and 241.54 ± 15.54 GAE/mg dry extract, has been observed for *Psidium guayaquilense* and *Acnistus arborescens*, respectively. On the other hand, antibacterial activity of the ethanolic extracts was evaluated using the disk diffusion agar method against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Vibrio parahaemolyticus* ATCC 17802. *P. rostratum*, *P. guayaquilense*, *P. cf. rostratum*, *R. floribunda* and *S. ecuadorensis* were the most active extracts against the bacterial assays with minimal inhibitory concentration (MIC) values ranging between 20 and 100 ppm. According to literature, it is most likely that this is the first report on phytochemical screening, total phenolic content and antibacterial activity of ethanolic extracts of these species.
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1. Introduction

Ecuador is one of the countries with highest biodiversity in the world (Jørgensen and León-Yánez, 1999) and possess high endemism, documenting about 5400 plant species (Neill and Ulloa-Ulloa, 2011). The floristic diversity of the dry rainforest of Ecuador and Perú is very interesting placing these countries as the most important natural reserve in the world (Dinerstein et al., 1995). In Ecuador, dry forests are mostly located in the center and south, west from the Andes, in the provinces of Imbabura, Esmeraldas, Manabí, Guayas, El Oro and Loja and are very important ecosystems (Aguirre, 2012).

In Ecuador, there is a strong tradition for the use of medicinal plants especially by the indigenous people (Naranjo and Escaleras, 1995). Several ethnic groups use ancestral knowledge to cure infectious diseases caused by parasites, bacteria and viruses, skin diseases, inflammations, tumors, poisonings by snakes and scorpions, bronchial conditions, fever and pain. Regarding ethnobotanical use of these species there are numerous reports

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in the literature (Tene et al., 2007; de la Torre et al., 2008, 2012; Torri, 2013; Armijos et al., 2016; Ballesteros et al., 2016), but scientific information on the phytochemical or biological activity is insufficient or non-existent.

In this investigation, thirteen medicinal plants were collected in the Guayas province (Table 1) with the purpose of obtaining phytochemical information. Additionally, quantification of phenols and the antibacterial activity of the alcoholic extracts against *Staphylococcus aureus*, *Escherichia coli* and *Vibrio parahaemolyticus* has been reported. *Psidium guayaquilense* Landrum & Cornejo (Landrum and Cornejo, 2016), *Simira ecuadorensis* (Standl.) Steyerm. and *Ruellia floribunda* Hook., have restricted distribution only to Ecuador, while *Capparidastrum petiolare* (Kunth) Hutch., *Colicodendron scabridum* (Kunth.) Seem. and *Psidium rostratum* Mc Vaugh are located in both countries, Ecuador and Perú. On the other hand, *Gustavia angustifolia* Benth have been reported for Ecuador and Colombia, while the rest of the species under investigation have a wider distribution to South America, Central America and the Caribbean.

To the best our knowledge this is the first report on phytochemical analysis, quantification of phenols and antibacterial activity of these species.

2. Material and methods

2.1. Plant material

Fresh leaves of thirteen different plants species were collected in September 2016 from Guayas province in Ecuador. Botanical identification was carried out by MSc. Xavier Cornejo.

2.2. Extraction

Dried and ground plant materials were extracted separately with ethanol using a soxhlet. The mixture were filtered and concentrated under reduced pressure at 40 °C. The obtained extracts were stored in a refrigerator at −4 °C until the performance of analysis.

2.3. Phytochemical screening

Crude extracts were phytochemically evaluated to determine the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, tannins, anthraquinones according to standard methods (Harborne, 1973). Any change of colours or the precipitate formation was used as indicative of positive response to these tests.

2.3.1. Testing for alkaloids

Each extract (10 mg) was dissolved in 2 mL of hydrochloric acid 5%, after mixing and filtering, three aliquots were taken. Drops of Wagner, Mayer, Bouchardat and Dragendorff reagents were added to each. A red-brown precipitate (Wagner), yellowish-white precipitate (Mayer), brown precipitate (Bouchardat) and red–orange precipitate (Dragendorff) indicated the presence of such metabolites.

