Chapter 3

Advances in Studies of *Vernonanthura patens* (Kunth) H. Rob. Growing in Ecuador

P.I. Manzano, M. Miranda, M.F. Quijano and L. Monzote

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/59866

1. Introduction

Asteracea family (Asteraceae) includes more than 23,000 species and is the group of Angiosperms with the richest biodiversity existing in the world. *Vernonia* (*Vernonanthura*) is the most representative gene among 1000 species [1 - 4].

Various chemical components have been reported for the genus: sesquiterpene lactones of glaucolide type, hirsutinolide, vernomargolide, eudesmanolide, cardinanolide, and elephantopus, reported for most species, with some exceptions [5-11]. Moreover, diterpenes derived from ent-kaurano and kaurano, pentacyclic triterpenoids from oleanane and ursane, phytosterols, and flavonoids have been isolated and identified [12-15], while the antimicrobial and molluscicidal pharmacological activities have been the most investigated [15-17].

Within the genus *Vernonia*, *Vernonanthura patens* has been one of the less studied species. It is a shrub that grows wild in Ecuador and is widely used by people in rural areas, who attribute various medicinal properties among them: analgesic, anti-inflammatory, anticancer, and antileishmanial. However, medicinal use, studies on the chemical composition, and pharmacological activities until 2010 were very few. The first information collected on the chemical composition of the species reported the absence of sesquiterpene lactones [18], which constituted a chemical marker of gender. Nevertheless, in 1986, the aerial parts were studied, isolated and 10 lactones of a species were identified, existing contradictions on the composition [19]. Jakupovic and Schmedia-Hirschmann, With respect to biological activity, less information exists. The first, was the effect demonstrated by a methanol extract of the leaves against *Artemia salina*, the inertness to the potato disc tumor produced by the introduction of *Agrobacterium tumefaciens*, and the lack of cytotoxicity against cell lines V79 [20]. The antimalarial activity of

aqueous extract of leaves has been described and a mean inhibitory concentration (IC_{50}) of 38.7 ± 10.1 μg/ml against *Plasmodium falciparum* has been reported [21], *whereas* others authors have reported that an aqueous extract of leaves, got antileishmanial activity against strains of *Leishmania amazonensis* with an IC_{50} >100 μg/ml, being these the only reports found for the species [22].

1.1. Studies in the species *V. patens* in the Ecuadorian coast

In an earlier chapter of this book, has been published the results for the fractionation of a methanol extract of the species, the study of fractions as antifungal against strains of *Fusarium oxysporum* and *Penicilium notatum*, and the chemical characterization by gas chromatography/mass spectrometry of the fraction that showed that activity. The hexane fraction presented antifungal activity against these strains, and 33 compounds were possible to identify. Three pentacyclic triterpenoids (lupeol, epilupeol, and lupeol acetate), presented as major components, were also isolated and characterized for fractions[23]

Unlike other species of *Vernonanthura* and the own *V. patens* living in other regions of South America, the one that grows in Ecuador presents as major components pentacyclic triterpenoids and no sesquiterpene lactones. These results forced to perform a genetic characterization of the genes from the plastid rbcL and matK of leaf chloroplast DNA fraction, and it confirmed that the species was *V. patens* [24, 25]

This research group has continued with studies to verify the identity of the species, obtaining the identification of 53 compounds from leaves of a methanolic extract within the terpene compounds are highlighted (oxygenated sesquiterpenoids and triterpenoids), aliphatic hydrocarbons, fatty acids, and their methyl and ethyl esters and sugars [26, 27].

In others studies, two types of waxes of leaves and fat fractions of stems and flowers were isolated and were identified 29 fatty acids and 8 triterpenoids as components of the lipid fractions of these organs, which had not been previously reported (Tables 1 and 2) [28]. Figure 1 presents some of the chemical structures identified in this species.

Regarding biological studies, the antileishmanial evaluation of ethanolic extract of the leaves and stems was performed, proving the traditional use of the leaves of the species as antileishmanial. The ethanol extract of stems was highly toxic. Leaves extract showed a higher selectivity index than pentamidine, used as reference drug [29].

	Compounds	Percent relative abundance			
		H_SC_B	H_SC_N	T_SC	F_SC
1	Nonanedioic acid dimethyl ester	0.37	-	0.01	-
2	Tetradecanoic acid	0.31	0.26	0.03	0.23
3	Pentadecanoic acid	0.31	0.24	0.06	0.15
4	Acid, 9-hexadecenoic (ISOM)	0.33	0.45	0.04	-

http://dx.doi.org/10.5772/59866

	Compounds	Percent relative abundance			
		H_SC_B	H_SC_N	T_SC	F_SC
5	Hexadecanoic acid	74.24	57.74	13.31	47.50
6	Hexadecanoic acid ethyl ester	-	-	-	0.26
7	(Z)-9-hexadecenoic acid	0.22	-	-	-
8	2-Hexadecenoic acid	-	0.29	-	-
9	Heptadecanoic acid	0.21	0.27	0.06	0.11
10	8,11-Octadecadienoic acid	-	-	-	7.52
11	7, 8,11-Octadecatrienoic acid	-	6.1	-	-
12	11-Octadecenoic acid	1.15	2.6	-	-
13	8-Octadecenoic acid	-	-	0.40	-
14	Octadecanoic acid	2.36	1.71	0.43	1.32
15	Nonadecanoic acid	0.05	-	-	-
16	9,12-Octadecadienoic acid	-	1.82	1.41	0.56
17	9,11-Octadecadienoic acid	-	0.33	0.18	-
18	9,12,15-Octadecatrienoic acid	-	0.31	-	2.14
19	9,13,15-Octadecatrienoic acid	-	0.72	-	
20	Eicosanoic acid	3.26	2.66	0.17	0.88
21	Heneicosanoic acid	0.38	0.34	-	-
22	Docosanoic acid	3.86	2.88	0.40	3.70
23	Tricosanoic acid	1.2	0.90	0.11	0.56
24	Tetracosanoic acid	3.69	2.70	0.54	7.24
25	Pentacosanoic acid	0.93	0.71	0.11	0.46
26	Hexacosanoic acid	2.79	2.17	0.95	7.32
27	Heptacosanoic acid	0.24	0.17	-	0.19
28	Octacosanoic acid	0.87	0.89	0.79	2.25
29	Triacontanoic acid	0.10	0.33	0.37	0.47

H_SC_B = saponifiable white wax fraction leaves; H_SC_N = saponifiable orange wax fraction leaves; T_SC = saponifiable wax fraction stems, F_SC = saponifiable wax fraction flowers.

