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Original Research Article

## Preliminary phytochemical screening for antioxidant activity and content of phenols and flavonoids of 18 species of plants native to western Ecuador

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### ABSTRACT

The phytochemical screening, total phenolics and flavonoids content and antioxidant activity of plants native to western Ecuador were investigated to provide the basic information for further studies towards the discovery of new compounds. The species studied were *Adenostemma platyphyllum* Cass., *Castilla elastica* subsp. *gummifera* (Miq.) C.C. Berg., *Cochlospermum vitifolium* (Willd.) Spreng., *Ectozoma pavonii* Miers, *Erythrochiton giganteus* Kaastra & A.H. Gentry, *Erythroxylum patens* Ruiz ex O.E.Schulz, *Ficus brevibracteata* W.C. Burger, *Ficus tonduzii* Standl., *Grias ecuadorica* Cornejo & S.A. Mori, *Handroanthus billbergii* subsp. *ampla* (Bureau K. Schum.) S.O. Grose, *Morisonia americana* L., *Operculina codonantha* (Benth.) Hallier f., *Passiflora macrophylla* Spruce ex Mast., *Podandrogynne jamesonii* (Briq.) Cochrane, *Pradosia montana* T.D. Penn., *Tecoma castaneifolia* (D.Don) Melch, *Urera baccifera* (L.) Gaudich. ex Wedd., and *Xanthosoma sagittifolium* (L.) Schott. The highest percentage of inhibition of DPPH• was found in *E. patens* (92.41%). Most of the extracts evaluated exhibited antioxidant activity.

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### 1. Introduction

In plants, many bioactive compounds derived from secondary metabolism, function to protect from pathogens, insects, and other types of environmental stress. Alkaloids, flavonoids, tannins, saponins, terpenes, sterols, and quinones are among these compounds (Tripathi and Mishra, 2015). These phytochemicals have various pharmacological activities, such as antimicrobial, anti-inflammatory, antimutagenic, healing and analgesic, which is why they form an important part of the formulation of pharmaceutical products and are used as raw materials for the development of new drugs (Eliaser et al., 2018; Shaikh and Patil, 2020). One of the most widely used biological properties of plant compounds is antioxidant activity. Antioxidants are defined as molecules capable of inhibiting oxidative stress at the cellular level (Rivas et al., 2015). In plants, flavonoids and tannins are capable

of neutralizing dangerous free radicals and, with an antioxidant power greater than some compounds such as vitamins A, E and  $\beta$ -carotene (Abdel-Lateif et al., 2016). These phytochemicals promote human health and are recognized as promising remedies against cardiovascular disease, cancer, Alzheimer's disease, Parkinson's disease, and atherosclerosis (Rondón et al., 2018a). The identification of the chemical compounds present in plants is important for the prediction of their possible pharmacological potential and in the discovery of new substances with pharmaceutical or cosmetic applications. The growing interest in therapeutic effects of antioxidants and the reported side effects of synthetic antioxidants, have motivated the search for natural sources of molecules with high activity and less toxic effects (Abdel-Lateif et al., 2016). Ecuador is one of the 17 countries in the world with very high biodiversity, harboring around 17,000 species of vascular plants, of which, a third are variously used by the

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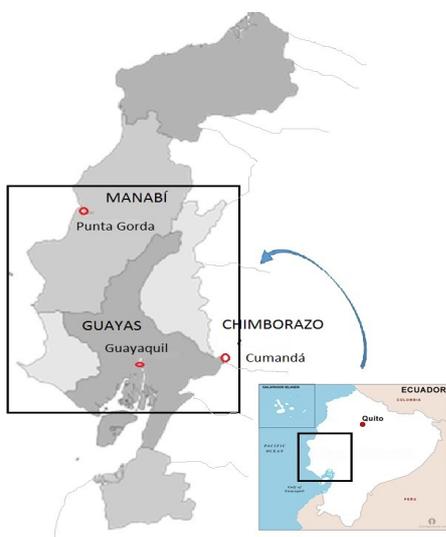
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population, mainly for medicinal applications. However, there have been no studies of chemical composition or possible biological activities for most of these species (De la Torre et al., 2012; Rondón et al., 2018b). The plant species studied in this investigation are *Adenostemma platyphyllum* Cass., *Castilla elastica* subsp. *gummifera* (Miq.) C.C. Berg., *Cochlospermum vitifolium* (Willd.) Spreng., *Ectozoma pavonii* Miers, *Erythrochiton giganteus* Kaastra & A.H. Gentry, *Erythroxylum patens* Ruiz ex O.E.Schulz, *Ficus brevibracteata* W.C. Burger, *Ficus tonduzii* Standl., *Grias ecuadorica* Cornejo & S.A. Mori, *Handroanthus billbergii* subsp. *ampla* (Bureau & K. Schum.) S.O. Grose, *Morisonia americana* L., *Operculina codonantha* (Benth.) Hallier f., *Passiflora macrophylla* Spruce ex Mast., *Podandrogynae jamesonii* (Briq.) Cochrane, *Pradosia montana* T.D. Penn., *Tecoma castaneifolia* (D.Don) Melch, *Urera baccifera* (L.) Gaudich. ex Wedd. and *Xanthosoma sagittifolium* (L.) Schott. These species are used in the traditional Ecuadorian medicine, mainly by the country's indigenous nationalities (Table 1) (De la Torre, 2008). *P. montana*, *E. pavonii*, *E. giganteus*, *G. ecuadorica* and *P. jamesonii* are endemic plants of western Ecuador, and according to the literature, no reports of phytochemical studies for these herbal species appear to exist. This research presents a phytochemical screening, estimation of phenol and flavonoid content and antioxidant activity of 18 species collected in western Ecuador. The aim of this investigation is also to provide basic information for further studies towards the discovery of new compounds with pharmacological and antioxidant applications as support for ethnomedicinal applications in Ecuador.

