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Antioxidant properties of medicinal plants used in the Southern Ecuador

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Abstract
In this research, 64 organic extracts from 37 species, belonging to 23 botanical families of plants used in the folk medicine of southern Ecuador was study. The extracts analysed were obtained with different solvents (methanol, *n*-hexane, dichloromethane, ethyl acetate). The antioxidant activity was determined by two methods: DPPH and $ABTS^{\cdot+}$. The phenolic content of the extracts was determined using the Folin-Ciocalteau colorimetric technique. The species that showed the highest antioxidant activity, according to the IC_{50} values and the total phenolic content were *Hypericum lancioides*, *Piper pseudochurumayo*, *Ludwigia peruviana*, *Sarcorhachis sydowii*, *Garcinia macrophylla*, *Clusia alata*, *Huperzia crassa* and *Fuchsia hybrida*. The results obtained suggest that the good antioxidant activity described for these species could play an important role in the medicinal properties claimed for the plants under study and some of these plants could be useful in the food and pharmaceutical industries.

Keywords: DPPH, $ABTS^{\cdot+}$, folk medicine, antioxidant

1. Introduction
Ecuador is considered one of the top ten countries with the highest biodiversity in the world, added to this; the country keeps strongly ancestral traditions, especially in the use of medicinal plants, to treat disease and ailments [1]. It is estimated that still today, 80% of the Ecuadorian inhabitants depends of folklore medicine on the primary attention of healthy problems [2].
The southern area of Ecuador comprises three provinces, El Oro, Loja and Zamora Chinchipe, this region is rich in flora that varies according to the different climates, is considered among the richest and most diverse in the world, consisting of a wide range of vegetation (Figure 1) [3, 4].
It is a region where ethnics groups live, like the Saraguros, that use a series of plants as therapeutic agents, for fighting common diseases and also in spiritual ailments or supernatural diseases such as *susto* (fright), *vaho de agua* (water vapor), *mal aire* (bad air), *mal de ojo* (evil eye) [5, 6].
The growing interest to find new natural resources with antioxidant properties is due to the well-known healthy benefits that they produce. The relation between the oxidative stress generated by the reactive oxygen species, and a series of pathologies and diseases such as, cancer, cataracts, diabetes, asthma, atherosclerosis, cardiovascular problems and ageing processes are well documented [7–9]. Furthermore, in the food industry the oxidation process is the principal cause of food deterioration, for these mentioned reasons there is an increasing necessity in the food industry to find new natural sources of antioxidants for replacing the synthetic ones believing that they present not only antioxidant capacities but also health benefits and less secondary effects. Different *in vitro* studies of vegetables and medicinal plants indicated that some constituents of them are capable of produce protective effects against the oxidative stress in biological systems [10–13].
Considering the ancestral use of plants by the inhabitants of Ecuador [14], the advantage of the rich culture and the high biodiversity in the southern part of Ecuador, a clear approach to identify antioxidant natural products was to investigate a large number of plants with strong reputation under the Saraguro ethnia [15]. A number of 37 species of medicinal plants, belonging to 23 different botanical families, used in the provinces of Loja and Zamora Chinchipe were screened to know the potential antioxidant capacity and the total phenolic content, on 64 organic extracts obtained with different solvents. The species studied have different uses in traditional medicine, from those for common ailments to those that are used in supernatural diseases; Table 1 shows a description of these plants.
Finally, the worldwide study of endemic resources provides a broad contribution to conservation, likewise, it is very important for scientists, academics, conservationists and conservation planners in Ecuador to have information on the cultural use of many endemic

plants by local ethnic groups that allows to know, the level of exploitation of the resource and estimate its potential use sustainable.

Fig 1: Southern area of Ecuador

2. Materials and Methods

2.1 Chemicals and reagents

Methanol, *n*-Hexane, Dichloromethane, Ethyl acetate, Sulfuric acid, hydrochloric acid, Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Folin–Ciocalteu reagent, Sodium bicarbonate, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Gallic acid, 2,2'-Azino-bis (3-ethylbenthiazoline-6-sulfonic acid) (ABTS), Potassium persulfate, Trolox (6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and Dragendorff reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2 Plant materials

Wild plant material (37 plants, see Table 1) were collected from different locations in the provinces of Loja and Zamora-Chinchipe. The plant material was authenticated by Bolívar Merino, botanic curator of the Herbarium-Loja of the Universidad Nacional de Loja. A voucher specimen of each plant species was deposited in the Herbarium of the Universidad Técnica Particular de Loja (HUTPL). The plants collections were realized according to the license of the Ministry of Environment of Ecuador-MAE (Nº001-IC-FLO-DBAP-VS-DRLZCH-MA) and (Nº047-IC-FLO-DPL-MA). The plant raw materials were cleaned from degraded parts and dried at 37º C. Plant parts were ground to a fine powder using a laboratory mill. Powdered materials were maintained at room temperature in sealed bags until use.

2.3 Extracts preparation for antioxidant analysis and total phenolic content

Extracts were obtained from 37 plants species (Table 1) with similar procedure for each material. Two types of extraction were developed. The first extractions were performed by dynamic maceration during 4 hours at room temperature of chosen plants, using different solvents: Hexane, Dichloromethane, Ethyl acetate and Methanol in increasing order of polarity. Filtrates under vacuum and concentrated by drying at reduced pressure. This procedure was repeated by three times with each solvent. The second extraction was to obtain the alkaloidal fractions (only 20 species showed in table 2 were submitted to this procedure), plant material was extracted by maceration with methanol-water 8:2 v/v, this procedure was repeated until the Dragendorff test [16] gave a negative result. The liquid solvent was evaporated by heating at reduced pressure in a rotary evaporator the extracts were acidified with H_2SO_4 (2% v/v) until pH 11.