Table 1. Compounds identified in the fatty acid fractions of leaves, stems and flowers of *Vernonanthura patens*

All these studies have contributed to knowledge of the species growing in the Ecuadorian coast and serve as reference for other studies.

Pico	Compound	Relative abundance %			
		H_IC_B	H_IC_N	T_IC	F_IC
1	β-Amyrin	2.45	15.52	28.5	19.8
2	α-Amyrin	-	-	-	66.83
3	Lupeol	97.5	88.48	-	0.49
4	α-Amyrin + lupeol	-	-	55.59	-
5	Glutinol	-	-	2.70	-
6	Taraxasterol acetate	-	-	1.93	-
7	Taraxasterol	-	-	-	7.9
8	Neoganmacer 22(29)-en-3.ol	-	-	-	4.9

H_IC_B and H_IC_N, unsaponifiable leaves fractions; T_IC, unsaponifiable stems fractions; F_IC, unsaponifiable flowers fractions

Table 2. Compounds identified in the unsaponifiable fractions *Vernonanthura patens*

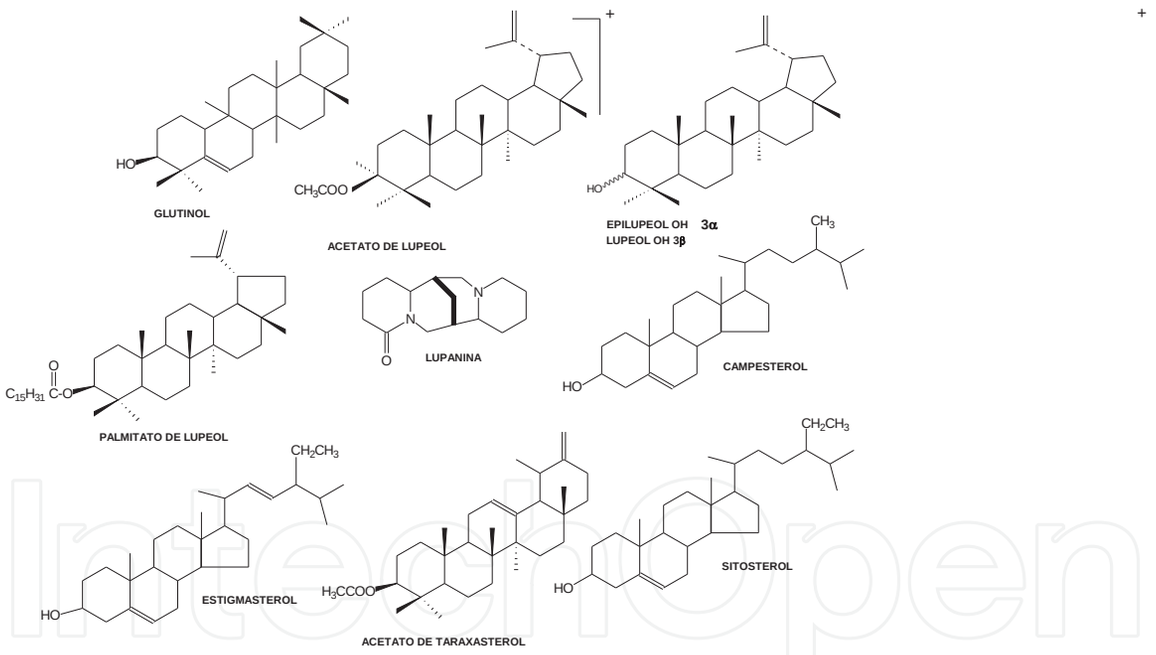

Figure 1. Some compounds isolated from *Vernonanthura patens.*

2. Materials and methods

Leaves, flowers, and stems of the species in the phenological stage of flowering were used and collected around the Biotechnology Research Center of Ecuador located at Km. 30.5 via Perimeter province of Guayas, Ecuador. A sample of the plant material was taken for botanical

http://dx.doi.org/10.5772/59866

identification, which was botanized at the National Herbarium of Ecuador (QCNE), Quito, with the key CIBE37a.

2.1. Extraction

The extraction from the aerial parts of the species (leaves, flowers, and stems) was performed in water and ethanol by triplicate in an ultrasonic bath VWR of 35 KHz power [30]. In all cases, 10 g of sample was extracted in the solvent (water or ethanol), in the following time intervals: 5, 15, 30, 45, and 60 min.

2.2. Determination of antioxidant activity

The determination of antioxidant activity was performed based on the stability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to alcoholic and aqueous extracts obtained from the aerial parts. A total of 800 μl of 0.1 N DPPH and 200 μl of the extracts were taken, and the absorbance was measured at 517 nm after 30 min using 200 μl of ethanol and 800 μl of DPPH as control. Determinations were performed in triplicate.

2.3. Determination of total polyphenols

The total polyphenol content was measured by the Folin-Ciocalteu method in a spectrophotometer (BioTek), at 760 nm using gallic acid monohydrate (CAS 149-91-7) as patron of the calibration curve. Results were expressed as milligrams of gallic acid per gram of sample (mg GA/g sample). A total of 250 μl of sample and 350 μl of Folin-Ciocalteu 1 N were mixed; 5 min after, 350 μl of 20% of sodium carbonate was added. After the 90-min incubation at room temperature, the absorbance was measured at 760 nm. Determinations were performed in triplicate.

2.4. Chromatographic profile of the extracts by HPLC

Chromatographic profiles by high-resolution liquid chromatography (HPLC) were performed in all extracts obtained at different extraction times to determine whether the extraction time produces chemical changes in the extracts. HPLC analysis was performed with Perkin Elmer Series 2000 HPLC with TotalCrom Software operating system. The phenolic compounds were detected at 280 nm with a flow rate of 1 ml/min. C18 column was used at a temperature of 30°C. Separations were carried out in a pumping system by varying the proportion of 2.5% (v/v) acetic acid in water (mobile phase A) and 70% methanol in water (mobile phase B). The solvent gradient elution program was as follows: 10% to 26% B (v/v) in 10 min, to 70% B at 20 min, and finally to 90% B at 25 to 31 min. The injection volume for all samples was 10μL.

2.5. Statistical analysis

A factorial design 2 × 3 × 5 was used involving the categorical factors: extraction solvent (water and alcohol), plant organ (leaves, flowers, and stems), and extraction time (5, 15, 30, 45, and 60 min), with antioxidant activity and total polyphenol content as response variables.

2.6. Antileishmanial activity

This test was performed with aqueous extracts obtained as described.

