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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF SEVEN MEDICINAL PLANTS SPECIES FROM ECUADOR

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Abstract

Seven plant species, used as traditional medicine, from two regions of Ecuador have been subjected to qualitative and cuantitative phytochemistry screening in order to provide information about possible chemical compounds present in these species that will contribute to future researchs. Chuquiraga jussieui J.F. Gmel., *Pseudognaphalium elegans* (Kunth) *Kartesz* (Asteraceae), *Gustavia pubescens* Ruiz & Pav. ex *Berg* (Lecythidaceae), Aeghiphila alba Moldenke (Lamiaceae), Cleome spinosa Jacq. (Cleomaceae), *Phyllantus acuminatus* Vahl (Phyllantaceae) and *Croton rivinifolius* Kunth (Euphorbiaceae) were the species selected for this investigation. Phytochemical determination was oriented to search for alkaloids, flavonoids, tannins, triterpenoids, steroids, saponins and anthraquinones. Total phenols and flavonoids content were measured on the crude ethanol extracts of all species assayed being Pseudognaphalium elegans and *Chuquiraga jussieu*i the species with higher content of this class of compounds with 1362.08 ± 2.10 and 1979.07 ± 3.10 mg E Cat / 100g dry extract and 130.69 ± 5.70 and 244.18 ± 9.80 mg E Querct / 100 g dry extract, respectively. Results showed a close relation to the % inhibition of DPPH and IC50mg E Trolox/mL observed where *P. elegan*s exhibited (56.4%;) 41.85 (± 0.07) and *C. jussieui* (58.2 %) 41,02 (± 0.05). This is the first report on the chemical screening, total phenolics, total flavonoids and antioxidant activity of these species. According to references consulted *Croton rivinifolius* is endemic from Guayas province and has no reports either for phytochemical composition or antioxidant activity.

Key words: Phytochemical screening, medicinal plants, secondary metabolites, flavonoids, total phenols, antioxidant capacity

Introduction

In Ecuador, the use of medicinal plants is a widespread practice, especially among habitants of rural areas who often find a wide variety of these plants in popular markets. This practice comes from ancestral knowledge [1,2]. It is assessed that approximately 5100 vegetable plant species are used for different reasons; however 3188 are used for medicinal purposes such as infections, wounds, respiratory ailments, digestive disorders and inflammations, among others. According to references, 75 % of these medicinal species are native, 5% are endemic, and 11% are introduced being Asteraceae, Fabaceae, Rubiaceae, Solanaceae, and Araceae the families more commonly used [3,4]. The wide diversity of the flora of Ecuador has encouraged investigations on the search for new therapeutic alternatives since most of these species are yet to be studied.