Antioxidant capacity

Antioxidant capacity was determined by two methods, the $ABTS^{\cdot+}$ assay according to Arnao *et al*. (2001) with slightly modifications, and the DPPH free radical-scavenging done under the procedure of Brand-Williams *et al*. (1995) with some modifications described by Thaipong *et al*. (2006), in both cases Trolox, a well-known synthetic antioxidant, was used as standard reference.

DPPH radical scavenging activity

The change in absorbance produced by reducing DPPH radical was used to measure the radical-scavenging activity. This assay was performed based on the technique of Brand-Williams *et al*. (1995) with some modifications described by Thaipong *et al*. (2006). Briefly, to prepare the radical stock solution, 24 mg of DPPH was dissolved in 100 mL of Methanol. The working solution was prepared by diluting 10 mL of the radical stock solution with 45 mL of Methanol to obtain a reading of 1.1 ± 0.02 absorbance units at a wavelength of 515 nm in a UV spectrophotometer. The working solution was prepared fresh daily. For quantification of antioxidant capacity a standard curve of Trolox was prepared at different concentrations between 0.25 to 800 µM Trolox. All determinations were performed by triplicate.

From each extract, a solution of 1000 ppm (w/v) was prepared and 150 µL were mixed with 2850 µL of DPPH working solution in an amber vial, then the mixture solution was shaken for three minutes and allowed to react for 24 hours at room temperature protected from light. The final absorbance was measured at a wavelength of 515 nm. The results are expressed as micromoles of Trolox equivalent per milligram of plant extract (µM TE/mg extract).

$ABTS^+$ radical scavenging activity

The $ABTS^+$ assay was performed using the procedure described by Arnao *et al*. (2001) with some modifications by Thaipong *et al*. (2006). Two stock solutions were prepared: 7.4 µM ABTS and 2.6 µM potassium persulfate. $ABTS^+$ radical cation was produced after mix both solutions in equal quantities and allowed to react protected from light during 12 hours to obtain the ABTS standard solution. For the study of extracts, 1 mL of the ABTS standard solution was diluted with 60 mL of Methanol to obtain an absorbance of 1.1 ± 0.02 in a spectrophotometer UV to 734 nm. A standard curve of Trolox was made with concentrations between 0.25 to 800 µM Trolox. From each concentration as well with each extract at standard concentration (1000 ppm), 150 µL were added to 2850 µL of the ABTS working solution, then was allowed to react by 2 hours protected from light, after this the absorbance was measured at 734 nm. The results are expressed as micromoles of Trolox equivalents per milligram of extract (µM TE/mg extract). All determinations were done by triplicate.

Total phenolic determination

Total phenolic content was measured using the Folin-Ciocalteu colorimetric method Folin & Ciocalteu (1927) with some modifications described by Kong and Lee (2010) and Thaipong *et al*. (2006). Different solutions were prepared, Folin-Ciocalteu (0.25 N), Sodium carbonate (1N), and Gallic acid solutions between 0 to 1 mg GA/mL. Plant extracts (150 µL) at a standard concentration of 1000 ppm (w/v), were mixed with 150 µL of Folin-Ciocalteu reagent and 2400 µL of water, the mixture was stirred by two minutes and allowed to react by 3 minutes; then 300 mL of Sodium carbonate were added and allowed to react for 2 hours protected from light. Finally, the absorbance was measured at 725 nm. The total phenolic content of extract was calculated from the standard curve of Gallic acid, the results are expressed as milligrams of Gallic acid equivalent per gram of extract (mg GAE/g extract).

2.4 Statistical analysis

All determinations were performed in triplicate, and all results were calculated as mean and standard deviation (SD) using the Microsoft Excel 2010. All standard curves had good correlation coefficients, $r^2 \geq 0.99$.

Table 1: Therapeutical application of medicinal plants species from Loja and Zamora Chinchipe