2.6.1. Aqueous extract

Extraction was carried out by decoction of plant materials in proportion of 10% in water during 20 min. The aqueous extract was evaporated to 87ºC and 400 mmHg. Finally, the concentrated extract was lyophilized to 120×10^{-3} mbar and 47ºC below zero.

2.6.2. Reference drug

Pentamidine (Richet, Buenos Aires, Argentina) diluted in sterile distilled water was used for *L. amazonensis*. *L. amazonensis* (MHOM/77BR/LTB0016) was kindly provided by the Department of Immunology, Oswaldo Cruz Foundation (FIOCRUZ), Brazil. Parasites were routinely isolated from mouse lesions and maintained as promastigotes at 26ºC in Schneider's medium (Sigma, St. Louis, MO, USA) containing 10% heat-inactivated fetal bovine serum (HFBS) (Sigma, St. Louis, MO, USA), 100 mg of streptomycin/ml, and 100 U penicillin/ml. The parasites were not used after the tenth passage.

2.6.3. Antileishmanial activity

The activity of the extracts against intracellular amastigotes was evaluated as described previously. The peritoneal macrophages were harvested and plated at 106/ml in 24-well Lab-Tek (Costarâ, USA) and incubated at 37ºC under an atmosphere of 5% CO_2 for 2 h. Nonadherent cells were removed by washing with prewarmed phosphate-buffered saline (PBS). Stationary-phase *L. amazonensis* promastigotes were added at a 4:1 parasite/macrophage ratio, and the cultures were incubated for further 4 h. The cell monolayers were washed three times with prewarmed PBS to remove free parasites. Then 999 ml of RPMI completed medium and 1 ml of the different products dissolved in DMSO were added in duplicate for further 48 h. The cultures were then fixed in absolute methanol, stained with Giemsa, and examined under a light microscope. The number of intracellular amastigotes was determined by counting the amastigotes in 100 macrophages per each sample. In addition, the percentage of infected macrophages was calculated. The results were expressed as percent of reduction of the infection rate in comparison to that of the controls, where the infection rates were obtained by multiplying the percentage of infected macrophages by the number of amastigotes per infected macrophages [31]. The IC_{50} value was determined from the linear regression of concentration–response curves.

The IC_{50} of the extracts for the viability of mouse peritoneal macrophages was determined. Twenty-two macrophages were collected from peritoneal cavities of normal BALB/c mice in ice-cold RPMI 1640 medium (Sigma, St. Louis, Mo, USA) supplemented with antibiotics and seeded at 30,000 cells/well. The cells were incubated for 2 h at 37ºC in 5% CO_2. Nonadherent cells were removed by washing with PBS, and then 1 μl of product solution was added to 200 μl medium containing 10% HFBS and antibiotics. Macrophages treated with 1 μl DMSO were included as controls. The cytotoxicity was determined using the colorimetric assay with 3-[4,5-

http://dx.doi.org/10.5772/59866

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA). MTT solutions were prepared at 5 mg/ml in saline solution, filtered, and sterilized at the moment of use, and 15 μl was added to each well. After incubation for an additional 3 h, the formazan crystals were dissolved by addition of 100 μl DMSO. The optical density was determined using an EMS Reader MF Version 2.4-0, at a test wavelength of 560 nm and a reference wavelength of 630 nm [32]. The IC50 was obtained by fitting a sigmoidal Emax model to dose–response curves. Selectivity indexes were calculated by dividing the IC50 for peritoneal macrophage of BALB/c mice by the IC50 for *L. amazonensis* amastigotes. The IC50 of the extracts for the viability of mouse peritoneal macrophages was determined [31].

3. Results and discussions

3.1. Determination of antioxidant activity

The percentage of antioxidant activity by the method of DPPH of the aqueous and the alcoholic extracts of different plant organs, which were obtained at different times of ultrasonic extraction, were determined and differences in time of extraction were observed.

The highest percentage of antioxidant activity was obtained by employing an extraction time of 5 min for the aqueous extracts of leaves and flowers. However, the highest percentage of activity was obtained at 15 min of extraction for the aqueous extract of stems.

Alcoholic extracts of leaves and stems showed no activity at any time of extraction; in contrast, a greater activity at 45 and 60 min of extraction was observed in flowers of *V. patens* with no differences of significance between these times ($p > 0.05$) (Table 3, Figure 2).

Aerial part of the plant	Extraction time				
	5 min	15 min	30 min	45 min	60 min
Leaves	80.98 ± 1.7 Aa	48.36 ± 6.69 Ba	51.68 ± 3.14 Ba	54.16 ± 4.95 Ba	44.72 ± 4.33 Ba
Flowers	82.85 ± 3.79 Aa	73.85 ± 5.83 ABb	62.63 ± 2.69 BCb	68.05 ± 8.77 BCa	56.26 ± 5.38 Cb
Stems	35.10 ± 9.18 Ab	78.76 ± 6.09 Bb	59.14 ± 5.65 Cab	61.35 ± 2.84 Ca	54.60 ± 2.97 Cb
Flowers (ethanol)	34.24 ± 6.36 Ab	28.07 ± 3.29 Ac	69.34 ± 3.09 Bb	79.42 ± 3.29 BCb	80.82 ± 2.60 Cc

Mean values in the same column or row followed by the same capital or lowercase letters, respectively, are not significantly different ($p > 0.05$).

Table 3. Antioxidant activity (%) of aerials parts of *Vernonanthura patens*

There is only one antecedent of antioxidant activity for the *Vernonia* genre, for the species *Vernonia tweedieana*, with no previous studies for *V. patens* [33].