In present work, qualitative and quantitative phytochemical analysis as well as antioxidant activity was evaluated in seven species collected from La Sierra and the coast, two regions of Ecuador. *Chuquiraga jussieui* J.F. Gmel., *Pseudognaphalium elegans* (Kunth) *Kartesz* (Asteraceae), *Gustavia pubescens* Ruiz & Pav. ex O. Berg (Lecythidaceae), *Aeghiphila alba* Moldenke (Lamiaceae), *Cleome spinosa* Jacq. (Cleomaceae), *Phyllantus acuminatus* Vahl (Phyllantaceae) and *Croton rivinifolius* Kunth (Euphorbiaceae) are species used in traditional medicine, being the latter endemic from Guayas province. According to the literature consulted, there are only few reports for these taxa and some of those are lacking of chemical composition or biological activity. A brief description of the species under investigation is summarized bellow in order to have some information of each. *Aegiphila* (Lamiaceae) genus is well represented in the tropical and subtropical regions of Central and South America. So far, there are only few phytochemical studies regarding *Aegiphila ihotzkyana* and *A. obtuse* [5]. *Cleome spinosa* (Cleomaceae), commonly called spider flower, is an annual ornamental plant, native to South America. There are about 200 species identified for this genus but information about chemical composition is limited [6]. Only few reports related to flavonoids have been published for *C. viscosa* (7), *C. droserifolia* [8] and *C. arabica* L. (9). On the other hand, *Gustavia* L. (Lecythidaceae) genus is comprised by approximately 46 species restricted to the Neotropics, especially around northern areas of South America [10]. In Ecuador 13 species of this genus have been reported from which 10 are distributed mainly in the coastal region. *Gustavia pubescens* Ruiz & Pav. ex O. Berg known as "membrillo", is a native species reported only for the Guayas province [11]. So far, there are no reports regarding G. pubescens however, antioxidant activity and chemical constituents have been investigated for *G. augusta* [12], leishmanicidal activity on *G. elliptica* [13] and chemical constituents of *G. hexapelata* [14]. On the other hand, Croton genus (Euphorbiaceae) comprises approximately 1200 species widely distributed in tropical and subtropical regions of the world [15]. These species are well known for its variety of diterpenes [16] which exhibit a wide range of biological activities [17]. *Croton rivinifolius* is a shrub endemic to western Ecuador and it grows between 0 to 500 meters above sea level [18]; according to folk medicine it has been used to ease pain. The genus *Pseudognaphalium* (Asteraceae) comprises 90 species mainly distributed en South, Central and North America, although some of these are present in Asia and Africa, as well [19]. It comprises annual or perennials plants that grow between 2000 to 3200 meters above sea level (masl). Some of these species are used as hemostatic, antitussive, expectorant, antibacterial, antifungal and to alleviate inflammations. In South America, specifically in Los Andes, *Pseudognaphalium elegans* (Kunth) Kartesz, (this name includes *Gnaphalium poeppigianm* DC., and the basyonym *G. elegans* Kunth) and *G. purpureum* L are recommended for the cure of cancer [20] and this biological activity has been associated to the flavonoids present on this species [21]. The genus *Phyllanthus* (Phyllanthaceae) is widely distributed throughout most of tropical and subtropical countries. Due to the large number of secondary metabolites presents in this species, it has been used in folk medicine to treat different ailments. Many of these ethnobotanical applications have been studied and reported; such as antibacterial, antioxidant, anti-HIV-1 [22], anti-viral and cytotoxic activity [23], antidiabetic and antidiarrheal activity [24]. In Ecuador, Phyllantus species grow mainly in cloud forest between 1000 to 3000 masl but may also occur in the Amazonian lowlands and also in the dry forest of Manabi and Guayas provinces [25]; 14 of these species have been reported in Ecuador from which *P. acuminatus* is popularly known as Cha'palaach [7, 26]. *Chuquiraga jussieui* (Asteraceae) locally known as Chuquiragua, is a well-known Andean species, it grows at 3000 masl and it is used for hummingbirds as food. According to popular medicine, Chuquiragua tea might be used as diuretic, tonic for liver and stomach, cough, to treat malaria

fever, molar pain, inflammations, and even as antiseptic for the urinary tract and prostate [27]. Present investigation aims to describe the phytochemical screening of ethanol extracts of seven species, previously described, as well as to evaluate the antioxidant activity, total phenol and flavonoids content. Ever since there are only few reports for *Pseudognaphalium* elegans and no previous investigations on the phytochemical composition and antioxidant activity for *Chuquiraga jussieui*, *Gustavia pubescens*, *Aeghiphila alba*, *Cleome spinosa*, Phyllantus acuminatus and *Croton rivinifolius*, results might be of interest for further studies focused on the isolation of secondary metabolites with potential antioxidant activity. Furthermore, results of this investigation are considered a contribution to the natural products research.