S. No	Scientific name	Family	Common names	Therapeutical applications
1	*Abutilon striatum* Dicks ex Lindl	Malvaceae	Malva goma	Headache [1].
2	*Alibertia* sp.	Rubiaceae	Suu	Anti-inflammatory, analgesic and antimicrobial [18, 19].
3	*Baccharis obtusifolia* Kunth.	Asteraceae	Shadan o chilca redonda	Anti-inflammatory, rheumatism, skin fungal infections and stomachache [20].
4	*Bejaria resinosa* Mutis ex L.F.	Ericaceae	Payama	Arthritis, kidney pain, relaxant, heart problems.
5	*Clusia alata* Triana & Planch.	Clusiaceae	Duco	Anti-inflammatory, antimicrobial, antifungal and anti-HIV activity [21].
6	*Coreopsis venusta*	Asteraceae	NC	Analgesic [22].
7	*Datura stramonium* L.	Solanaceae	Chamico	Asthma, analgesic, sore throat, toothache, antiparasitic, and Parkinson's disease [23, 24].
8	*Euphorbia weberbaueri* Mansf.	Euphorbiaceae	Látex	Emetic properties, cathartic and purgative [25].
9	*Garcinia macrophylla* Mart.	Clusiaceae	Shora	External inflammation [26].
10	*Guazuma ulmifolia* Lam	Sterticuliaceae	Guázimo	Malaria, antidiuretic, diarrhea, kidney disorders, inflammatory, influenza and skin conditions [1, 27].
11	*Huperzia columnaris* B. Øllg.	Lycopodiaceae	Waminga oso kari	"Susto" (fright)," vaho de agua" (water vapor) [6].
12	*Huperzia kuesteri* (Nessel) B. Øllg.	Lycopodiaceae	Waminga	Treatment of fever, contusions, distensions, inflammations, schizophrenia, diuretic, blood loss and as a regulator of menstruation [28].
13	*Huperzia tetragona* (Hook. & Grev.) Trevis.	Lycopodiaceae	Trencilla rojo	Purgative [6].
14	*Huperzia compacta* (Hook.) Trevis.	Lycopodiaceae	Waminga roja-verde	Purgative to cleanse the stomach and liver and "mal aire" (bad air) [6].
15	*Huperzia crassa* (Humb. & Bonpl. Ex Willd.) Rothm.	Lycopodiaceae	Waminga amarilla	"Susto" (fright) [6].
16	*Huperzia espinosana* B. Øllg.	Lycopodiaceae	Waminga oso warmi	"Susto" (fright), "mal aire" (bad air) [6].
17	*Lycopodium complanatum* L.	Lycopodiaceae	NC	Antipruritic, decongestant, diuretic and stomach, analgesic, antirheumatic, carminative, tonic, antibacterial, antifungal and antiviral anticholinesterase [29, 30].
18	*Hypericum lancioides* Cuatrec.	Hyperiaceae	Ornamo	It has antidepressant effects, antioxidant, antimicrobial and antiviral properties [31].
19	*Jamesoniella rubricaulis* (Nees) Grolle	Jungermaniaceae	NC	It has antibiotic activity inhibiting the growth of microorganisms [32].
20	*Solanum betaceum* Cav.	Solanaceae	Tomate de árbol	Sore throats, flu, swollen tonsils and tonsils [33].
21	*Oreopanax andreanus* Marchal	Araliaceae	Puma-maqui	Astringent, healing, anti-septic and disinfectant [3].
22	*Oreopanax eriocephalus* Harms	Araliaceae	Maqui maqui	Anti-inflammatory and antibacterial [34].
23	*Siparuna eggersii* Hieron.	Monimiaceae	Monte de Oso	Strokes, diabetes, fractured bones, rheumatism and Kidney problems [1].
24	*Otholobium mexicanum* (L.f.) J.W. Grimes	Fabaceae	Teculen	Antidiabetic, astringent, balsamic, hemostatic, carminative, emmenagogue and vulneraria, stomach pain, indigestion, flatulence, intestinal infections, female contraceptive and "susto" (fright) [1, 35].
25	*Oxalis tuberosa* Molina	Oxalidaceae	Oca	Anti-inflammatory, healing, fever, earache and dermatitis [36,37].
26	*Phyla dulcis* (Trevir.) Moldenke	Verbenaceae	Buscapina	Cramps, diarrhea in children, infection, stomach pain and "susto" (fright) [1].
27	*Fuchsia hybrida* hort. Ex Siebert & Voss	Onagraceae	Pena pena	Relaxant and disinfectant [1].

28	*Sarcorhachis sydowii* Trel	Piperaceae	NC	Antifungal.
29	*Lupinus semperflorens* Hartw. ex Benth.	Fabaceae	Taure de cerro	Fever and stomach pain [2].
30	*Ludwigia peruviana* (L.) H. Hara	Onagraceae	Mejorana de Campo	Hepatic pain, diuretic and kidney problems [1] .
31	*Tropaeolum tuberosum* Ruiz & Pav	Tropaeolaceae	Mashua	Kidney disorders, liver pain, skin eczemas and prostate disorders [38].
32	*Stereocaulon ramulosum* (Sw.) Rausch.	Stereocaulaceae	NC	External infections, antibiotic activity [39].
33	*Macrocarpaea lenae* J.R. Grant	Gentianaceae	Tabaco de cerro	"Mal de ojo" (evil eye).
34	*Piper pseudochurumayo*	Piperaceae	Ámbar ámbar	Respiratory infections, analgesic, antirheumatic, diuretic, digestive, anti-ulcer, dermatological, antidiarrheal, anthelmintic [40].
35	*Prestonia mollis* Kunth	Apocynaceae	Bejuco del cáncer	Cancer, disinfectant and healing of wounds [1] .
36	*Huperzia brevifolia* (Grev. & Hook.) Holub	Lycopodiaceae	Waminga verde	"Susto" (fright)," vaho de agua" (water vapor) and "mal aire" (bad air) [6].
37	*Renealmia alpinia* (Rottb.) Maas.	Zingiberaceae	Cumbià	NC

NC unknown

3. Results and Discussion

3.1 Antioxidant activity and total phenolic

A total of 67 different solvent extracts (Hexane, Dichloromethane, Ethyl acetate, Methanol and alkaloidal extracts) of the 37 species of medicinal plants, traditionally used in southern region of Ecuador for various diseases and disorders, were analysed by ABTS, DPPH and total phenols content. From the 37 medicinal plants, 20 were chosen to prepare alkaloidal extracts, all these species belonging to genera and family well recognized for the presence of alkaloids. Analysis of these 20 alkaloidal extracts indicated that the total phenolic content varied considerably in these extracts with values that ranging from a maximum value of 5222.9 mg GAE/g extract to a minor value of 20.9 mg GAE / g extract (Table 2).