2.3.2. Testing for flavonoids

2.3.2.1. Shinoda test. 1 mL of absolute ethanol and 3 drops of concentrated hydrochloric acid were added to 0.5 mL of diluted extract in isopropyl alcohol. Formation of red color indicated the presence of aurones and chalcones. In cases where no colour change was observed, pieces of metallic magnesium was added. The formation of orange, red or magenta coloration indicated the presence of flavones and flavonols, respectively.

2.3.2.2. Sodium hydroxide (10%) test. 3 drops of sodium hydroxide 10% were added to 1 mL of diluted extract in isopropyl alcohol. Formation of yellow-red, coffee-orange, purple-red or blue coloration indicated the presence of xanthones and/or flavones, flavonols, chalcones and anthocyanins, respectively.

2.3.3. Testing for saponins

2.3.3.1. Foam height test. 1 mL of distilled water were added to 10 drops of the extract dissolved in isopropyl alcohol (20 mg/mL) in a test-tube, shaken vigorously to froth, and then allowed to stand for 10 min. Saponin content was measured as follows: no froth (absence); froth less than 3 mm high (poor); froth 6 mm high (moderate) and froth greater than 8 mm high (abundant).

Table 1
Species collected in the Guayas province, Ecuador.

N°	Scientific Name	Common Name	Family	Uses[a]	Voucher Specimen[b]
1	*Acnistus arborescens* (L.) Schltdl.	Cojojo	Solanaceae	Inflammation, apiarian	*Cornejo 1786*
2	*Albizia multiflora* (Kunth) Barneby & J.W. Grimes	Compoño	Fabaceae	inflammation	*Cornejo 2885*
3	*Capparidastrum petiolare* (Kunth) Hutch.	Chirimoya de montaña	Capparaceae	The fruits are used to elaborate refreshing beverages	*Cornejo 7596*
4	*Colicodendron scabridum* (Kunth) Seem.	Zapote de perro	Capparaceae	A glue is obtained from cut wood	*Cornejo & Bonifaz 5852*
5	*Gronovia scandens* L.	Comezonera, ortiga	Loasaceae	Not found	*Cornejo 5647*
6	*Gustavia angustifolia* Benth.	Membrillo	Lecythidaceae	Not found	*Cornejo 164*
7	*Piscidia carthagenensis* Jacq.	Barbasco de agua dulce, matasarna	Fabaceae	Sedative, poison fish, fungicide	*Cornejo & Bonifaz 5378*
8	*Psidium rostratum* Mc Vaugh	Guayaba	Myrtaceae	Not found	*Cornejo. M.Rondón & S. Moncayo 8820*
9	*Psidium guayaquilense* Landrum & Cornejo	Guayaba	Myrtaceae	Not found	*Cornejo & Landrum 8690*
10	*Psidium* cf. *rostratum* Mc Vaugh	Guayaba	Myrtaceae	Not found	*Cornejo,Gallardo & Maissen 8852*
11	*Salicornia fruticosa* L.	Not found	Amaranthaceae	Food Energy drink	*Cornejo & Gallardo 8845*
12	*Simira ecuadorensis* (Standl.) Steyerm.	Colorado	Rubiaceae	The wood is used to elaborate skewers, locally known as¨chuzos	*Cornejo 161*
13	*Ruellia floribunda* Hook.	Pega pollo	Acanthaceae	Pesticide	*Rubio et al. 1877*

[a] Only referring to the use by the Ecuadorian population.
[b] Voucher specimens deposited in the Herbario GUAY, Faculty of Natural Sciences, University of Guayaquil, Ecuador.

2.3.4. Testing for quinones and anthraquinones

2.3.4.1. Borntrager's test. 3 mL of each extracts were treated with 3 mL of chloroform and the chloroform layer were separated. To this 5% potassium hydroxide dissolution were added. Occurrence of red color in alkaline phase indicated the presence of quinones. Those samples showing yellow color with Green fluorescence where treated with one drop of 6% hydrogen peroxide, formation of red color was considered positive for anthrones derivatives.