## 2. Experimental

### 2.1. Study area

The study area includes four localities in western Ecuador (Fig. 1). No phytochemical studies have



**Fig. 1.** Map of western Ecuador, the red dots indicate the collection sites.

been conducted for species within these areas. The Reserva Natural Punta Gorda in the province of Manabí (0°38'25" S, 80°28'7" W, altitude 4 m), that harbors secondary dry thorn scrub/very dry deciduous coastal forest ecosystems. The Bosque Protector Cerro Colorado (2°4'27" S, 79°53'56" W, altitude 104 m) and the campus Mapasingue of Faculty of Natural Sciences, University of Guayaquil (2°8'49" S, 79°54'59" W, altitude 5 m), are among the few remnants of Pacific deciduous dry forests within the city of Guayaquil. This ecosystem is so characterized because more than 75% of plant species lose their leaves during the dry season (May to December) (Aguirre, 2006). The Cumanda canton, located in the western foothills of the Andes, in the province of Chimborazo (2°12'S, 79°07'W, altitude 460 m), harbors secondary wet forest remnants and ravines. These fragments are limited by grasslands.

### 2.2. Plant material

The plant species sampled in the present study are listed in Table 1. The specimens were deposited at the Herbarium of the Faculty of Natural Sciences of the University of Guayaquil (GUAY), Guayaquil, Ecuador. The taxonomical identifications were carried out by Xavier Cornejo, curator of GUAY Herbarium by using the keys to the species provided in Flora of Ecuador (Harling and Andersson, 1986-1998). Taxonomist from other herbaria as Andrés Orejuela (Solanaceae), Ted Cochrane (Cleomaceae), John Wood (Convolvulaceae), and Yero Kuethe (Passifloraceae), were also consulted.

### 2.3. Preparation of extract

Plant material was dried at 40 °C for 3 days and then pulverized. The dried and finely ground aerial plant parts (100 g) were vigorously stirred and separately extracted with 500 mL of ethanol 98% (v/v) in a hotplate magnetic stirrer (Thermo Scientific™ Cimarec+™) at room temperature for 48 h. The mixture (250 mL) was filtered and concentrated under reduced pressure using a rotary evaporator (Buchi R-3, Switzerland, 60 °C, 250 RPM). The obtained extracts were stored at refrigerator at -4 °C pending analysis.

### 2.4. Phytochemical screening

Crude extracts were phytochemically evaluated to determine the presence of alkaloids (ALK), flavonoids (FLAV), saponins (SAP), anthraquinones (ANTRAQ), steroids (STER), terpenoids (TERP), and tannins (TAN), according to standard methods (Harborne, 1998; Rondón et al., 2018b). Any change in color or the formation of a precipitate was taken to indicate a positive response.

### 2.5. Determination of total phenolic content

Total phenolic content of each extract was determined using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). 0.5 mL of properly diluted extract solution (concentration 5 mg/mL) was mixed with 0.25 mL of

**Table 1**  
List of collected plants and the respective localities from western Ecuador.

Species	Family	Vernacular name	Ethnobotanical use in Ecuador <sup>a</sup>	Locality of collection	Voucher specimen
<i>Adenostemma platyphyllum</i> Cass.	Asteraceae	Mama Juana	Antitussive, analgesic, snake bites and scorpion stings	Cumandá	X. Cornejo 4561 (GUAY)
<i>Castilla elastica</i> subsp. <i>gummifera</i> (Miq.) C.C. Berg.	Moraceae	Árbol de caucho	Timber, rubber, food for animals (fruit)	Cumandá	X. Cornejo 7114 (GUAY)
<i>Cochlospermum vitifolium</i> (Willd.) Spreng.	Bixaceae	Bototillo	Timber	Guayaquil, Mapasingue	X. Cornejo 2399 (GUAY)
<i>Ectozoma pavonii</i> Miers	Solanaceae	No data	No data	Cumandá	X. Cornejo 9168 (GUAY, NY)
<i>Erythrochiton giganteus</i> Kaastra & A.H. Gentry	Rutaceae	No data	Snakebites, childbirth <sup>b</sup>	Cumandá	X. Cornejo s.n. (GUAY)
<i>Erythroxylum patens</i> Ruiz ex O.E.Schulz	Erythroxylaceae	Mama cuca	Timber	Cumandá	C. Bonifaz 4145 (GUAY)
<i>Ficus brevibracteata</i> W.C. Burger	Moraceae	Higuerón	The bark is used to elaborate bags	Cumandá	X. Cornejo 9170 (GUAY)
<i>Ficus tonduzii</i> Standl.	Moraceae	Higuerón	Dewormer (latex), flu	Cumandá	C. Bonifaz 595 (GUAY)
<i>Grias ecuadorica</i> Cornejo & S.A. Mori	Lecythidaceae	Membrillo	No data	Cumandá	X. Cornejo 9124 (GUAY)
<i>Handroanthus billbergii</i> subsp. <i>ampla</i> (Bureau & K. Schum.) S.O. Grose	Bignoniaceae	Guayacán	Timber	Guayaquil, Mapasingue	X. Cornejo 5161 (GUAY)
<i>Morisonia americana</i> L.	Capparaceae	Zapote de perro	The fruit is used to feed animals; timber	Reserva La Gorda	X. Cornejo 9349 (GUAY)
<i>Operculina codonantha</i> (Benth.) Hallier f.	Convolvulaceae	Michoacán	Edible (tuber)	Guayaquil, Mapasingue	X. Cornejo 2386 (GUAY)
<i>Passiflora macrophylla</i> Spruce ex Mast.	Passifloraceae	Cacao de monte	Headaches (leaves)	Cumandá	X. Cornejo 1794 (GUAY)
<i>Podandrogynne jamesonii</i> (Briq.) Cochrane	Cleomaceae	No data	No data	Cumandá	X. Cornejo 9169 (GUAY, NY)
<i>Pradosia montana</i> T.D. Penn.	Sapotaceae	Caimitillo	Edible (fruit)	Guayaquil, Cerro colorado	X. Cornejo 2394 (GUAY)
<i>Tecoma castaneifolia</i> (D.Don) Melch	Bignoniaceae	Moyuyo de montaña	Timber	Guayaquil, Mapasingue	X. Cornejo 2401 (GUAY)
<i>Urera baccifera</i> (L.) Gaudich. ex Wedd.	Urticaceae	Ortiga	Inflammatory diseases, rheumatoid arthritis, snake bite (leaves), anti-hemorrhagic, anti-diarrhoea (root)	Cumandá	C. Bonifaz 595 (GUAY)
<i>Xanthosoma sagittifolium</i> (L.) Schott	Araceae	Camacho	Edible (leaves)	Cumandá	X. Cornejo s.n. (GUAY)