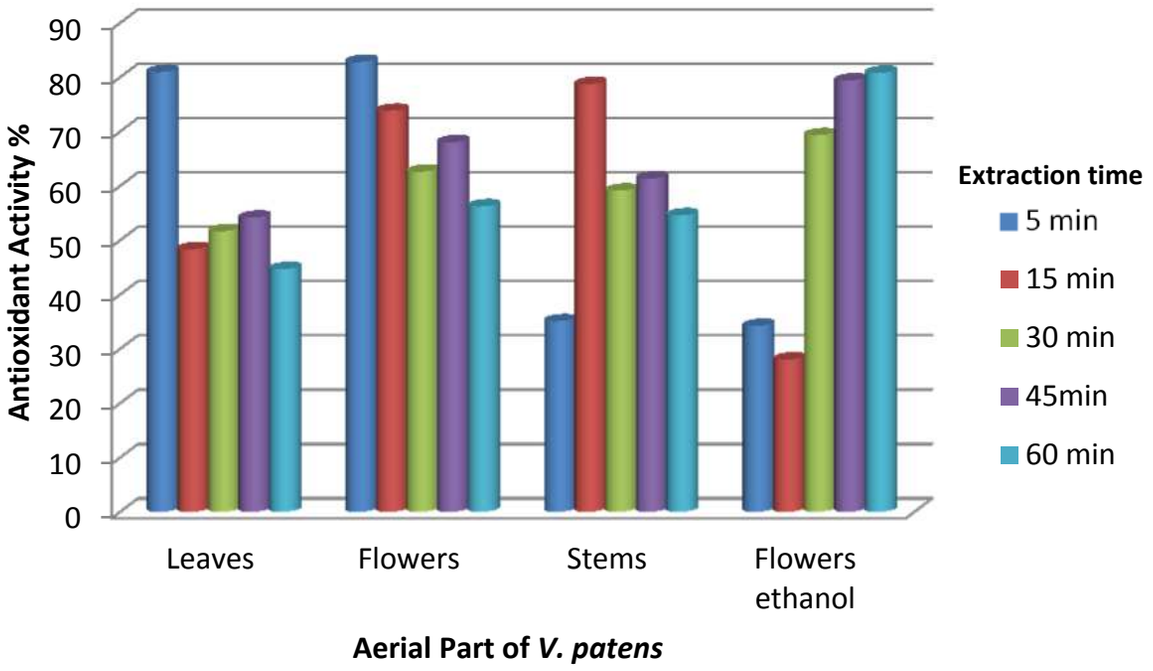

Figure 2. Antioxidant activity of aerials parts of *Vernonanthura patens* according to extraction time.

3.2. Determination of total polyphenols

The part of the plant, the extraction time, and the solvent employed have influence in the content of polyphenols.

No polyphenol could be quantified when ethanol was used as solvent, possibly because this solvent removes other colored chemical compounds such as chlorophyll, carotene, and other pigments that interfere with the spectrophotometric determination of polyphenols in the extract.

When water is used as solvent, there is a direct relationship between the extraction time and the concentration of polyphenols; that is, the longer the extraction, the higher the concentration independent of the plant organ used (Figure 3).

In all cases, the highest percentage of total polyphenols was obtained at 45 min of aqueous extraction without significant differences ($p > 0.05$) with 60 min of extraction. At this time of extraction (45 min), the leaves of the species exhibited the highest percentage of polyphenols, followed by flowers. Stems exhibited the lowest percentage of polyphenol (Table 4).

There are several reports regarding the presence of phenolic compounds in the different plant organs for the *Vernonanthura* (*Vernonia*) genre. Flavonoids have been designated as constituents of gender [34]. Flavonoids, tannins, and phenolics are reported for leaves of *V. cinerea* [35], whereas others authors have reported the presence of flavonoids [14]. For flowers of the same species, flavonoids, tannins, and phenolic compounds have been identifiedas well [35]. Flavonoids and tannins has been detected for leaves of *Vernonia amigdalina*, [36, 37].

http://dx.doi.org/10.5772/59866

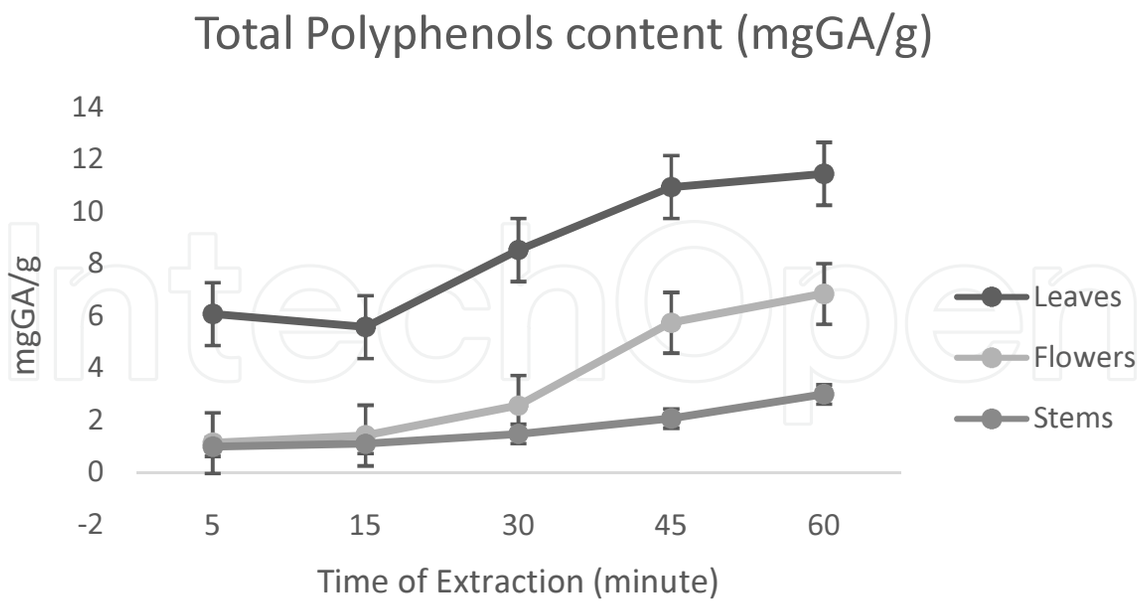

Figure 3. Polyphenol concentration *vs* time for different plant organs.

Aerial part of the plant	Extraction time				
	5 min	**15 min**	**30 min**	**45 min**	**60 min**
Leaves	6.07 ± 1.7 Aa	5.57 ± 0.22 Aa	8.53 ± 1.2 Aa	10.95 ± 0.91 Ba	11.46 ± 1.94 Ba
Flowers	1.12 ± 0.12 Ab	1.41 ± 0.17 Ab	2.55 ± 0.67 Ab	5.74 ± 0.06 Bb	6.85 ± 0.96 Bb
Stems	0.98 ± 0.06 Ac	1.09 ± 0.14 Ac	1.47 ± 0.22 ABc	2.06 ± 0.48 BCc	2.99 ± 0.49 Cc

Mean values in the same column or row followed by the same capital or lowercase letters, respectively, are not significantly different ($p > 0.05$)

Table 4. Total polyphenol content (mg GA/g) of aerial parts of *Vernonanthura patens* using water as solvent.

Phenolic compounds, tannins, and flavonoids among other compounds in the leaves, stems, and flowers of the species *V. patens* of Ecuadorian coast have been reported [38]. This may explain the total polyphenol content found in different plant organs studied, although in not a very high proportion.

3.3. Chromatographic profile of the extracts by HPLC

Considering the fact that the extraction method using ultrasound sometimes causes changes in the chemical composition of the extracts, a chromatographic profile was performed to the aqueous extracts obtained at 5 and 45 min of extraction.