Table 2: Antioxidant activity and total phenolic content of alkaloidal extracts of 20 medicinal plant species from Loja and Zamora Chinchipe

Scientific name	Parts used	Antioxidant Capacity[a]		Total phenol[b]
		ABTS µM TE / mg Ext	DPPH µM TE / mg Ext	mg GAE / g Ext
Huperzia crassa	Aerial parts	765.5 ± 2.9	512.7±1.6	20.9 ± 2.9
Huperzia compacta	Aerial parts	768.4 ± 1.6	616.2 ± 0.4	42.8 ± 2.0
Huperzia espinosana	Aerial parts	766.9 ± 3.1	811.5 ± 16.3	41.6 ± 0.7
Huperzia kuesteri	Aerial parts	524.4 ± 6.4	546.4 ± 0.7	NI
Huperzia brevifolia	Aerial parts	769.8 ± 1.3	723.2 ± 8.9	63.4 ± 1.1
Huperzia tetragona	Aerial parts	760.2 ± 4.4	805.9 ± 4.4	NI
Huperzia columnaris	Aerial parts	766.5 ± 2.7	832.2 ± 5.5	56.4 ± 2.7
Erythroxylum coca	Leaves	765.5 ± 2.7	460.1 ± 6.7	48.5 ± 0.5
Lycopodium complanatum	Leaves	747.5 ± 7.9	370.8 ± 7.2	52.2 ± 1.3
Datura stamonium L	Leaves	581.8 ± 24.7	257.8 ± 17.2	40.2 ± 0.4
Macrocarpaea lenae	Leaves	434.9 ± 13.0	213.4 ± 15.6	46.4 ± 3.6
Phyla dulcis	Leaves, stem	837.2 ± 1.4	888.2 ± 19.2	5222.9 ± 114.2
Alibertia sp.	Leaves, stem	654.6 ± 17.5	310.8 ± 9.8	58.0 ± 2.0
Bejoria resinosa	Leaves, stem	843.3 ± 4.9	900.7 ± 16.1	80.3 ± 10.6
Abutilon striatum	Leaves, stem	533.4 ± 25.7	219.1 ± 20.4	43.0 ± 6.0
Tropaeolum tuberosum	Leaves, stem	364.8 ± 18.9	258.2 ± 38.0	45.9 ± 1.8
Fucsia hybrid	Leaves, stem	836.4 ±13.0	909.7 ± 12,6	1762.6 ± 1.0
Jamesoniella rubricaulis	Leaves, stem	814.1 ± 2.0	903.08 ± 3.4	169.8 ± 25
Sarcohachis sydowill	Leaves, stem	791.0 ± 0.4	946.3 ± 14.9	104.5 ± 4.4
Oxalis tuberosa	Tuber	724.9 ± 6.9	461.8 ± 24.1	25.8 ±0.9

All date shown as means ± SD from alkaloidal extracts, n=3. NI not identified

[a] Date are expressed as micro moles of trolox equivalents (µM TE) per mg extract

[b] Date are expressed as milligrams of gallic acid equivalents (mg GAE) per g extract

In this group, the major number of species belongs to the Lycopodiaceae family, most of these species are used in Ecuador in treatment of *supernatural* or *magical* diseases [6,15,41], and the medicinal action is attributed to the alkaloid content, usually found in phytochemical studies of these species. The highest and remarkable phenolic content was found in *P. dulcis* 5222.9 mg GAE/g of extract, followed from *F. hibrida* 1762.6 mg GAE/g of extract. Other plants considered with high levels of phenolics were *J. rubricaulis* 169.8, *S. sydowii* 104.5, *B. resino*sa 80.3, *H. brevifolia* 63.4 *Alibertia* sp. 58.0 *and H. columnaris* 56.4. While, *Huperzia tetragona* shows the lowest content of total phenols.

The results of antioxidant activity in the alkaloidal extracts by the ABTS test gave values between 364.8 to 843.3 µM TE/mg extract, these are high values if comparing with those reported in literature, being the extract of *B. resinosa* 843.3 the most active, followed in decreasing values by *P. dulcis* 837.2, *F. hybrida*. 836.4, *J. rubricaulis* 814.1, the alkaloid extracts from *H. brevifolia* 769.8, *H. compacta* 768.4, *H. columnaris* and *H. crassa* 766.5, and *O. tuberosa* 724.9 µM TE/mg extract. In general the alkaloid extracts of *Huperzia* spp. (Lycopodiaceae) show the highest values, of antioxidant activity by the ABTS assay.

Regarding to the antioxidant activity of the non-alkaloidal extracts measured by the DPPH assay, the higher activity was found in *S. sydowii* 946.3 µg Trolox/mg, followed by *F.*

hybrida 909.7, *J. rubricaulis* 903.05, *B. resinosa* 900.7, *P. dulcis* 888.2. To the alkaloid extracts, the higher activity value of DPPH were, *H. columnaris* 832.2, *H. espinosana* 811.5, *H. tetragona* 805.9, *H. brevifolia* 723.2, *H. compacta* 616.2, being the *M. lenae* the specie with the lowest value of antioxidant activity with 213.4 µM TE/mg extract, as shown in Table 2. Only with few exceptions the antioxidant activity measured by DPPH method were lower that with the ABTS, however only with limited exceptions the species with higher activity in both tests are in the same group. The above discussed results are very interesting, because these plants rich in alkaloids also presented high antioxidant activity, suggesting that research should be focus in the identification of new classes of natural products with benefits to stop the oxidative process in cells and organs.