<sup>a</sup>(De la Torre, 2008); <sup>b</sup>(Doyle et al., 2012)

Folin-Ciocalteu reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 10 min at room temperature, 1.25 mL of sodium carbonate solution (0.075% w/v) was added. The solutions were mixed and allowed to stand for 30 min at room temperature. The absorbance was measured with a Microplate Reader (Multiskan Go Thermo Scientific, Japan) at 760 nm. A standard calibration curve was prepared, using a gallic acid solution (30, 50, 80, 100, 120, 150, 180 and 210 µg/mL). Results were expressed as milligrams of gallic acid equivalents per gram dry extract (mg GAE/g dry extract). Folin-Ciocalteu reagent, sodium carbonate and gallic acid were purchased from Sigma-Aldrich.

## 2.6. Determination of total flavonoid content

Total flavonoid content was estimated using the aluminium chloride method (Zhishen et al., 1999). 0.25 mL of properly diluted extract (concentration 1 mg/mL) was mixed with 1 mL of distilled water and 0.075 mL NaNO<sub>2</sub> (5% w/v). Additionally, 0.075 mL AlCl<sub>3</sub> (10% w/v) was added 5 min later and left to react for another 6 min, after which 0.5 mL of NaOH solution (1 M) was added. The mixture was made up to 2.5 mL with distilled water. The solution was mixed carefully, and the absorbance measured using a Microplate Reader (Multiskan Go Thermo Scientific, Japan) at 510 nm. A calibration curve was plotted using quercetin as standard flavonoid reference (20, 40, 60, 80, 100, 200, 300, 400 and 500 µg/mL). Results were expressed as milligrams of quercetin equivalents per gram dry extract (mg QE/g dry extract). Sodium nitrite, aluminium chloride, sodium hydroxide and quercetin were purchased from Sigma-Aldrich.

## 2.7. Determination of antioxidant activity

The DPPH• radical scavenging capacity of each plant extract was determined according to the method of Brand-Williams et al. (1995) with modifications. A solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH•; 6 x 10<sup>-2</sup> mM) in methanol was prepared, and 2.8 mL of this solution was mixed with 0.2 mL of each extract previously dissolved in methanol at concentrations of 0.2, 0.5, 0.75, 1.25, 2.5 and 5.0 mg/mL. The mixture was kept in the dark at room temperature for 30 min. The absorbance was measured using a Microplate Reader (Multiskan Go Thermo Scientific, Japan) at 517 nm. A solution of 2.8 mL of DPPH• and 200 µL of methanol was used as negative control while ascorbic acid at the concentration of 176 µg/mL was used as standard antioxidant reference. The 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), methanol and ascorbic acid were purchased from Sigma-Aldrich. Results were expressed as inhibition percentage (%) and calculated as described by Goupy et al. (1999) and Murillo et al. (2007) using the following equation (Eqn. 1):

$$\%I = [\text{Abs DPPH}\bullet - \text{Abs sample} / \text{Abs DPPH}\bullet] \times 100 \quad (\text{Eqn. 1})$$

The concentration required to obtain 50% of the maximum capacity of free radicals scavenging (IC<sub>50</sub>) was calculated by linear regression in the species that obtained a %I ≥ 50.

## 2.8. Statistical analysis

All experiments were carried out in triplicate and results expressed as mean ± standard deviation. Using the statistical program STATGRAPHICS Centurion, version 17, the correlation between phenols, flavonoids and the antioxidant activity in extracts were analyzed and the significant differences between groups determined by one-way analysis of variance (ANOVA) using the Tukey test, with a confidence level of 95%. Values ≤ 0.05 were submitted statistically different.

## 3. Results and Discussion

### 3.1. Phytochemical screening

The qualitative analysis indicating the presence or absence of alkaloids, flavonoids, saponins, anthraquinones, steroids, triterpenes and tannins of the selected plant species collected in western Ecuador is presented in Table 2. All phytochemical groups evaluated in the screening were present in *M. americana*, *H. billbergii* subsp. *ampla*, *T. castaneifolia* and *O. codonantha*. The chemical compounds with highest prevalence among the plant species examined were flavonoids and terpenes. Alkaloids were moderate to abundant in 11 of the evaluated species. Anthraquinones and tannins were also present in species studies. Anthraquinones were detected in all species except *A. platyphyllum*, and *U. baccifera*, while only *P. montana* was found to have no tannins. Saponins were the chemical compounds with lowest prevalence within the evaluated plants, with *C. vitifolium*, *G. ecuadorica*, and *O. codonantha* being species that show moderate presence of this compound. Secondary metabolites are compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense against herbivores and pathogens. Secondary metabolites are produced in response to both biotic and abiotic stress conditions (Ramakrishna and Ravishankar, 2011). Secondary metabolites have also been defined as compounds whose biosynthesis is restricted to certain groups of plants. They possess specific enzymes capable of synthesizing distinctive metabolites only under suitable conditions (Pichersky and Gang, 2000). Thus, these compounds often differ among individuals of the same plant population in terms of their quantity and types. The presence of specific constituents in a certain species or family has been used to help with systematic determination and with groups of secondary metabolites being used as markers for botanical classification (chemotaxonomy) (Pagare et al., 2015). In the present work, these differences in metabolite production can be observed (Table 2), where species such as *H. billbergii* subsp. *ampla*, *M. americana*, *O. codonantha* and *T. castaneifolia* present all the metabolites evaluated, while *A. platyphyllum* and *U. baccifera*, present a few. The majority presence of flavonoids, terpenes, anthraquinones, and tannins in all species evaluated may be due to the fact that these compounds belong to

**Table 2**

Phytochemical screening of ethanolic extract of 18 species of plants from western Ecuador.