2.3.4.2. Ammonium hydroxide test. One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in isopropyl alcohol. After two minutes, formation of red color indicated the presence of anthraquinone.

2.3.4.3. Sulfuric acid test. One drop of concentrated sulfuric acid was added to 10 mg of each extract dissolved in isopropyl alcohol. Formation of red color indicated the presence of quinones.

2.3.5. Testing for steroids and / or triterpenoids

2.3.5.1. Salkowski test. 2 mL of chloroform and 1 mL concentrated sulfuric acid were added to 10 drops of the extract dissolved in isopropyl alcohol, slowly until double phase formation. The presence of a dish-brown color in the middle layer was indicative of steroidal ring.

2.3.5.2. Lieberman Bouchard test. 1 mL of anhydrous acetic acid and 3 drops of concentrated sulfuric acid were added to 2 mL of the extract dissolved in isopropyl alcohol. After 5 min a blue-green color middle layer was indicative of sterols, but pink, red, magenta or violet color revealed the presence of terpenoids.

2.3.6. Testing for tannins

10 mg of each extract were dissolved in 1 mL of ethanol, then 2 mL of distilled water was added followed by 4 drops of ferric chloride aqueous solution 10% w/v. Formation of a blue or green color indicated the presence of phenols.

2.4. Determination of total phenolic content

Total phenolic contents of each extract were determined using a Folin-Ciocalteu colorimetric method (Singleton et al., 1999). 1 mL properly diluted of each extract solution were 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 calibration curve was prepared, using a standard solution of gallic acid (5, 10, 20, 25, 30, 40 and 50 μg). Results are expressed as mg of gallic acid equivalents (GAE)/100 g dry weight (dw) extract. Data is reported by means of at least two replications.

2.5. Determination of antibacterial activity

The antimicrobial activity was carried out according to the disc diffusion assay against one *Gram* positive (*Staphylococcus aureus* ATCC 25923) and two *Gram* negative (*Escherichia coli* ATCC 25922, *Vibrio parahaemolyticus* ATCC 17802) bacteria. The strains were mantained in agar at room temperature. Each bacterial inoculum (2.5 mL) was incubated in Müeller-Hinton broth at 37 °C for 18 h. The bacterial inoculum was diluted in sterile 0.85% saline to obtain turbidity visually comparable to a McFarland N° 0.5 standard (106–8 CFU/mL). All extracts were dissolved in ethanol at 200 ppm. As a positive control, antibiotic disks were used. The ethanol was used as negative control. 20 μL of each solution were placed onto individual sterile 6.35 mm filter disk and allowed to dry at room temperature. Each disk was placed on the surface of Muller-Hinton agar previously inoculated.

The minimal inhibitory concentration (MIC) were determined only with microorganisms that displayed inhibitory zones. MIC were determined by the standard Clinical and Laboratory Standards Institute dilution method (CLSI, 2016) of the extract with ethanol at 150, 100, 50, 40, 30, 20 ppm pipetting 20 μL of each dilution onto a filter paper disc. MIC was defined as the lowest concentration that inhibited visible bacterial growth. The experiments were repeated at least twice.

3. Results

3.1. Phytochemical screening

Phytochemical screening of ethanolic extracts of thirteen medicinal plants collected from Guayas province was carried out using various chemical assays in order to identify either the presence or absence of secondary metabolites such as alkaloids, coumarins, phenolic compounds, flavonoids, glycosides, quinones, saponins, tannins, steroids and triterpenoids. Table 2 summarizes secondary metabolites present in all extracts assayed. All the extracts resulted positive to Dragendorff test observing an orange precipitate. *Colicodendron scabridum*, *Gronovia scandens*, *Gustavia angustifolia* and *Ruellia floribunda* revealed presence of alkaloids in all tests used. Phenolic compounds were also quite abundant, possibly in the form of tannins which was evidenced when treated

Table 2
Phytochemical screening of alcoholic extracts achieved from thirteen species collected from Guayas provinces, Ecuador.