Methods

Plant material

Fresh leaves of *Chuquiraga jussieui* J.F. Gmel. and *Pseudognaphalium elegans* (Kunth) Kartesz were collected in May 2015 from La Sierra, while the other species were collected in July 2015 from Guayas province in western Ecuador. Botanical identification was carried out by Xavier Cornejo, a curator of GUAY Herbarium, Faculty of Natural Sciences, University of Guayaquil, Ecuador. Voucher specimens were deposited under the following codes: *Pseudognaphalium elegans* (Kunth) Kartesz (MERA03); *Chuquiraga jussieui* J.F. Gmel. (MERA04); *Gustavia pubescens* Ruiz & Pav. ex O. Berg (Cornejo 8713); *Phyllantus acuminatus* Vahl (Cornejo s.n); *Aeghiphila alba* Moldenke (Cornejo 2790); *Cleome spinosa* Jacq. (Cornejo 7003); and *Croton rivinifolius* Kunth (Cornejo s.n).

Chemical and reagents

Folin–Ciocalteu (FC) reagent, sodium nitrite, aluminium chloride, sodium hydroxide, sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl (DPPH_), catechin, quercetin, trolox, methanol and ethanol were supplied by Sigma–Aldrich (St. Louis, MO, USA). All chemicals and reagents used in the study are of analytical grade.

Extraction

Every plant material was dried at 40 °C for 3 days, and then pulverized. Crude plant extracts were prepared by Soxhlet extraction method. About 50 g of powdered plant was uniformly packed into a thimble and extracted with 250 mL of ethanol. The mixture were filtered and concentrated under

Phytochemical Screening

Crude extracts were phytochemically evaluated to determine the presence of chemical constituents using standard procedures, which are described below:

Testing for Alkaloids

Each extract (10mg) was dissolved in 2mL of hydrochloric acid 5%, after mixing and filtered, three aliquots were taken. Drops of Wagner, Mayer, Bouchardat and Dragendorff reagents were added to each. A red- brown precipitate (Wagner), yellowish-white precipitate (Mayer), brown precipitate (Bouchardat) and red–orange precipitate (Dragendorff) indicated the presence of such metabolites (28).

Testing for Flavonoids

Shinoda test

1mL of absolute ethanol and 3 drops of concentrated hydrochloric acid were added to 10 drops of diluted extract in isopropyl alcohol. Formation of red colour indicated the presence of aurones and chalcones. In cases where no colour change was observed, pieces of metallic magnesium were placed. The formation of orange, red or magenta colour indicated the presence of isoflavones, flavonols and flavones, respectively [28].

Test with 10% sodium hydroxide

3 drops of sodium hydroxide 10% were added to 1 mL of diluted extract in isopropyl alcohol. Formation of yellow-red, coffee-orange, purple-red or blue colour indicated the presence of xanthones and / or flavones, flavonols, chalcones and anthocyanins, respectively [28].

Testing for Saponins

Foam height test (without sodium bicarbonate)

1 mL of distilled water was added to 10 drops of the extract dissolved in isopropyl alcohol (20mg/mL) in a test-tube, shaken vigorously to froth, and then allowed to stand for 30 minutes. Saponin content was measured as follows: no froth (absence); froth less than 3 mm high (poor); froth 6mm high (moderate) and froth greater than 8 mm high (abundant) [28].

Foam height Test (with sodium bicarbonate)

1 mL of distilled water and 1 drop of sodium bicarbonate saturated solution were added to 5 drops of the extract dissolved in isopropyl alcohol (20 mg/mL) in a test-tube and shaken vigorously during 3 minutes. Formation of honey combs shaped foam

indicated the presence of saponins [29].

Testing for Quinones and Anthraquinones

Borntraguer test

10 mg of each extract were dissolved in 3 mL of distilled water and filtrated. After filtration 3 mL of 5% potassium hydroxide solution were added to each. The mixture was heated to boiling for 3 minutes. Alkaline solution was allowed to cool down and then extracted with 3 mL of chloroform. Organic phase was separated and shaken with 2 mL of 5 % potassium hydroxide solution. Occurrence of red colour in alkaline phase indicated the presence of quinones. Those samples showing yellow colour with Green fluorescence where treated with one drop of 6 % hydrogen peroxide, formation of red colour was considered positive for anthrone derivatives [28].