Species	ALK			FLAV		SAP	ANTRAQ		STER/TERP		TAN
	WR	MR	DR	SHIN	NaOH 10%	FOAM	H <sub>2</sub> SO <sub>4</sub>	BR	SALK	L-B	FeCl <sub>3</sub>
<i>Adenostemma platyphyllum</i>	-	-	-	++	++	+	-	-	+	++	+++
<i>Castilla elastica</i> subsp. <i>gummifera</i>	-	-	-	+	+	+	+	-	+	++	+
<i>Cochlospermum vitifolium</i>	+	-	++	-	+++	++	-	+++	++	++	+++
<i>Ectozoma pavonii</i>	++	+++	+++	++	+++	-	++	-	+	++	+
<i>Erythrochiton giganteus</i>	+	+	+	++	++	-	+	-	+	++	+++
<i>Erythroxylum patens</i>	+	++	+	++	++	-	++	-	+	++	++
<i>Ficus brevibracteata</i>	+	+	+	-	++	-	++	+	+++	++	+++
<i>Ficus tonduzii</i>	+++	+++	+++	+	+	-	+	-	-	++	+
<i>Grias ecuadorica</i>	-	+	-	+	+	++	++	++	-	++	+++
<i>Handroanthus billbergii</i> subsp. <i>ampla</i>	++	++	+++	++	++	+	+	+	+++	+++	++
<i>Morisonia americana</i>	+	+++	+++	++	++	+	++	++	+	++	+
<i>Operculina codonantha</i>	++	++	+++	+++	+++	++	+++	+++	++	+++	+++
<i>Passiflora macrophylla</i>	-	-	-	+++	+	-	++	+	+	++	++
<i>Podandrogynne jamesonii</i>	+	-	++	+	+	+	+	+	+	++	+
<i>Pradosia montana</i>	+	+	+++	-	+	-	-	++	+	+	-
<i>Tecoma castaneifolia</i>	-	+	++	+	++	+	++	++	++	+	++
<i>Urera baccifera</i>	-	-	+	+	++	+	-	-	+	+	+
<i>Xanthosoma sagittifolium</i>	+++	+++	+++	+++	++	-	+	-	+++	++	+

**Keys:** (-) Absence; (+) Poor; (++) Moderate; (+++) Abundant **WR:** Warner reactive, **MR:** Mayer reactive, **DR:** Reindorf reactive, **SHIN:** Shinoda reactive, **FOAM:** Foam test, **BR:** Borntrager reactive, **SALK:** Salkowsky reactive, **L-B:** Liebermann-Burchard reactive

the groups of secondary metabolites with the greatest distribution in nature. These compounds are the plant's first line of defense against various pollutants (Ludwiczuk et al., 2017; Niaz and Khan, 2020). While other compounds such as the alkaloids, are restricted to some botanical families (Ramawat et al., 2009). The alkaloids are found in 20% of the species of vascular plants, most frequently in the herbaceous dicot and relatively a few in monocots and gymnosperms vascular plant species (Pagare et al., 2015). Saponins were the least present chemical among the species. These compounds are considered as

constitutive chemical defenses of plants, increasing in concentration only in response to stimuli (Díaz, 2009). Saponins seem to be rather rare in Moraceae (Berg et al., 2006). This is in agreement with those reported for the analyzed species of this family. Many of the species were growing under similar environmental conditions, so the presence of all classes of metabolites evaluated in certain plants could be related to their genetic characteristics, an aspect that is reflected in the differentiated absorption of nutrients and the conversion of substances synthesized by photosynthesis (García and Medina, 2006). Thus, the absence of

certain compounds in some species could be due to genetic factors since they do not possess the enzymes necessary for the production of these compounds. In the scientific literature, few reports were found on the phytochemical or biological activity of species evaluated. The chemical composition of *A. platyphyllum* collected in the tropical rain forest at Indonesia was characterized using pyrolysis-gas chromatography/mass spectrometry and proximate analysis (Fauzan et al., 2018). The result showed that it contained 9.18% of water, 17.84% of protein, 6.33% of fat, and 19.94% of ash. The secondary metabolites present were alkaloids, phenolic compounds, terpenoids and steroids. In the present paper, *A. platyphyllum* was found to contain the same metabolites except alkaloids. Alkaloids are compounds present in the Asteraceae family. This difference could be due to the sensitivity of the chromatographic technique employed by Fauzan. Alkaloids were absent in the leaves of *C. elastica* subsp. *gummifera*. The presence of alkaloids has been reported in the latex of this species (Marinho and Texeira, 2018), so the absence could be due to the difference between the plant parts analyzed. There are studies of the presence of cardenolic glycosides in the seeds and latex of *C. elastica* (Brauchli et al., 1961). These compounds have also been reported in other species of the Moraceae family and are possibly responsible for the toxicity found in their leaves (Witharana et al., 2014). Sterols, aromatic compounds, apocarotenoids, flavonoids and lignans groups, have been isolated from extracts of *C. vitifolium* in Mexico. Flavonoids and sterols were also present in the *C. vitifolium* extract from Ecuador. These compounds have been reported in other *Cochlospermum* species, for which they are considered chemotaxonomically significant within the genus (Aguilar-Guadarrama and Rios, 2018). In this preliminary phytochemical screening, *T. castaneifolia* extract showed the presence of all the metabolites evaluated. This result is in agreement with the metabolites reported for species of the Bignoniaceae family, mainly phenolic compounds (Choudhury et al., 2011), which confer diverse biological activities. Reis et al. (2020) performed the screening and identification of components in ethanol extracts of *T. castaneifolia* leaves and trunk by ultra-high performance liquid chromatography (UPLC-DAD-UV-MS). The compounds detected were the phenylethanoids verbascoside and isoverbascoside, the lignin olivil and paulownin, the phenylpropanoid crenoside and martynoside and showed *in vitro* antiviral activity against the *Zika virus*. *T. castaneifolia* extracts have also shown cytotoxic activity against MCF-7 cell line (Vidhya and Fleming, 2015). In the present study, the chemicals present in *U. baccifera* were alkaloids, flavonoids, saponins, sterols and terpenes. These compounds have also been reported in other *U. baccifera* specimens. The crude extract of *U. baccifera* leaves present a low polyphenol ( $61.55 \pm 1.54$  mg GAE/mL extract), flavonoid ( $41.54 \pm 0.53$  mg QE/mL extract), flavonol ( $15.47 \pm 0.72$  mg QE/mL extract), condensed tannins ( $1.95 \pm 0.04$  mg CE/mL extract) and alkaloids ( $2.34 \pm 0.02$  mg alkaloids