In the last years, a number of researches have been carried out in identification of medicinal plants with high content of alkaloids. Chemical compounds which showed to be useful in treatment of brain diseases such as Alzheimer disease, dementia, Parkinson and similar disorders. One of the more important properties considered in these screened plants had been the antioxidant capacity, due to alkaloids like Huperzine, present in several species of *Huperzia*. It is also recognized that many species of Lycopodiaceae family have been used in the traditional medicine to treat these diseases [41,42]. The results here obtained with the Lycopodiacea species are higher than those reported in other species of this family [43,44]. *B. resinosa*, "*pena de cerro",* one of the screened species with higher values in all assays, is a well renowned medicinal plant, it is used as wound healing, as purgative and also to prevent heart failures [45], the flowers specifically had been used to treat arthritis, kidney pain, pulmonary and heart ailments [1]. *B. resinosa*, belonging to Ericaceae family rich in antioxidant compounds such as *Arbustus unedo* (strawberry) [46], *Phyla dulcis*, is known as "*buscapina*", name of a known commercial drug, used as analgesic and for colic. Infusions of this plant have different uses such as relieve of stomachache, diarrheas, infections as to cure the "*espanto*" [1], some species in this genus and in this family had been reported with anticancer and antioxidant activities [47, 48].

Moreover *Huperzia* species are endemic of Ecuador; they are used mostly in ancestral medicine to treat diseases such as *supernatural horror* and vapor of water. From *H. columnaris*, a maceration in aguardiente is used during the *limpias*, a kind of ritual where the *yachak* (healers) blowing the body of the person with this preparation [6]. *H. kuesteri*, has many medicinal uses such as: to treat fevers, contusions, inflammations, diuretic, schizophrenia, myasthenia gravis and as regulator of menstruation [28]. *H. tetragona*, is used as purgative. *H. compacta*, is believed clean the liver and stomach, and also is useful to treat *el mal de aire* (bad air). *H. crassa* and *H. espinosana* commonly are used to cure supernatural diseases such as *shuka*, *espanto*, *mal de aire* (bad air) [6]. Two species of this genus were investigated by AChE inhibitors and antioxidant activity, offering interesting results [49].

A number of 14 extracts of methanol were analysed; the total phenolic content estimated by the Folin-Ciocalteau method, varied widely among all the analysed species, the values ranging from 4578.2 mg GAE/g extract to 55.3 mg GAE/g extract as shown in Table **3**. Similar to alkaloidal extracts, several species showed remarkable levels of phenolic content. The highest value was found in the species *P. pseudochurumayo* 4578.2, followed by *L. peruviana* 4130.2, *S. eggersii* 1572.3, *B. obtusifolia* 1008.7 mg GAE/g extract. Other species with high content were *C. alata*, *G. macrophylla* 666.9 and *G. ulmifolia* 566.8 mg GAE/g extract.

The determination of antioxidant activity by ABTS revealed that species with high values were *C. alata* 791.4, *O. mexicanum 790.9*, *G. ulmifolia* 790.8, *G. macrophyla* 790.7, *Otholobium mexicanum* 790.5, *P. pseudochurumayo* and *L. semperflorens* 790.1, *B. obtusifolia* 789.8, *S. eggersii* 788.1, and µM TE/mg extract. Plants from the Clusiaceae family are recognized with good antioxidant activities, some *Hypericum* species gave better values with DPPH assay that the obtained with the well-studied *H. perforatum* [50–52]. *H. triquitifolium*, showed similar activities that the found in this study [53]. Species belonging to the Fabaceae, are commonly used in the traditional medicine of South American countries, some have demonstrated food antioxidant activity [54, 55].

Table 3: Antioxidant activity and total phenolic content of methanol extracts of 14 medicinal plant species from Loja and Zamora Chinchipe

Scientific name	Parts used	Antioxidant Capacity[a]		Total phenol[b]
		ABTS µM TE / mg Ext	DPPH µM TE / mg Ext	mg GAE / g Ext
Clusia alata	Leaves	791.4 ± 0.5	961.8 ± 3.0	991.1 ± 34.6
Oreopanax eriocephalus	Leaves, stem	473.2 ± 4.7	665.6 ± 42.9	64.9 ± 3.1
Oreopanax andreanus	Leaves, stem	108.9 ± 24.9	75.7 ± 34.5	55.3 ± 2.7
Prestonia mollis	Leaves, stem	226.7 ± 5.6	423.1 ± 20.6	102.4 ± 2.9
Baccharis obtusifolia	Leaves, stem	789.8 ± 0.7	984.4 ± 4.5	1008.7 ± 221.3
Coreopsis venusta	Leaves, stem	790.5 ± 1.1	951.5 ± 23.3	462.6 ± 213.9
Garcinia macrophylla	Leaves, stem	790.7 ± 1.4	869.8 ± 15.4	666.9 ± 109.0
Ludwigia peruviana	Leaves, stem	582.4 ± 29.0	982.8 ± 10.4	4130.2 ± 76.8
Piper pseudochurumayo	Leaves, stem	790.1 ± 1.3	949.3 ± 11.8	4578.2 ± 61.8
Siparuna eggersii	Leaves, stem	788.1 ± 1.4	937.9 ± 34.1	1572.3 ± 70.1
Guazuma ulmifolia	Leaves, stem	790.8 ± 0.7	856.7 ± 5.6	566.8 ± 159.5
Lupinus semperflorens	Leaves, stem	790.1 ± 2.0	317.3 ± 30.2	118.7 ± 1.6
Otholobium mexicanum	Leaves, stem	790.9 ± 1.1	918.1 ± 5.6	97.6 ± 4.7
Euphorbia weberbaueri	Latex	489.3 ± 3.6	152.5 ± 17.0	90.1 ± 2.4