Sample	WR	MR	DR	BR	SR	NaOH 10%	$FeCl_3$ 5%	Foam-1	L-BR	SR*	BR*	NH_4OH 10%	H_2SO_4
Acnistus arborescens	+	–	+++	++	–	–	++	–	–	++	–	–	+
Albizia multiflora	–	–	+++	+	+	–	++	–	++	++	–	–	–
Capparidastrum petiolare	–	–	++	++	–	+	++	–	+	+	–	–	–
Colicodendron scabridum	+++	+++	+++	++	–	–	–	–	–	–	+	–	–
Gronovia scandens	+	+	+++	++	–	–	++	–	+	+++	–	–	–
Gustavia angustifolia	++	++	+++	++	+	++	++	–	–	–	–	–	+
Piscidia carthagenensis	–	–	+++	–	–	++	+++	+++	++	+	–	–	–
Psidium guayaquilense	–	–	+++	+	–	+++	+++	–	–	+++	+++	+++	+++
Psidium rostratum	–	–	+++	++	+++	++	+++	+++	++	–	–	++	+++
Psidium cf. rostratum	–	–	+++	+	–	+++	+++	–	–	+++	+	+	–
Ruellia floribunda	+	+	++	–	++	+++	++	+++	+++	+++	+	+++	+++
Salicornia fruticosa	–	–	+++	+	–	+++	+++	+	++	–	+++	+++	+++
Simira ecuadorensis	–	–	+++	–	–	++	++	++	+	+	–	++	++

Key: (–) Absence, (+) Poor, (++) Moderate, (+++) Abundant. WR: Wagner reactive; MR: Mayer reactive; DR: Dragendorff reactive; BR: Bouchardat reactive; SR: Shinoda reactive; SR*: Salkowski reactive; Foam-1: Foam (out sodium bicarbonate); L-BR: Liebermann-Burchard reactive; BR*: Borntrager reactive.

Table 3
Antibacterial activity of ethanolic extracts of species from Guayas provinces, Ecuador.

Microorganism	Inhibition Zone (mm)					Inhibition Zone (mm)		
	P. rostratum	*P. guayaquilense*	*P. cf rostratum*	*R. floribunda*	*S. ecuadorensis*	Reference antibiotics		
						OX	TMS	TCT
Staphylococcus aureus ATCC 25923	16	14	12	15	NI	23		
Escherichia coli ATCC 25922	17	18	NI	18	NI		25	
Vibrio parahaemolyticus ATCC 17802	18	17	14	17	12			26

*Inhibition Zone, diameter measured in mm, disc diameter 6 mm, average of two consecutive assays.
The disks were impregnated using a concentration of 200 ppm of each extract.
OX: Oxacilin® Oxoid™ (1 μg); TMS: Trimethoprim/Sulphametoxazole® 1:19 Oxoid™ (25μg); TCN: Tetracycline® Oxoid™ (30 μg) NI: No inhibition.

Table 4
Minimum inhibitory concentration of ethanolic extract of species from Guayas.

Microorganism	*P. rostratum*	*P. guayaquilense*	*P. cf rostratum*	*R. floribunda*	*S. ecuadorensis*
	Minimal inhibition concentration (μg/mL)				
Staphylococcus aureus ATCC 25923	50	50	50	20	–
Escherichia coli ATCC 25922	30	100	–	40	–
Vibrio parahaemolyticus ATCC 17802	50	50	50	50	40

MIC: Minimal inhibition concentration, concentration range 20–150 ppm.

the sample with a solution of ferric chloride and the formation of a dark precipitate was observed. Triterpenes and steroids were relatively frequent in the ethanolic extracts while saponins, quinones and anthraquinones were the least abundant.