equivalents/mL extract) contents and significant quantity of oxalic acid ( $0.44 \pm 0.05$  mg/g). The extracts showed genotoxic effects (Gindri et al., 2014). Flavonoids such as diosmetin, luteolin, vicenin-2 and apigenin with gastroprotective effects have been reported in the leaves of *U. baccifera* (Benvenuti et al., 2020). *X. sagittifolium* had a moderate presence of flavonoids, terpenes, sterols, tannins and alkaloids. Souza (2018) found flavonoids, saponins, steroids, triterpenes and tannins in the phytochemical analysis performed on the aqueous extract of *Xanthosoma sagittifolium* (L.) Shott from Brazil, while the alkaloids were absent. Alkaloids are compounds commonly found in the Araceae family (Dring et al., 1995). The presence of alkaloids in this study, could be due to the fact that the collection was carried out during the time of the year when there was no rainfall, which could have influenced the higher production of these compounds. In addition, this condition could be the cause of the absence of saponins in the present investigation. It has been reported that drought conditions decreased the content of saponins (Ramakrishna and Ravishankar, 2011). *X. sagittifolium* tubers are edible in other countries and have lipids, proteins, carbohydrates,  $\beta$ -carotene, lycopene, and vitamins B and C in their compositions (Souza, 2018). The leaves are used to prevent diseases such as osteoporosis (Nishanthini and Mohan, 2012), although there are no reports of medicinal uses in Ecuador. The concentrations of various secondary plant products are strongly dependent on the growing conditions and have impact on the metabolic pathways responsible for the accumulation of the related natural products (Ramakrishna and Ravishankar, 2011). Variations in chemical composition between species from different regions may be due to different factors such as the presence of different chemotypes, the local geoclimate, seasonal changes, external conditions such as light, temperature, humidity, state of development (Ortiz and Chaves, 2017). Phytochemical screening performed on plant extracts revealed the presence of compounds with potential phytotherapeutic effects that might also possess various biological and physiological properties (Tripathi and Mishra, 2015). Flavonoids are polyphenolic compounds with antioxidant, antithrombotic and anti-inflammatory properties (Quiñones et al., 2012). The presence of flavonoids in *A. platyphyllum*, *U. baccifera*, and *P. macrophylla* can be associated as anti-inflammatory and analgesic applications in the traditional medicine of Ecuador (De la Torre, 2008). The extract of *E. pavonii*, a species endemic to western Ecuador, showed presence of alkaloids and flavonoids. Alkaloids are characteristic compounds of the Solanaceae family (Jerzykiewicz, 2007). *Erythrochiton giganteus* and *Erythroxyllum patens* have a moderate presence of alkaloids, flavonoids, tannins and terpenes. Flavonoids and benzoic derivatives have been isolated from the genus *Erythrochiton* (Baj et al., 2017). The genus *Erythroxyllum* is known for the presence of tropane-type alkaloids, terpenes, and flavonoids among



its compounds (Gonzalez et al., 2005; Restrepo et al., 2019). The tests carried out on *F. brevibracteata* and *F. tonduzii* reveal an important presence of alkaloids, triterpenes and, tannins. Alkaloids with anticancer activity have been found in other *Ficus* species (Abubakar et al., 2015). The use of *F. tonduzii* as vermifuge (De la Torre, 2008) could be attributed to the high presence of alkaloids in its extract. A significant quantity of tannins, triterpenes, and anthraquinones were reported in *G. ecuadorica*, a tree endemic to the wet forests of western Ecuador. There are no previous reports of its biological activities, although two triterpenes and a tannin with proven cytotoxic activity have been isolated from *G. neuberthii*, another species of Lecythidaceae, endemic to the Amazonia of Peru, Ecuador and southeastern Colombia (Cornejo and Mori, 2012; Guamán-Ortiz et al., 2020). The ethanolic extracts of *M. americana*, a species of the Capparaceae family, show a moderate presence of saponins, alkaloids, flavonoids, anthraquinones, terpenes, and tannins. No scientific reports of chemical composition or biological activities of species of this genus were found. A previous study carried out in Ecuador indicates that *Capparidastrium petiolare* and *Colicodendrum scabridum*, species belonging to Capparaceae, have a moderate presence of alkaloids, tannins, and anthraquinones (Rondón et al., 2018b), so these compounds could be common within that botanical family. *O. codonantha*, species of Convolvulaceae family, showed an important amount of alkaloids and saponins in its phytochemical composition. Ergolin-type alkaloids have been found in plants of the Convolvulaceae family and have been used to treat migraines and Parkinson's diseases (Markert et al., 2008). Saponins have been reported in some species of the Convolvulaceae family (Mascarenhas, 2017). *Podandrogynne jamesonii* presented a significant amount of alkaloids in its composition. This species is endemic to western Ecuador (Cochrane, 1997) and data on its phytochemical characterization have not been found in the literature. The analysis carried out on *P. montana*, a species endemic to western Ecuador and NW Peru, shows an important presence of alkaloids and anthraquinones. These compounds have also been identified in *P. huberi*, a congeneric native to the Amazonian region in Brazil, which is traditionally used to treat gastritis (Dos Santos et al., 2016). *P. montana* showed a low presence of flavonoids. Flavonoids have been reported in another species of *Pradosia* collected in the Amazon (Dos Santos et al., 2016), while *P. montana* was collected in a completely different ecosystem (deciduous dry forest). Reports indicate that drought stress reduced the concentration of polyphenols in *Brassica rapa* L (Ku et al., 2019) and *Merremia aegyptia* (Salgado et al., 2020). The absence of flavonoids and tannins could be due to this environmental factor. Phytochemical studies reveal that the extracts of many species of Bignoniaceae contain metabolites such as saponins, tannins, flavonoids, quinones, alkaloids among others (Choudhury et al., 2011). These data coincide with the compounds reported on the present