All date shown as means ± SD from methanol extracts, n=3

[a] Date are expressed as micromoles of trolox equivalents (µM TE) per mg extract

[b] Date are expressed as milligrams of gallic acid equivalents (mg GAE) per g extract

The results of antioxidant activity of methanolic extracts by DPPH assay showed that the extract from the species *B. obtusifolia* was the most active with a value of 984.4 µM TE/mg extract, followed very close of *L. peruviana* 982.8, *C. alata* 961.8, *Coreopsis venusta* 951.8, *P. pseudochurumayo* 949.3, *S. eggersii* 937.9, and *O. mexicanum* with 918.1 and the specie with the smallest antioxidant activity was found in *Euphorbia weberbaueri* with 152.5 µM TE / mg extract.

Similar to the results found in the alkaloids extracts, the values obtained in antioxidant activity with DPPH assay were higher that the found with ABTS in methanol extracts and mostly the same species gave better activity.

The analyses of Hexane, Dichloromethane, and Ethyl acetate extracts were done with 14 species and as expected the values in polyphenol content and antioxidant activity, in general, decline with the polarity of the used solvent (Table 4). However it was not easy to establish a clear trend in the three tests with these extracts, due to differences with each analysed species. The polyphenol content showed that the species with high content were *Abutilon striatum* (EtOAc) 2415.3 µM TE/mg extract, *J. rubricaulis* (EtOAc) 169.0, *Fuchsia* sp. (EtOAc) 155.0, *B. resinosa* (EtOAc) 141.0, and *Alibertia* sp. (CH_2Cl_2) 93.9 µM TE/mg extract.

In the ABTS results, values are very disperse among solvents and also among species, the highest value was found in *Fuchsia* sp. (EtOAc) 839.1 µM TE/mg extract, and with close values for *B. resinosa* (EtOAc) 834.4, *J. rubricaulis* (EtOAc) 808.1, *Alibertia* sp. (CH_2Cl_2) 693.9 and *P. dulcis* (EtOAc) 692.6 µM TE/mg extract.

The values found for DPPH assay (Table 4) indicated that *B. resinosa* (EtOAc) 1045.6 µM TE/mg extract shows the better antioxidant activity followed by *F. Hybrida* (EtOAc) 951.8, *J. rubricaulis* (EtOAc) 877.2, *P. dulcis* (EtOAc) 589.2, *B. resinosa* (EtOAc) 578.7 and *H. lancioides* (EtOAc) 486.2. It was evident that many of these medicinal plants species displayed strong antioxidant activities, and not only in extracts obtained with polar solvents like methanol but also in lipophilic extracts, were are not usually, the more important to see compounds with this action.

Table 4: Antioxidant activity and total phenolic content of hexane, dichloromethane and ethyl acetate extracts of 9 medicinal plant species from Loja and Zamora Chinchipe

Scientific name	Parts used	Solvent	Antioxidant Capacity[a]		Total phenol[b]
			ABTS µM TE / mg Ext	DPPH µM TE / mg Ext	mg GAE / g Ext
Hypericum lancioides	Leaves, stem	Hexane	311.1 ± 5.8	256.0 ± 9.3	29.3 ± 2.2
		Dichloromethane	501.9 ± 11.3	424.6 ± 14.4	12.6 ± 2.4
		Ethyl acetate	523.3 ± 3.5	486.2 ± 7.0	48.8 ± 2.9
Alibertia sp.	Leaves, stem	Hexane	58.6 ± 20.6	68.5 ± 15.1	12.6 ± 1.2
		Dichloromethane	693.9 ± 23.9	220.3 ± 24.1	93.9 ± 3.7
		Ethyl acetate	526.0 ± 31.9	360.3 ± 18.9	46.9 ±1.1
Phyla dulcis	Leaves, stem	Hexane	108.9 ± 20.1	42.5 ± 19.4	1.8 ± 1.3
		Dichloromethane	469.3 ± 3.2	57.1 ± 11.5	6.5 ± 1.5
		Ethyl acetate	692.6 ± 47.8	589.2 ± 12.6	53.1 ± 6.3
Solanum betaceum	Seeds	Hexane	194.9 ± 5.9	68.2 ± 14.7	9.7 ± 1.6
		Dichloromethane	43.4 ± 4.6	104.3 ± 25.8	58.1 ± 2.9
		Ethyl acetate	498.9 ± 6.3	1045.6± 11.0	141.0 ± 6.0
Bejoria resinosa	Leaves, stem	Hexane	171.2 ± 19.0	111.8 ± 6.1	25.5 ± 2.6
		Dichloromethane	342.4 ± 14.6	172.5 ± 9.8	54.3 ± 0.9
		Ethyl acetate	834.4 ± 0.8	578.7 ± 5.0	22.9 ± 6.1
Abutilon striatum	Leaves, stem	Hexane	58.9 ± 2.1	44.7 ± 4.6	18.3 ± 0.9
		Dichloromethane	190.7 ± 16.4	87.6 ± 8.2	22.8 ± 2.6
		Ethyl acetate	271.6 ± 5.8	164.9 ± 24.3	2415.3 ± 110.0
Fucsia hybrid	Leaves, stem	Hexane	116.5 ± 2.2	88.3 ± 2.8	16.9 ± 1.9
		Dichloromethane	130.3 ± 15.8	NI	19.3 ± 2.9
		Ethyl acetate	839.1 ± 1.0	951.8 ± 17.5	155.0 ± 4.0
Stereocaulom ramulosum	Leaves, stem	Hexane	685.1 ± 23.2	224.6 ± 8.2	80.2 ± 1.8
		Dichloromethane	659.0 ± 18.7	218.7 ± 8.5	72.6 ± 4.5
		Ethyl acetate	467.6 ± 18.5	167.7 ± 11.9	61.8 ± 2.1
Jamesoniella rubricaulis	Leaves, stem	Hexane	97.2 ± 8.2	320.5 ± 9.5	26.1 ± 2.4
		Dichloromethane	149.7 ± 9.8	69.97 ± 7.6	15.7 ± 2.2
		Ethyl acetate	808.1 ± 4.6	877.2 ±9.4	169.0 ± 3.1
Oxalis tuberosa	Tuber	Hexane	505.3 ± 17.9	111.1 ± 2.3	32.0 ± 3.5
		Dichloromethane	81.8 ± 3.9	78.9 ± 0.8	11.1 ± 1.9
		Ethyl acetate	75.1 ± 6.2	94.7 ± 18.1	19.1 ± 1.2