3.2. Antibacterial activity

Antibacterial activity of thirteen species were screened against *Staphylococcus aureus*, *Escherichia coli* and *Vibrio parahaemolyticus*, and results obtained are summarized in Table 3. Only five extracts showed a significantly growth inhibition of bacterial strains producing an inhibition zone ranging from 12 to 18 mm, showing MIC values from 20 to 100 ppm (Table 4). The best results observed were to ethanolic extract of *R. floribunda* is active against *S. aureus*; *P. rostratum* against *E. coli* and *S. ecuadorensis* against *V. parahaemolyticus* with MIC values of 20, 30, 40 ppm, respectively. Ethanol extracts of the other analyzed species were inactive against all bacteria strains and concentrations assayed.

3.3. Total phenolic content

The phenolic content found in the extracts were determined using the linear regression equation using gallic acid as standard ($y = 0.0663x-0.6409$) $r^2 = 0.9924$, showing at range of 941.97 ± 30.69 and 241.54 ± 15.54 mg GAE/g dry extracts. The results are show in Table 5. All extracts used in the quantification were those that showed presence of tannins using $FeCl_3$ according to the test performed in the phytochemical screening (Table 2).

Table 5
Total Phenolic Content.

Sample	TP mg GAE/100 g dry weight*
Acnistus arborescens	241.54 ± 15.54
Albizia multiflora	375.53 ± 19.37
Gronovia scandens	358.51 ± 18.93
Gustavia angustifolia	309.44 ± 17.59
Piscidia carthagenensis	930.93 ± 30.51
Psidium guayaquilense	941.97 ± 30.69
Psidium rostratum	591.34 ± 24.31
Ruellia floribunda	379.11 ± 19.47
Salicornia fruticosa	696.30 ± 26.3.8
Simira ecuadorensis	346.28 ± 18.60

Values are means (n = 2) ± SD. The results were found to be statistically significant $P < 0.05$.

4. Discusión

4.1. Phytochemical screening

Alkaloids, tannins, steroids, triterpenes, saponins and anthraquinones are known due to the important biological activity attributed to this class of compounds. Most of the extracts analyzed revealed the presence of these in amounts ranging from abundant to poor or absence.

Aerial part of *Acnistus arborescens* (Solanaceae) showed major content of alkaloids and tannins, while steroles were present in less proportion. Some species of Solanaceae, are knows due to the biosynthesis of different kinds of alkaloids which present very important pharmacologic properties (Jerzykiewicz, 2007). However, alkaloids isolated from *A. arborescens* has not been reported. Whitanolides with cytotoxic activity on different tumor cell lines are the compounds frequently reported in these species (Cordero et al., 2009; Batista et al., 2016). Ethanolic extract of the leaves of *Capparidastrum petiolare* and fruits of *Colicodendrum scabridum*, two species belonging to Capparaceae family, showed moderate to abundant presence of alkaloids. To date there are no scientific reports on the chemical composition or biological activity of any species belonging to these genera although some novel alkaloids have been isolated from fruits and aerial parts of some Cappaceae (Yang et al., 2010; Foster et al., 2016).

The extract of the aerial parts of *Gronovia scandens*, was rich in alkaloids and terpenoids. Very few phytochemical analyzes on this genus have been performed probably due to geographic restriction of it.

Previous report on *G. scandens* and other species of the Loasaceae family evidence the accumulating bioactive iridoids and secoiridoids (Rodríguez et al., 2002; De Luca et al., 2014).

Gustavia genus comprise 45 species native to tropical areas, especially from South America. Some triterpenes, sterols, betulinic acid, xanthones and Gustastatin, a new compound with anticancer activity have been isolated from *Gustavia* species (Pettit et al., 2004; Rodríguez et al., 2008). These *Gustavia* species are used in the traditional medicine against leishmaniasis and anthelmintic activities for which they are of biological interest. In Ecuador have

been reported 13 species of this genus, 9 of them are mainly distributed in the coast region. In a previous study we reported presence of tannins, saponins, triterpenes, sterols and flavonoids of the aerial part of *Gustavia pubenscens*, an endemic specie from Ecuatorian rainforest (Rondón et al., 2015) while that *G. angustifolia* collected in the dry forest exhibited abundant presence of alkaloid.