investigation for *T. castaneifolia* and *H. billbergii* subsp. *ampla*. No reports of chemical composition of *H. billbergii* subsp. *ampla* have been found, however, quinones such as lapachones and naphthoquinones have been isolated from *H. serratifolius* (Costa et al., 2017). The considerable presence of secondary metabolites in this preliminary study would support some of the ethnomedicinal uses reported for the studied species of plants in Ecuador (Table 1).

### 3.2. Quantification of phenols and flavonoids

Phenolic compounds are the most widely distributed metabolites in plants and presence of this class of compounds in a phytochemical study is of particular interest due to the multiple biological properties phenols possess, e.g., anti-cancer, anti-inflammatory, antimicrobial and antioxidant as well as their direct benefits in the prevention of multiple diseases (Vuolo et al., 2019). The Folin-Ciocalteu method is one of the most often used methods to determine the total content of phenols in plants or food (Roginsky and Lissi, 2005). The phenolic content found in the extracts was determined using the linear regression equation using gallic acid as standard ( $y = 0.0667x + 0.0503$ ;  $R^2 = 0.9978$ ) and the results are shown in Table 3. *P. montana* did not present tannins or flavonoids in its phytochemical screening, so quantification of these compounds was not performed. The values of the total phenol content varied between the samples and ranged from  $27.14 \pm 0.02$  mg GAE/g to  $4.65 \pm 0.09$  mg GAE/g dry extract. The plants showing the highest amount of phenols were *C. vitifolium* and *F. tonduzii*, with  $27.14 \pm 0.02$  and  $26.76 \pm 0.05$  mg GAE/g dry extract, respectively. Among the evaluated plants, *H. billbergii* subsp. *ampla* and *T. castaneifolia* extracts showed a very low phenolic content with  $4.65 \pm 0.09$  and  $697 \pm 0.17$  mg GAE/g extract, respectively. The quantification of phenols of some of the species in this research has already been reported. Previous studies carried out on specimens of *C. vitifolium* in Mexico (González-Gómez et al., 2006), *U. baccifera* in Brazil (Gindri et al., 2014) and *A. platyphyllum* in Indonesia Mexico (Fauzan et al., 2018) showed phenol contents of 285.8 mg GAE/g extract, 61.55 mg GAE/mL extract and 74.2 mg/g, respectively, which were higher than those reported in this investigation. However, the phenol content of the *X. sagittifolium* leaf extract was higher (10.70 mg GAE/g dry extract) than that of the corm extract of this species, which was 3.2 mg GAE/g extract (Nishanthini and Mohan, 2012). The differences found in the phenol content may be due to the fact that they come from different organs of the plant. The location of a phenolic compound in plant tissue reveals the physiological function in the plant. Since they are stored in important locations, they act as signaling or plant defense mechanism (Souza, 2018). High levels of phenol have been reported in other species of the genus *Ficus* (range 63.61-131.38 mg GAE/g extract) (Abdel-Hameed, 2009; Ayoub et al.,

**Table 3**

Content of total flavonoids and total phenols in the extracts of the evaluated plants from Ecuador.

Species	Total flavonoids	Total phenols
	(mg QE/g dry extract)	(mg GAE/g dry extract)
<i>Adenostemma platyphyllum</i>	476.02 ± 12.35a	9.89 ± 0.02a
<i>Castilla elastica</i> subsp. <i>gummifera</i>	454.13 ± 7.91a	7.11 ± 0.15b
<i>Cochlospermum vitifolium</i>	610.32 ± 10.15b	27.14 ± 0.02c
<i>Ectozoma pavonii</i>	175.79 ± 5.35c	8.64 ± 0.03d
<i>Erythrochiton giganteus</i>	303.50 ± 2.07d	11.15 ± 0.01e
<i>Erythroxyllum patens</i>	880.65 ± 8.63e	16.37 ± 0.14f
<i>Ficus brevibracteata</i>	386.55 ± 15.10f	12.79 ± 0.07g
<i>Ficus tonduzii</i>	456.81 ± 6.90a	26.76 ± 0.05h
<i>Grias ecuadorica</i>	418.05 ± 1.70g	16.43 ± 0.03f
<i>Handroanthus billbergii</i> subsp. <i>ampla</i>	254.32 ± 0.95h	4.65 ± 0.09i
<i>Morisonia americana</i>	173.27 ± 1.73c	11.72 ± 0.11j
<i>Operculina codonantha</i>	273.97 ± 5.35hk	9.40 ± 0.34k
<i>Passiflora macrophylla</i>	282.90 ± 4.76dk	14.57 ± 0.03l
<i>Podandrogynne jamesonii</i>	405.09 ± 24.99fg	18.57 ± 0.19m
<i>Pradosia montana</i>	-	-
<i>Tecoma castaneifolia</i>	217.67 ± 1.38i	6.97 ± 0.17b
<i>Urera baccifera</i>	354.51 ± 3.91j	14.33 ± 0.05l
<i>Xanthosoma sagittifolium</i>	296.62 ± 2.38dk	10.70 ± 0.00n

Values represent the mean of three repetitions ± standard deviation.