Some changes in the chromatographic profile of the aqueous extract of leaves at 45 min are observed (Figure 4), especially in the baseline, although the highest chromatographic peak intensity observed at about 56.9 min has no variation.

Chromatographic profile changes are minor in the aqueous extract of the flowers; a major chromatographic peak around 56.6 min is also seen (Figure 5).

Nevertheless, the aqueous extract of the stems suffered no change in the chromatographic profile at different extraction times analyzed (5, 15, and 45 min). The retention time of the major peak was found at 56.9 min (Figure 6).

Only the study for alcoholic extracts of flowers was done because it was the only one that showed antioxidant activity. Minimal changes were observed in the chromatographic profile of 5 and 45 min extraction, which may be due to the transmission of the ultrasonic waves in the ethanol solvent, which are lower than when water is used [38, 39]. A chromatographic peak around 57 min was also observed in this extract (Figure 7).

The results lead us to believe that in all plant organs of the species obtained with water and the alcoholic extract of the flowers, there is a major component or a mixture thereof, which eluted at a similar retention time by HPLC, in the studied conditions, which may or may not be responsible for the antioxidant activity found for these compounds. However, it is important to note that no correlation between the polyphenol content of the extracts and the antioxidant activity was found. In all cases, the higher the percentage of antioxidant activity, the lower the percentage of total polyphenolics (Figure 8).

These results indicate that the antioxidant activity of aqueous extracts of the species is not due solely to the presence of polyphenolic compounds that remain to determine the chemical composition of these extracts to determine which one or more compounds are influential in this activity.

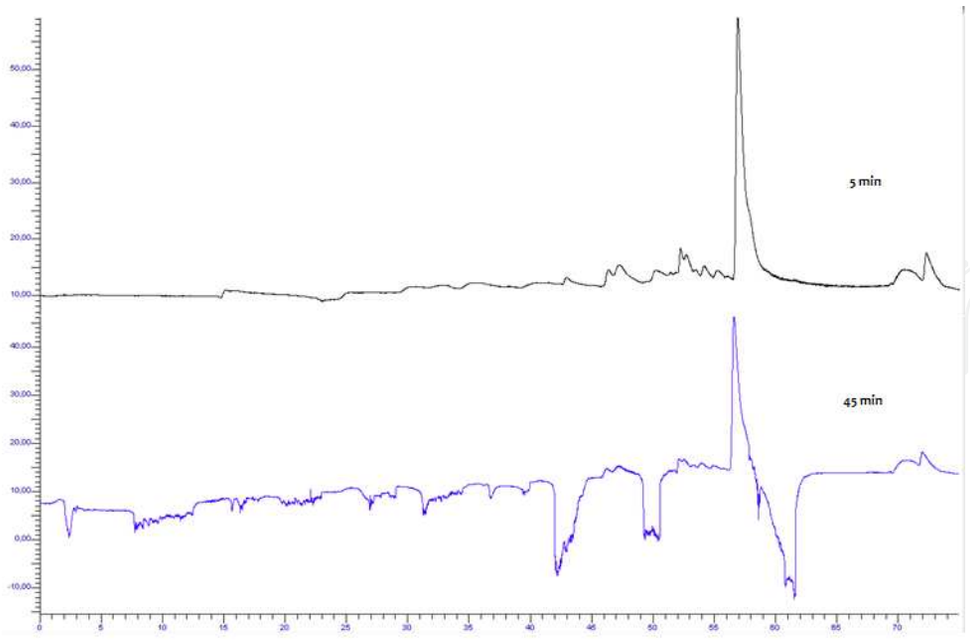

Figure 4. HPLC chromatographic profile of the aqueous extract of the leaves of *Vernonanthura patens* obtained with 5 and 45 min of ultrasound extraction

http://dx.doi.org/10.5772/59866

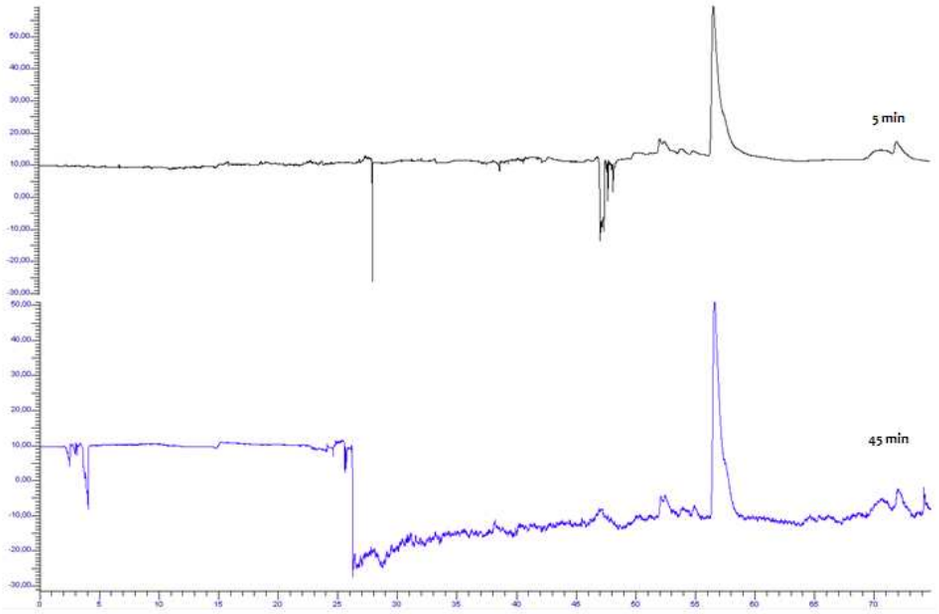

Figure 5. HPLC chromatographic profile of the aqueous extract of the flowers of *Vernonanthura patens* obtained with 5 and 45 min of ultrasound extraction