Constituents from aerial parts of *Piscidia carthagenensis* (Fabaceae), were mainly alkaloids, saponins, tannins, flavonoids and terpenoids. The presence of abundant amounts of saponins in the ethanol extract of *P. carthagenensis* can give an explanation for the uses of this species as poison for the fishes.

Psidium rostratun, *P. guayaquilense* and *P. cf. rostratun* (Myrtaceae) showed abundant presence of tannins using ferric chloride test. This result is in accordance to the observed for other species of *Psidium* genus. Particularly, *Psidium guajava*, which has an excellent reputation as remedy against diarrhea and dysentery due to the high content of tannins (Gutiérrez et al., 2008). Additionally, the *Psidium* species used in this investigation have a high content of alkaloids, steroids and quinone compounds, which are speculated to account for the pharmacological effects observed in previous studies in *Psidium* species (Alvarenga et al., 2013; Feng et al. 2015; Morais-Braga et al., 2016).

Salicornia fruticosa, reveled alkaloids, flavonoids, tannins, anthraquinones, quinones and less proportion of triterpenes and saponins. *S. fruticosa* grows near to the salt marshes from Guayas province, and ethanol extract showed a high salt content due to its condition as halophyte plant.

Very few investigations on *Simira* genus has been reported. Interesting alkaloids and diterpenes are present in *Simira maxonni* and *S. eliezeriana*, respectively (Hasbun et al., 1989; Araújo et al., 2011). To the best of our knowledge this is the first report on the chemical composition of the alcoholic extract of *S. ecuadorensis* which showed abundant presence of alkaloids which is in accordance to the Rubiaceae species while tannins, flavonoids, quinones, anthraquinones, terpenes and saponins were observed in minor proportion.

4.2. Antibacterial activity

In present study thirteen ethanolic extracts from species collected in various locations in Ecuador were evaluated to determine the antibacterial activity, some of these are commonly consumed by the Ecuatorian population. Table 3 and 4 show that, *Psidium rostratum*, *P. guayaquilense*, *P. cf rostratum*, *Ruellia floribunda* and *Simira ecuadorensis* exhibited antibacterial activity that might be attributed to the presence of phenolic compounds (phenolic acid, flavonoids, tannins, coumarins, quinones) and anthraquinones present in the ethanolic extracts.

However, this activity showed by phenolic compounds present in *Psidium* species have been well documented in many scientific papers already published. Tannins obtained from acetonic extract of *Psidium guineense* reveled growth inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC values of 4.01 and 8.20 mg/mL, respectively (Rodríguez et al., 2014). Ethanolic extracts of *P. rostratum*, *P. guayaquilense* and *P. cf. rostratum* showed 50 ppm against *S. aureus* used in this investigation.

Additionally, phenolic compound are recognized for their potentials chemopreventive properties especially by their antioxidant activity (Huang et al., 2010). These characteristics should be considered for future research on these three *Psidium* species.

Ruellia floribunda was active against all bacterial used, while *S. ecuadorensis* only showed inhibition against *V. parahaemolyticus* at moderate concentrations. The microorganism *E. coli* is known to be multi-resistant to several drugs, however it was inhibited by the ethanolic extracts of *R. floribunda*, *Psidium rostratum* and *P. guayaquilense*.

According to the references consulted there are no reports on the antibacterial activity of *P. rostratum, P. guayaquilense, P. cf. rostratum, R. floribunda* and *S. ecuadorensis*, thus, authors recommend to broaden the spectrum of bacteria for future research.

Surprisingly, *P. carthagenensis* showed no activity against the bacteria tested despite having a high content of phenols in the extract (Table 5). An antagonist effect of the other constituents of *P. carthagenensis* extract cannot be ruled out.

4.3. Total phenolic content

Numerous investigations have demonstrated the benefits of phenolic compounds for human health. In this regard, antioxidant, anticancer, anti-inflammatory effect and the way these compounds exert their therapeutic action have also been analyzed (Nicholson, 1992; Rice-Evans et al., 1997). Fruits, seeds and some edible vegetables are rich in these class of compounds. Nevertheless; phenolic compounds with pharmacological properties have been isolated from leaves of various plant species. In order to provide more phytochemical information on these species used in Ecuador, quantification of total contents of phenolic compounds was carried out in those extracts that revealed the presence of this type of compounds using the $FeCl_3$ test in the phytochemical screening.