Test with ammonium hydroxide

One drop of concentrated ammonium hydroxide was added to 10 mg of each extract, previously dissolved in isopropyl alcohol. After two minutes, formation of red colour indicated the presence of anthraquinones [28].

Test with sulphuric acid

One drop of concentrated sulphuric acid was added to 10 mg of each extract dissolved in isopropyl alcohol. Formation of red colour indicated the presence of quinones [28].

Testing for steroids and / or triterpenoids

Salkowski test

2 mL of chloroform and 1mL of concentrated sulphuric acid were added to 10 drops of the extract dissolved in isopropyl alcohol, slowly until double phase formation. The presence of a reddish-brown colour in the middle layer was indicative of steroidal ring [28].

Lieberman Bouchard test

1 mL of anhydrous acetic acid and 3 drops of concentrated sulphuric acid were added to 2 mL of the extract dissolved in isopropyl alcohol. After 5 minutes a blue-green colour middle layer was indicative of sterols, but a pink, red, magenta or violet colour revealed the presence of terpenoids (28).

Testing for tannins

10 mg of each extract were dissolved in 1 mL of ethanol, and extracted with 3 ml of distilled water in boiling during 15 minutes.

Once allowed to fresh at room temperature, 0.2 mL of 10 % sodium chloride solution were added to the mixture and filtered. In addition, 4 drops of 10 % ferric chloride solution were also added. Precipitation observed was indicative of the presence of tannins [29].

DPPH• free radical scavenging assay

The antioxidant capacity of each extract was assessed using a method described by Lai (30). A solution of DPPH• (6 x 10-2 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 the concentration of 2.5 mg/mL. The reaction mixture was kept in the dark at room temperature for 30 min. The absorbance was measured on a spectrophotometer (Thermo Electron Corporation) at 517 nm. A solution of 2.8 mL of DPPH and 0.2 mL of methanol was used as negative control while Ascorbic acid at the concentration of 0.176 mg/mL was used as standard antioxidant reference. Results are expressed as percentage of inhibition (% INH) and are calculated following this equation:

% INH = [(Abs DPPH• – Abs sample/ Abs DPPH•) x 100], where Abs DPPH• represents the DPPH• radical + methanol absorbance while Abs sample refers to the absorbance of DPPH• radical + sample extract /or ascorbic acid. A calibration curve was prepared, using a standard solution of trolox (0.5, 1.0, 1.5, 2.0 µg). Data is reported by means of at least two replications

Determination of total phenolic content

Total phenolic contents of each extract were determined using a Folin-Ciocalteu colorimetric method [31]. 40 µL properly diluted of each extract solution were mixed with 1.8 mL of Folin-Ciocalteu reagent. The reagent was pre-diluted, 10 times, with distilled water. After standing for 5 min at room temperature, 1.2 mL of (7.5 % w/v) sodium carbonate solution were added. The solutions were mixed and allowed to stand for 1 h at room temperature. Then, the absorbance was measured with a spectrophotometer (Thermo Electron Corporation) at 765 nm. A calibration curve was prepared, using a standard solution of cathequin (2.2, 4.4, 6.6, 8.8 and 11.0 µg). Results are expressed as mg of cathequin equivalents (CE)/100 g dry weight (dw) extract. Data is reported by means of at least two replications

Determination of total flavonoids

Total flavonoid content was estimated according to the method described by Zhishen [32]. 1 mL of properly diluted extract was mixed with 4 mL of

distilled water and 0.3 mL NaNO2 (5% w/v) was added. 3 ml AlCl3 (10 % w/v) was also added 5 min later. After 6 min of reaction, 2 mL of NaOH 1 M solution was added and the mixture was completed up to 10 ml with distilled water. The solution was mixed carefully and the absorbance was measured at 510 nm. A calibration curve was plotted using quercetin as standard flavonoid reference (25, 50, 100, 150 and 200 µg). The results are expressed as mg of quercetin equivalents (QE)/100 g dry weight (dW) extract. Data is reported by means of at least two replications.