 Same letters indicate no significant differences between these extracts, according to the Tukey test ( $p < 0.05$ )

2019), higher than those of the species studied, e.g., *F. Tonduzii*. This plant is used in the traditional medicine of Ecuador to treat fever and flu (De la Torre, 2008) and these applications could be related to the high proportion of phenolic compounds found. *H. billbergii* subsp. *ampla* and *T. castaneifolia*, both species of the Bignoniaceae family, obtained a low concentration of phenols, which could be influenced by the dry season in which the collection was carried out. Both species belong to the Pacific dry deciduous forest ecosystem. Plant species adapted to xerophytic conditions have been reported to decrease their phenol content as a strategy against water stress (Salgado et al., 2020). Flavonoids contents were determined using the aluminum chloride colorimetric technique, which is based on the formation of aluminum-flavonoid complexes and is one of the most commonly used procedures in the evaluation of samples of medicinal or food plants (Pełal and Pyrzynska, 2014). The flavonoid content in plant extracts was determined using the quercetin calibration curve ( $y = 0.0004518x + 0.0668$ ;  $R^2 = 0.989$ ). The total flavonoid content of 18 plants from western Ecuador is presented in Table 3. Among the evaluated plants, a wide variation is observed in the content of total flavonoids, which range from  $880.65 \pm 8.63$  mg QE/g dry extract to  $173.27 \pm 1.73$  mg QE/g dry extract. *Erythroxyllum patens* and *C. vitifolium* had the greatest flavonoid content with  $880.65 \pm 8.63$  and  $610.32 \pm 10.15$  mg QE/g dry extract, respectively. The

smallest amounts of flavonoids were found in a *M. americana* with  $173.27 \pm 1.73$  mg QE/g dry extract and *E. pavonii* with  $175.79 \pm 5.35$  mg QE/g dry extract. Flavonoids have been shown to be an important chemical group within the composition of the genus *Erythroxyllum* (Gonzalez et al., 2005), while some flavonoids of high importance in the treatment of liver diseases have been isolated from the extracts of *C. vitifolium* (Aguilar-Guadarrama and Rios, 2018). However, no flavonoid quantification reports were found for these species using this methodology. In general, high values were observed in the flavonoid content of the extracts of the evaluated plants ( $> 100$  mg QE/g extract), which were higher than those found in previous studies for these species. In the *U. baccifera* extract, the flavonoid value was  $34.33$  mg QE/g extract (Benvenuti et al., 2020), while the extract from the corm of *X. sagittifolium* showed a flavonoid content of  $2.6$  mg QE/g extract (Nishanthini and Mohan, 2012). The high concentration of flavonoids from Ecuadorian plants would indicate that these species could serve as resources in flavonoid research and isolation. Statistically, significant differences were found in the content of total phenols and flavonoids between the extracts ( $p < 0.05$ ). Similar variations in phenol and flavonoids content have been observed between different medicinal plants (Sengul et al., 2009; Mustafa et al., 2010). This could be due to multiple factors that considerably influence the production of these polyphenolic compounds in plants, such as genetic



and biological species variations, geographical origin of the plant, part of the plant used, seasonal changes, soil types, extraction and drying methods used, among others (Babbar et al., 2011; Kumar and Pandey, 2013; Farag et al., 2020). The wide variations found in flavonoid and phenol content among similar species evaluated in different regions could also be due to the above factors.

### 3.3. Antioxidant activity

The DPPH• (1,1-diphenyl-2-picryl-hydrazyl) free radical

scavenging test is an acceptable, relatively simple and the most widely used method in evaluating the antioxidant activity of natural extracts due to its strong hydrogen donating capacity (Subedi et al., 2014). The results of the percentage of inhibition of DPPH• and the IC<sub>50</sub> value of the plant species collected in western Ecuador are presented in Table 4 at the highest concentration of the extracts used (5 mg/mL). Although more than half (> 50%) of sampled species presented antioxidant activity, the extracts of *E. patens* (92.41 ± 0.33%) and *H. billbergii* (90.53 ± 0.13%) contains the

**Table 4**

Antioxidant activity of the ethanolic extract of 18 species of plants native to western Ecuador.

Species	I%	IC <sub>50</sub> (mg/mL)
<i>Adenostemma platyphyllum</i>	77.50 ± 0.39 a	1.60
<i>Castilla elastica</i> subsp. <i>gummifera</i>	47.79 ± 0.93 b	-
<i>Cochlospermum vitifolium</i>	78.11 ± 0.20 ai	0.29
<i>Ectozoma pavonii</i>	62.83 ± 0.68 c	3.43
<i>Erythrochiton giganteus</i>	29.84 ± 1.99 d	-
<i>Erythroxyllum patens</i>	92.41 ± 0.33 e	0.14
<i>Ficus brevibracteata</i>	85.18 ± 0.22 f	1.30
<i>Ficus tonduzii</i>	12.30 ± 2.09 g	-
<i>Grias ecuadorica</i>	87.93 ± 0.33 h	0.74
<i>Handroanthus billbergii</i> subsp. <i>ampla</i>	90.53 ± 0.13 el	0.51
<i>Morisonia americana</i>	80.14 ± 0.99 i	1.58
<i>Operculina codonantha</i>	58.07 ± 0.45 j	4.44
<i>Passiflora macrophylla</i>	65.60 ± 0.53 k	3.76
<i>Podandroyne jamesonii</i>	84.39 ± 0.26 f	1.02
<i>Pradosia montana</i>	88.36 ± 0.73 hl	0.27
<i>Tecoma castaneifolia</i>	86.59 ± 0.08 fh	1.18
<i>Urera baccifera</i>	28.75 ± 0.36 d	-
<i>Xanthosoma sagittifolium</i>	44.99 ± 0.23 m	-
Ascorbic acid (control)	95.54 ± 0.98 n	-