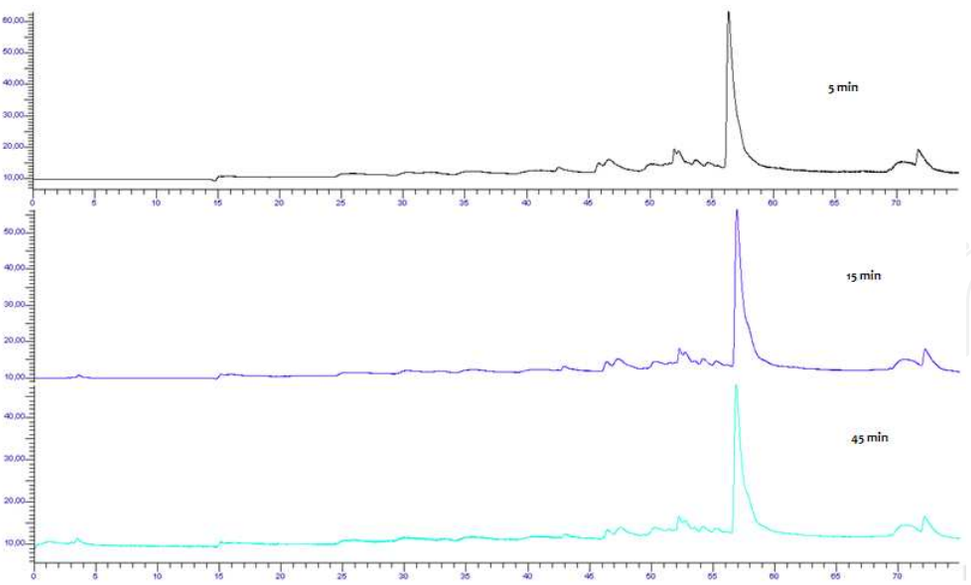

Figure 6. HPLC chromatographic profile of the aqueous extract of the stems of *Vernonanthura patens* obtained with 5, 15, and 45 min of ultrasound extraction

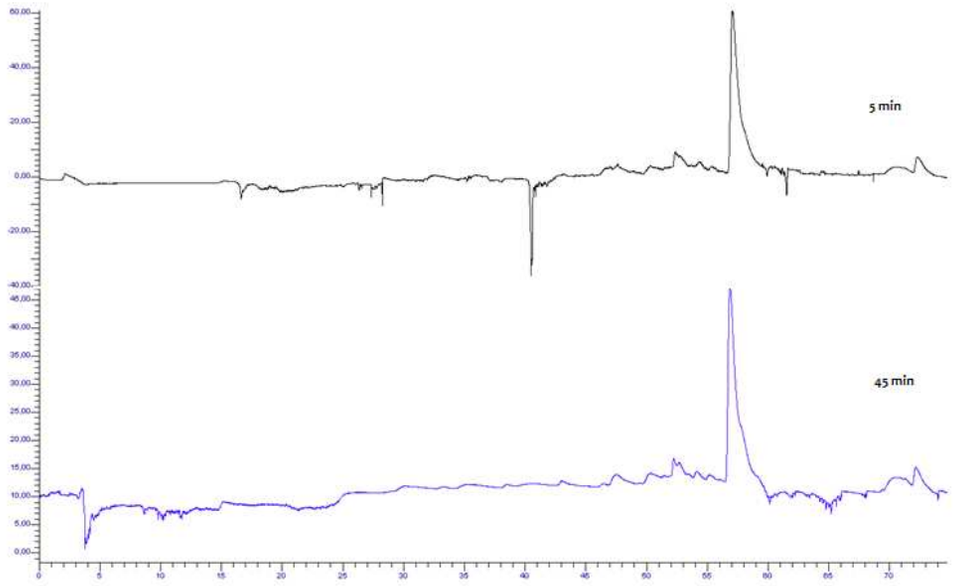

Figure 7. HPLC chromatographic profile of the ethanolic extract of the flowers of *Vernonanthura patens* obtained with 5 and 45 min of ultrasound extraction

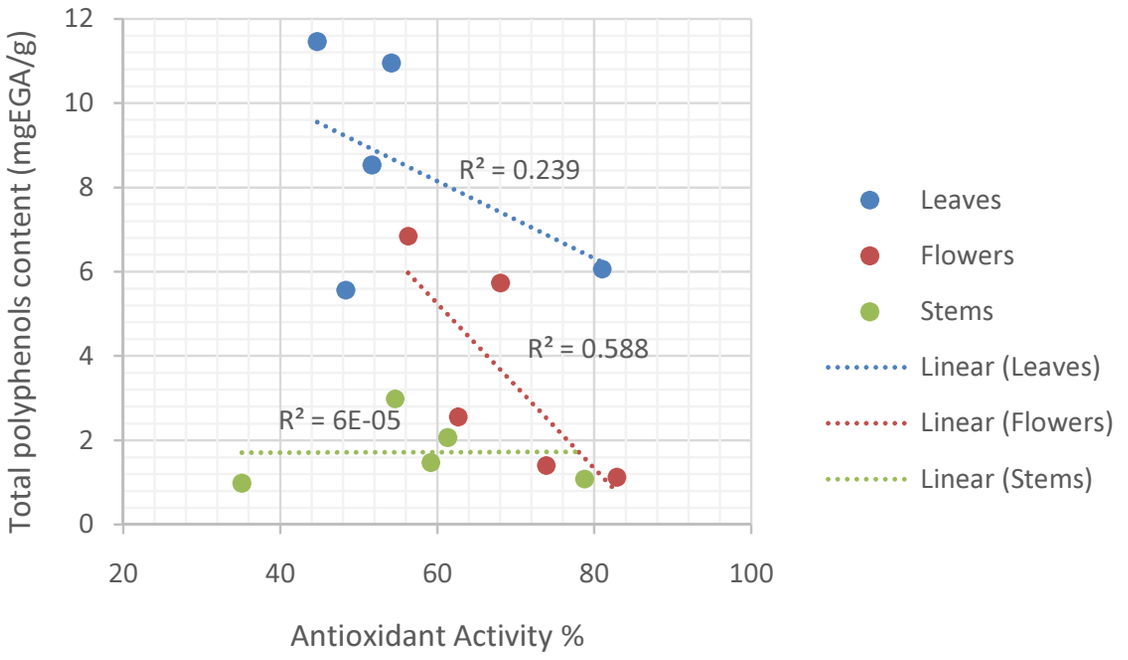

Figure 8. Total polyphenol content *vs* antioxidant activity of the leaves, flowers, and stems of *Vernonanthura patens*

3.4. Evaluation of antileishmanial activity

Aqueous extracts of leaves and stems showed activity and selectivity against *L. amazonensis* (Table 5). The activity and selectivity present in the aqueous extract of the stems showed a

http://dx.doi.org/10.5772/59866

different behavior to that reported for the ethanol extract of this plant organ [28]. The evaluation was reported as not detectable because it caused a minimal toxicity in the concentrations tested, destroying the cell monolayer.

The results obtained for the aqueous extract support the traditional use of the species and are highly relevant as these extracts would enhance the usefulness of a possible low-cost source for the development of an effective herbal drug for the treatment against Leishmania.