All date shown as means ± SD from hexane, dichloromethane and ethyl acetate extracts, n=3. NI not identified

[a] Date are expressed as micromoles of trolox equivalents (µM TE) per mg extract

[b] Date are expressed as milligrams of gallic acid equivalents (mg GAE) per g extract

A final analysis of the above results indicated that some of the species of this group of medicinal plants used in Ecuador, showed remarkable indices with the DPPH assay. In the 14 species analysed with methanol extracts 8 (57%) of them were found to be over de 700 µM TE/mg extract. From the 20 alkaloidal extracts 14 (70%) of this group had antioxidant activity with the ABTS upper 700 µM TE/mg extract, and the rest higher of 300 µM TE/mg extract.

When comparing the general results, with some reported in literature, it was found that some species of Rubiacea are reported with similar antioxidant activity of *Alibertia* sp. [56]. However some species in this study, like *Phyla dulcis* (Verbenaceae), *Datura stramonium* L. (Solanacea) and *Tropaeolum tuberosum* (Tropaeolaceae) showed superior antioxidant activity compared with species of the same family [38]. The comparison with studies of species from the same genera could be more interesting, but in general many of the screened plants had not been reported before with the properties herein described. Future investigations are encouraged with the purpose to identify the substances responsible of the antioxidant activities, of these medicinal plants which form part of this study. Regarding to the ecological status according to the Libro Rojo de plantas medicinales del Ecuador [25], we report the use and the

antioxidant activities from five endemic plants, all used in traditional medicine in the south-Ecuador. *H. columnaris*, *H. compacta*, *H. espinosana*, *O. andreanus* and *S. eggersii*. On the basis of the results of antioxidant test we propose the hypothesis that the endemic plants used in traditional medicine of Ecuador have an important role as antioxidant effect. It represents the starting point for future studies.

3.2 IC_{50} of DPPH and ABTS

After the analysis of the above mentioned results, it was evident that the methanol extracts are those with high activity. The ethyl acetate, dichloromethane and alkaloid extracts gave results very irregular, while also was evident that the hexane extracts offered the lowest values in all the assays performed. The samples considered with high levels of antioxidant activities (> 400 µM TE/mg extract) in DPPH and ABTS assays were choose to calculate the IC_{50}, defined as the concentration of extract necessary to give an inhibition of 50% of the initial concentration of DPPH or ABTS radical. A lower value of IC_{50} is indicative of a higher antioxidant capacity (Huang *et al*., 2005) (Table 5).

Table 5: Inhibitory activities (IC_{50}) on DPPH and ABTS assays of medicinal plant species from Loja and Zamora Chinchipe

Scientific name	Solvent	IC_{50} (µg extract/mL)[a]	
		ABTS	DPPH
Abutilon striatum	Alkaloidal extract	>1000	>1
Alibertia ssp.	Ethyl acetate	>1000	>1
	Dichloromethane	280	>1
	Alkaloidal extract	>1000	>1
Bacharis obtusifolia	Methanol	480	490
Bejoria resinosa	Alkaloidal extract	170	310
Clusia alata	Methanol	80	90
Coreopsis venusta	Methanol	180	>1
Datura stamonium	Alkaloidal extract	500	>1
Erythroxylum coca	Alkaloidal extract	120	>1000
Euphorbia weberbaueri	Methanol	160	>1000
Fucsia hybrida	Alkaloidal extract	90	160
	Ethyl acetate	120	150
Garcinia macrophylla	Methanol	50	80
Guazuma ulmifolia	Methanol	140	170
Hipericum lancioide	Ethyl acetate	250	>1000
	Dichloromethane	10	>1000
Hueprzia compacta	Alkaloidal extract	140	>1000
Huperzia brebifolia	Alkaloidal extract	110	400
Huperzia columnaris	Alkaloidal extract	140	190
Huperzia crassa	Alkaloidal extract	90	>1000
Huperzia espinosana	Alkaloidal extract	>1000	>1000
Huperzia kuesteri	Alkaloidal extract	500	>1000
Huperzia tetragona	Alkaloidal extract	170	410
Ludwigia peruviana	Methanol	80	90
Lupinus serpenflorens	Methanol	250	>1000
Lycopodium complanatum	Alkaloidal extract	140	380
Macrocarpaea lenae	Alkaloidal extract	120	270
Oreopanax eriocephalus	Methanol	500	>1000
Otholobium mexicanum	Methanol	470	150
Oxalis tuberosa	Hexane	460	>1000
	Alkaloidal extract	>1000	>1000
Phyla strigulosa	Alkaloidal extract	210	310
	Ethyl acetate	400	>1000
	Dichloromethane	>1000	>1000
Piper pseudochurumayo	Methanol	10	10
Prestonia mollis	Methanol	500	>1000
Renealmia alpinia	Dichloromethane	500	>1000
Sarcohachis sydowil	Alkaloidal extract	70	100
Siparuna eggerssi	Methanol	160	170
Solanum betaceum	Ethyl acetate	>1000	>1000
Stereocaulom ramulosam	Ethyl acetate	>1000	>1000