Concentration of extracts: 5 mg/mL Same letters indicate no significant differences between these extracts, according to the Tukey test ( $p < 0.05$ )

highest antioxidant capacity, followed by *P. montana* (88.36 ± 0.73%) and *G. ecuadorica* (87.93 ± 0.33%). The extracts of *F. tonduzii* and *U. baccifera* had the lowest percentages of inhibition with 12.30 ± 2.09% and 28.75 ± 0.36%, respectively. The antioxidant activity of ascorbic acid was 95.54%. Significant differences ( $p < 0.05$ ) were found between the antioxidant activity of the extracts and ascorbic acid, with a significance level of 5%. Free radicals are chemically unstable atoms that are produced in cells during metabolic processes and can damage cellular DNA, lipid cells, and proteins. They are known to be the underlying cause of oxidative stress, which is strongly implicated in the pathogenesis of various diseases such as cancer and diabetes (Lobo et al., 2010). The antioxidant activity measures the general ability of the extract to counteract reactive

oxygen species (ROS), and its potential to prevent diseases related to oxidative stress (Roy et al., 2019). The most notable of the phenolic compounds present in plants are their antioxidant properties. Hydroxyl groups, attached to a benzene ring, give them special characteristics. They are very susceptible to oxidation, prevent metals from catalyzing oxidation reactions, and act as chelators, forming complexes with di or trivalent metals (Gimeno Creus, 2004). There were no correlation between antioxidant activity and total phenolic content ( $R = -0.188$ ), and antioxidant activity and total flavonoids content ( $R = 0.154$ ), although other studies have demonstrated a strong correlation between these variables (Derakhshan et al., 2018; Mwamatope et al., 2020). It has been reported that the antioxidant activity

of plants is mainly due to the presence of phenolic compounds, including flavonoids (Borkataký et al., 2013), however, the antioxidant capacity in our plant samples can be attributed to the presence of other phytochemical compounds such as alkaloids, terpenes, ascorbic acid and various pigments that also possess total antioxidant capacity (Sengul et al., 2009; Ibarra et al., 2011; Gonzalez and Gómez, 2012). The presence of alkaloids could be the reason why *P. montana* extract obtained a high percentage of inhibition (88.36%) despite not presenting flavonoids in the screening carried out in the present research (Table 2). The negative correlation between phenolic compounds and antioxidant capacity has been previously reported (Sengul et al., 2009; Rondón et al., 2018a). Species such as *E. giganteus*, *F. tonduzii* and *U. baccifera*, despite having high levels of flavonoids, do not present antioxidant activity. The free radical neutralizing capacity of flavonoids depends on some factors, including the existence of sugars in their composition, the number of hydroxyl in the molecule and the presence of a free 3-OH group in the C ring (Bors et al., 1990; Hopia and Heinonen, 1999; Farag et al., 2020). Thus, the low antioxidant activity of these extracts could be due to the presence of glycosidated flavonoids that have the sugar fraction in that position within the molecule. For comparative studies, few previous reports of the antioxidant activity of the evaluated species were found, which were superior to those of the present investigation. The hydroalcoholic extract of the leaves of *U. baccifera* from Brazil presented DPPH scavenging activity with IC<sub>50</sub> value of 0.087 mg/mL (Benvenuti et al., 2020), while in the present study, the extracts of *U. baccifera* reported an inhibition percentage of less than 50%. The methanol extract of *X. saggitifolium* corm from India, reported a considerable antioxidant activity with an IC<sub>50</sub> value of 0.036 mg/mL (Nishanthini and Mohan, 2012), while the ethanol extract of *X. saggitifolium* leaves from Ecuador presented an inhibition percentage of 44.99%. The high polarity of the solvents used in obtaining the extracts of the species from Brazil and India could have caused a greater extraction of glycosidated flavonoids and a high antioxidant activity. In addition, the different location and function of the parts evaluated in *X. saggitifolium* may have influenced the variations found in their antioxidant capacity (Souza, 2018). Similarly to that reported in the present study, a strong antioxidant activity has been documented for the genera *Erythroxyllum* (Ranjitham et al., 2014), *Handroanthus* (Costa et al., 2020), *Pradosia* (Lorz et al., 2019), *Tecoma* (Ramírez-Ortiz et al., 2016), and *Ficus* (Sirisha et al., 2010), supporting the results obtained here. In general, the extracts of the evaluated plants exhibited a high antioxidant capacity, which may be related to their medicinal uses in Ecuador. Additional studies should be conducted to validate the traditional use of these plants.

#### 4. Concluding remarks

In the present study, the content of phenols,

flavonoids and the antioxidant activity of the ethanolic extracts of 18 species of plants native to western Ecuador were evaluated. In general, all plants show a high content of phenols and flavonoids and a great antioxidant capacity, although no correlation was found between these variables. The results of the work would contribute to broadening scientific knowledge about these species, which proved to be a promising source of phytochemical compounds and could serve for the isolation and identification of molecules with pharmaceutical potentials. In addition, these plants could have great importance in the therapies for diseases associated with oxidative stress. It is recommended to carry out *in vivo* and *in vitro* studies of the toxicity of the extracts and the identification of the compounds that would be related to their antiradical activity. The phytochemical characterization and antioxidant activity of these 18 species of plants collected in Ecuador is reported for the first time. This indicates the relevance of the information provided in this paper.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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