Extracts	Promastigote L. amazonensis (IC_{50} μg/ml) $\overline{X}(S)$	Cytotoxicity macrophages (CC_{50} μg/ml)	SI
Leaves	18.8 ± 0.2	200	11
Stems	23.7 ± 0.1	200	8
Pentamidine (positive control)	1.3 ± 0.1	11.7	9

Table 5. Antileishmanial activity and cytotoxicity of aqueous extracts of *Vernonanthura patens* against *L. amazonensis*

IC_{50}, half maximal inhibitory concentration, is expressed as the concentration of extract (mg/ml) that inhibits 50% of the parasite growth. CC50, median cytotoxic concentration, is expressed as the concentration of extract (mg/ml) causing 50% of parasite mortality. $\overline{X}(S)$, mean (standard deviation). SI, selectivity index: CC50 macrophages / IC50 Leishmania. SI ≤ 5 more selective for the cell, SI ≥5 more selective for parasite.

4. Conclusion

The extraction time ultrasound influences the concentration of polyphenolic compounds in all organs tested, when water is used as solvent. Ethanol extracts are unable to determine the concentration of phenolic compounds due to the possible interference of colored compounds extracted with this solvent. Aqueous extracts of the leaves and flowers and the alcoholic extract of flowers showed higher antioxidant activity than the aqueous extracts of the stems. Antioxidant activity in the alcoholic extracts of leaves and stems was not observed.

The HPLC chromatographic profiles of all extracts tested showed a majority chromatographic peak between 56 and 57 min, which could correspond to a mixture of compounds responsible for the antioxidant activity found.

No correlation between antioxidant activity and polyphenol content was found, so presumably they are not solely responsible for the antioxidant activity found.

Aqueous extracts of the leaves and stems of the species *V. patens* showed higher activity and selectivity than the alcoholic extracts, against *L. amazonensis*, corroborating the traditional use of the species.

5. Future directions

The results obtained in the chemical and biological study of the species *V. patens* show its potential as antimicrobial, antioxidant, and particularly antileishmanial, antiparasitic diseases of high incidence in Ecuador and other countries. However, inclusion in the therapeutic still requires further studies among which may be mentioned toxicology to demonstrate the safety of their preparations and as a development of a suitable dosage form to the pathology to be treated, aspects that should motivate future research of our work group.

Acknowledgements

This study was supported by grants from SENESCYT, Prometheus program, and ESPOL (Ecuador).

Author details

P.I. Manzano[1*], M. Miranda[2], M.F. Quijano[2] and L. Monzote[3]

*Address all correspondence to: pmanzano@espol.edu.ec

1 Escuela Superior Politécnica del Litoral, Facultad de Ciencias Naturales y Matemáticas (ESPOL-FCNM), Guayaquil, Ecuador

2 Escuela Superior Politécnica del Litoral, Centro de Investigaciones Biotecnológicas del Ecuador (ESPOL-CIBE), Guayaquil, Ecuador

3 Institute of Tropical Medicine Pedro Kouri, Havana, Cuba

References

[1] Rodríguez A. (2005). *Asteraceae*. In: Hammel BE, Zamora N, Grayum MH (eds.). Plant Manual of Costa Rica. Missouri Bot. Gard. Press, St. Louis & Inst. Nac. de Biodiversidad, Santo Domingo de Heredia, Costa Rica.

[2] Stevens PF. (2006). «Asteraceae». Angiosperm Phylogeny Website. Version 7, Mayo.

[3] Jeffrey C. (2007). Compositae: Introduction with Key to Tribes. 7 in Families and Genera of Vascular Plants. Flowering Plants, Eudicots, *Asterales*. Springer-Verlag, Berlin: J. W. Kadereit and C. Jeffrey. Vol. VIII: 61–68.

[4] Bremer K, Anderber A. (1994). *Asteraceae:* Cladistics & Classification. Universidad de Michigan, Timber Press. ISBN-10: 0881922757. ISBN-13: 978-0881922752.

[5] Borkosky S, Bardón A, Catalán C, Díaz J, Herz W. (1997). Glaucolides, hirsutinolides and other sesquiterpene lactones from *Vernonanthura pinguis*. Phytochemistry, 44(3): 465–470

[6] Bazon J, Callegari J, Vichnewski W, Dias D, Nagamiti K, Cunha W, Herz W. (1997). Cadinanolides and other constituents from *Vernonia fruticulosa* and *Vernonanthura discolor*. Phytochemistry, 44(8): 1535–1536.

[7] Hernández Z, Catalán C, Hernández L, Ramírez D, Joseph-Nathan P. (1999). Sesquiterpene lactones from *Stevia alpina* var. Glutinosa. Fitoquimica, 51(1): 79–82.

[8] Kotowicz C, Bardóna A, Catalán C, Cerda-García-Rojas C, Joseph-Nathan P. (1998). Glaucolides and hirsutinolides from *Vernonanthura squamous*. Phytochemistry, 47(3): 425–428

[9] Pollora GC, Bardón A, Catálan C, Gedris E, Herz W. (2004a). Elephantopus-type sesquiterpene lactones from a *Vernonanthura* species, *Vernonanthura nebularum*. Biochemical Systematics and Ecology, 31(4): 397–405.

[10] Pollora GC, Bardón A, Catalán C, Griffin Cl, Herz W. (2004b). Elephantopus-type sesquiterpene lactones from a second *Vernonanthura* species, *Vernonanthura lipeoensis*. Biochemical Systematics and Ecology, 32(6): 619–625.

[11] Kos O, Castro V, Murillo R, Poveda L, Merfort I. (2006). Ent-kaurane glycosides and sesquiterpene lactones of the hirsutinolide type from *Vernonia triflosculosa*. Phytochemistry, 67: 62–69

[12] Latha R, Geetha T, Varalaskshmi P. (1998). Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis. General Pharmacology, 31(4): 601–606.

[13] Abeysekera A, de Silva K, Silva S, Sirimanne V, Labadie R, Berg A, Van Den S, W Van Der. (1999). Inhibition of chemiluminescence generated by zymosan-activated polymorphonuclear leucocytes by phenolic constituents of *Vernonia cinerea*. Fitoterapia, 70: 317–319.

[14] Iwalewa O, Iwalewa J, Adeboye O. (2003). Analgesic, antipyretic, anti-inflammatory effects of methanol, chloroform and ether extracts of *Vernonia cinerea* Less leaf. Journal of Ethnopharmacology, 86: 229–239.

[15] Portillo A, Vila R, Freixaa B, Ferrob E, Parellac T, Casanovad J, Cañiguerala S. (2005). Antifungal sesquiterpene from the root of *Vernonanthura tweedieana*. Journal of Ethnopharmacology, 97(1): 49–52

[16] Bardón A, Borkoskya S, Ybarra M, Montanaroa S, Cartagena E. (2007). Bioactive plants from Argentina and Bolivia. Fitoterapia, 78(3): 227–231.