All date shown as means ± SD from IC_{50} in DPPH and ABTS assays of, n=3

[a] Date are expressed as micrograms of extract (µg extract) per mL

The calculated IC_{50} by ABTS gave more relevant species (Figure 2) *H. lancioides* and *P. pseudochurumayo* both species with 10 µg extract/mL, other species considered with moderate values were *G. macrophylla* 50 µg extract/mL, *L. peruviana* and *C. alata* with 80 µg extract/mL, *S. sidowii* 70 µg extract/ mL, *H. crassa* 90 µg extract/mL, *Fuchsia* sp. 90 µg extract/mL. The rest of species showed values too high to be considered in this discussion.

The results of IC_{50} calculated with DPPH, are very different of the ABTS. The species considered with significant IC_{50} was *P. pseudochurumayo* 10 µg extract/mL, other species with moderate concentrations are: *L. peruviana* 90 µg extract/mL, *G. macrophylla* 80 µg extract/mL, *C. alata* 90 µg

extract/mL. The Figure 3 shows the curves obtained with the seven species with better IC_{50}.

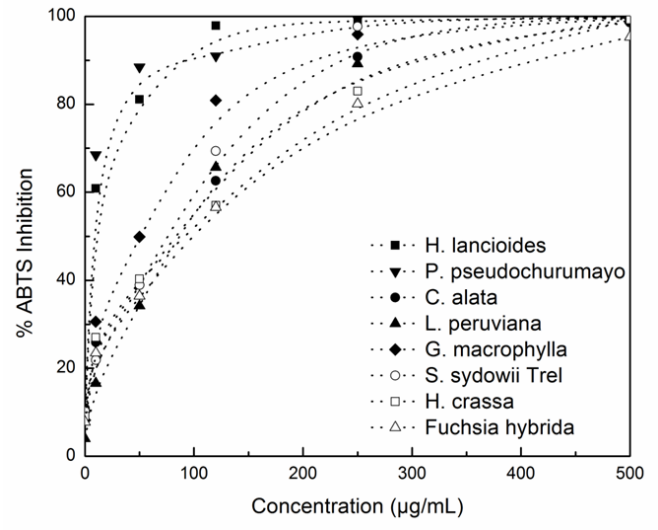

Fig 2: Graphic of IC_{50} by ABTS

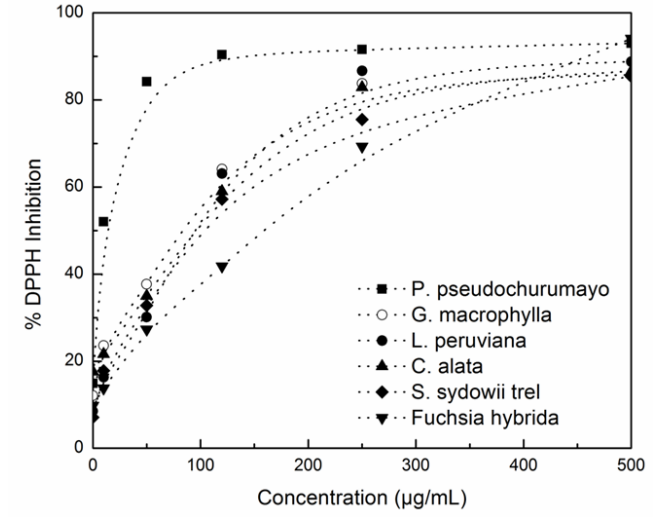

Fig 3: Graphic by IC_{50} DPPH

4. Discussion

The results of this research that account for the evaluation for free radical scavenging and determination of the total phenolic content of 37 medicinal plants, strongly support that some medicinal plants used in the south region of Ecuador are rich in phenolic constituents, and several species showed remarkable antioxidant activity. The compounds responsible of these actions could be the accountable of the therapeutic action, claimed in the ancestral uses of these medicinal plants. Some botanical families showed the best results, being the Piperaceae, Onagraceae, Clusiaceae, Ericaceae and Lycopodiaceae the outstanding in this study which included species from 23 different families. The effects of some species indicate that these plants may be considered as good source of antioxidants for nutraceuticals or ingredients that will be used in the food industry. It is noteworthy that several species belonging to the Lycopodiaceae family, showed excellent values in antioxidant activity, becoming in excellent candidates to investigate as new sources of antioxidants.

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