Piscidia carthagenensis showed a high phenolic content at 930.93 ± 30.51 mg GAE/g dry extract. To date, only one previous report on *P. cartaghenensis* collected from Amazonian Ecuatorian was found in the literature. In this investigation, authors analyzed antioxidant activity, antibacterial activity, xanthine oxidase inhibition and toxicity of some species including *P. carthagenensis*. However, no antioxidant activity was observed in the extract of this specie and no quantification of total phenolic content was reported (Guerrero et al., 2003).

Psidium guayaquilense and *P. rostratum* leaf extracts showed 941.97 ± 30.69 mg GAE/ g dry extract and 591.34 ± 24.31 mg GAE/g dry extract, respectively. Some results have been reported for other *Psidium* species. For example leaves of *Psidium cauotteanum*, *P. australe* and *P. cinereum* from Brazil, showed a phenolic content between 24.72 ± 3.28 to 598.25 ± 4 mg GAE/g dry extract (Takao et al., 2015); while leaves of *P. guajava* from China showed 458 ± 8.1 GAE mg/g (Chen et al., 2007). *P. guayaquilense* showed the highest phenolic content of all species analyzed. These results revealed important information about the phenolic content present in endemic *Psidium* species of Ecuador and may be considered as potential natural remedies that support the traditional use of these species in diarrhea. Additionally, previous study revealed that phenolic compounds present in the guajava leaves play role in the antioxidant activity through free radical-scavenging activity (Tachakittirungrod et al., 2007) Future studies should be oriented towards the analysis of the potential therapeutic of this species as antidiarrheal and antioxidant.

On the other hand, some *Ruellia* species present important content of phenolic compounds responsible of antioxidant activity (Wangia et al., 2016). *R. floribunda* showed a moderated presence of tannins 379.11 ± 19.47 GAE mg/g dry extract.

Albizia genus is recognized for the presence of phenolic compounds. *Albizia multifora* showed 375.53 ± 19.37 mg GAE/g extract dry. Recently, several tannins were isolated of *A. anthelmintic* which demonstrated cytotoxic activity against human ovarian carcinoma cell line (Hassanien et al., 2017).

Salicornia species are known by the phenolic content. For example, the phytochemical analysis of *S. brachiata* from India contains tannins and flavonoids in the alcoholic extract and antibacterial activity attributed to this class of compounds were also described. (Santhanakrishnan and Chandrasekaran, 2014). Particularly important was the report on study of the phenolic compounds of *S. fruticosa* from Egypt tested as natural antioxidants on preservation

of corn oil. The phenolic compounds of *S. fruticosa* determined by HPLC were identified as pyrogallol, cathequin, ellagic and B-OH benzoic (Elsebaie et al., 2014). Additionally, *S. fructicosa* from Ecuador have uses as legume for its nutritional content rich in sodium, potassium, magnesium and proteins.

5. Conclusions

The use of medicinal plants is a traditional practice in Ecuador thus, it is very important to evaluate the therapeutic use of plants through scientific methods and provide informations about the species that could be used in the future for their properties. In the present study, the majority of biological active constituents were present in ethanolic extracts of thirteen native species from Ecuador. However, alkaloids and tannins were the most abundant metabolites. The higher total phenolic contents were observed in *Psidium guayaquilense* and *Piscidia carthagenensis* with 941.97 ± 30.69 and 930.93 ± 30.51 mg GAE/ 100 g dry extract, respectively. According to the obtained results, these species might be considered a promising source of natural antibacterial drugs, specially *Psidium rostratum*, *P. guayaquilense*, *P. cf rostratum*, *Ruellia floribunda* and *Simira ecuadorensis* that showed the best result against *S. aureus*, *E. coli* and *V. parahaemolyticus* bacteria strains, which cause serious human infection diseases.

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