[17] Borkosky S, Ponce S, Gabriela Juárez G, González M, Bardón A. (2009). Molluscicidal sesquiterpene lactones from species of the tribe Vernonieae (Compositae). Chemistry and Biodiversity, 6(4): 513–519.

[18] Mabry TJ, Abdel-Baset Z, Padolina WG. (1975). Systematic implications of flavonoides and sesquiterpene lactones in species of *Vernonia*. Biochemical Systematics and Ecology, 2(3–4):185–192.

[19] Jakupovic J, Schmedia-Hirschmann G. (1986). Hirsutinolides, glaucolides and sesquiterpene lactones in species of *Vernonia*. Phytochemistry, 25(2): 145–158.

[20] Gupta M, Monge A. (1986). Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA intercalation. International Journal of Pharmacognosy, 34: 19–27.

[21] Fournet A, Barrios A. (1994). Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. Journal of Ethnopharmacology, 42: 19.

[22] Valadeau C, Pabon A, Deharo E, Albán-Castillo J, Estévez Y, Lores F, Rojas R, Gamboa D, Sauvain M, Castillo D, Bourdy G. (2009). Medicinal plants from the Yanesha (Peru): evaluation of the leishmanicidal and antimalarial activity of selected extracts. Journal of Ethnopharmacology, 123(3): 413–422

[23] Manzano Santana PI, Silva Osorio M, Sterner O, Peralta Garcìa EL. (2012). Phytochemical Studies of Fractions and Compounds Present in *Vernonanthura patens* with Antifungal Bioactivity and Potential as Antineoplastic, Phytochemicals—A Global Perspective of Their Role in Nutrition and Health, Dr Venketeshwer Rao (Ed.). ISBN: 978-953-51-0296-0, InTech.

[24] Santos E, Manzano P, Navarrete O, Hidalgo L, Miranda M, Peralta E. (2012a). *Vernonanthura patens* maturase K (matK) gene, partial cds; chloroplast. National Center for Biotechnology Information. GenBank: JX276535.1. http://www.ncbi.nlm.nih.gov/nuccore/JX286590.1

[25] Santos E, Manzano P, Navarrete O, Hidalgo L, Miranda M, Peralta E. (2012b). *Vernonanthura patens* ribulosa-1,5-bifosfato carboxilasa/oxigenasa subunidad grande (rbcL) de genes, cds parcial; cloroplasto National Center for Biotechnology Information. GenBank: JX276536.1. http://www.ncbi.nlm.nih.gov/nuccore/JX276535.1; http://www.ncbi.nlm.nih.gov/nuccore/JX276536.1

[26] Manzano Santana P, Miranda Martínez M, Abreu Payrol J, Silva M, Olov S, Peralta E. (2013a). Pentacyclic triterpenoids with antimicrobial activity from the leaves of *Vernonanthura patens* (Asteraceae). Emirates Journal of Food and Agriculture, 25(7). doi: 10.9755/ejfa.v25i7.15989.

[27] Manzano Santana PI, Orellana León T, Miranda Martínez M, Abreu Payrol J, Ruíz O, Peralta García EL. (2013b). Some pharmacognostic parameters of *Vernonanthura patens* (Kunth) H. Rob. (Asteraceae) endemic to Ecuador. Cuban Journal of Medicinal Plants, 18(1).

[28] Manzano Santana PI, Miranda Martínez M, de Oca Porto RM, Orellana León T, Abreu Payrol J, Peralta García EL. (2013c). Chemical study of lipid compounds of the leaves, stems and flowers *Vernonanthura patens* (Kunth) H. Rob. (Asteraceae). Cuban Journal of Medicinal Plants, 18(4).

[29] Manzano Santana PI, García M, Mendiola J, Fernández-Calienes A, OrellanaT, Miranda M, Peralta E, Monzote L. (2014). In vitro anti-protozoal assessment of *Vernonanthura patents* extracts. Pharmacology Online 1, 1–6.

[30] Lee L, Lee N, Kim Y, Lee C, Hong S, Jeon Y, Kim Y. (2013). Optimization of ultrasonic extraction of phenolic antioxidants from green tea using response surface methodology. Korea Food Research Institute, Vol. 18, pp. 13530–13535.

[31] Monzote L. (2010). Therapeutic potential of essential oil of *Chenopodium ambrosoides* and some of its constituents against leishmania. PhD thesis in Pharmaceutical Sciences, Institute of Tropical Medicine "Pedro Kouri," Havana.

[32] Sladowski D, Steer SJ, Clothier RH, Balls M. (1993). An improve MTT assay. Journal of Immunological Methods, 157(1–2): 203–207.

[33] Basso R. Secondary metabolites in *Vernonia tweedieana* Baker. Master in Pharmaceutical Sciences. Federal University of Santa Maria. Center for Health Sciences. Postgraduate Program in Pharmaceutical Sciences. Dissertation, 2006.

[34] Carvalho M, Costa P, Abreu H. (1999). Flavones from *Vernonia diffusa*. Journal of the Brazilian Chemical Society, 10(2): 163–166.

[35] Latha R, Geetha T, Varalaskshmi P. (1998). Effect of *Vernonia cinerea* less flower extract in adjuvant-induced arthritis. General Pharmacology, 31(4): 601–606.

[36] Awe S, Makinde J, Olajide O. (1999). Cathartic effect of the leaf extract of *Vernonia amygdalina*. Fitoterapia, 70(2): 161–165.

[37] Akinpelu D. Antimicrobial activity of *Vernonia amigdalina* leaves. Fitoterapia, 70(4): 432–434.

[38] Manzano Santana PI, Miranda M, Abreu Payrol J, Silva M, Hernández V, Peralta E. (2013d). Gas chromatography-mass spectrometry study from the leaves fractions obtained of *Vernonanthura patens* (Kunth) H. Rob. International Journal of Organic Chemistry, 3: 105–109. doi:10.4236/ijoc.2013.32011 Published Online June 2013 (http://www.scirp.org/journal/ijoc).

[39] Soares MI, Péres VF, Dariva C, Zini CA, Abad FC, Martinez MM, Caramão EB. (2006). Optimization of the sonication extraction method of *Hibiscus tiliaceus* L. flowers. Ultrasonics Sonochemistry, Apr 13(3): 242–250.

[40] Peres VF, Saffia J, Melecchi MI, Abadc FC, de Assis Jacques R, Martinez MM, Oliveira EC, Caramao EB. (2006). Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from *Piper gau*dichaudianum Kunth. Journal of Chromatography A, 1105(1–2): 115–118.

IntechOpen