Results and Discussion

Phytochemical screening of methanolic extracts of seven medicinal plants collected from La Sierra and Guayas, Ecuador, was carried out using various chemical assays in order to identify either the presence or absence of secondary metabolites such as alkaloids, coumarins, phenolic compounds, flavonoids, glycosides, quinones, saponins, tannins, steroids and triterpenoids.

Table 1 summarizes secondary metabolites present in all extracts assayed. As might be observed, alkaloids were only detected in *Aegiphila alba, Cleome spinosa* and *Chuquiraga jussieui,* showing moderate to poor presence. References consulted have demonstrated the incidence of alkaloids in Cleome paradoxa, an alkaloid known as paradoxonina, has also been isolated [33]. Flavonoids and related compounds were detected in all samples assayed. According to Shinoda´s test, presence of aurones and chalcones are poor while NaOH 10% test showed abundant incidence of xanthones, flavones and flavonols in *Gustavia pubescens*, *Phyllantus acuminatus*, *Aegiphila alba*, *Cleome spinosa*, *Chuquiragua jussieui* and *Croton rivinifolius* but moderate to poor in *Pseudonagphalium elegans*. Previous investigations have reported the presence of these type of metabolites in *Aegiphila* [34]; *Croton* [35] and *Phyllanthus* [36] species and biological activities such as antioxidant and anticancer have also been related to these compounds. In addition, two new flavonols glycosides isomers *Cleomesides* A and B isolated from *Cleome chelidonii* (6a,6b, 6c, 8a) genus and two new flavone isomers from *Gnaphalium elegans* proved to be active on human cancer cells such as breast, pancreas and prostate [21]. Phenolic compounds were observed between moderate to abudant presence in *P. elegans*, C. jussieui, *G. pubescens* and *C. spinosa* while a poor incidence was detected in *Phyllantus acuminatus*, *Aegiphila alba* and *Croton rivinifolius*. Phenolic acids as salicylic, trans-cinamic, vanillic and cafeic acid have been identified, previously, in some *Gustavia* species [37] while a phytochemical screening of *Cleome* species proved the presence of flavonoids and phenolic derivatives, supporting the use of this genus for therapeutical purposes (8a, 33). Furthermore, phenolic acids have also been reported for *Gnaphalium* genus where antioxidant activity has been associated to these compounds [38].

Abudance of saponins were only observed in *Pseudognaphalium elegans* and *Chuquiraga jussieui* while moderate presence was detected in *Gustavia pubescens*. So far, there are no reports on the isolation of saponins from any of these three species, thus, present results are consider a contribution to the research on natural products. Interestingly, extracts of *C. rivinifolius*, *P. acuminatus*, *A. alba* and *C. spinosa* showed a complete absence of saponins in present investigation, however there are reports on the isolation of these type of metabolites from species of these genus [35, 39].

On the other hand, terpenoids were appreciated in all samples analyzed being particularly abudant in *Cleome spinosa*, while steroids were limited to *P. elegans*, C. jussieui and G. pubescens. Previous investigations have reported the isolation of diterpenes and triterpenes such as cleomelide and cleomaldic acid which also showed anti-cancer activity [40] as well as some steroids with antioxidant activity [41]. A review on the phytochemical and pharmacological properties of *Gnaphalium genus* described flavonoids, terpenes, anthraquinones and phenolics as the main compounds isolated from this genus [42]. Regarding anthraquinone nuclei, a moderated presence was observed in *Cleome spinosa* while *Pseudognaphalium elegans* and *Chuquiraga jussieui* showed only a limited presence. So far there are no reports in the literature about the isolation of quinones or anthraquinones from any of Cleome species.

Antioxidant Capacity

The antioxidant activity of *Pseudognaphalium elegans* (Kunth) Kartesz; *Chuquiraga jussieui* J.F Gmel.; *Gustavia pubescens* Ruiz & Pav. ex O. Berg; Phyllanthus acuminatus Vahl; *Aeghiphila alba* Moldenke; *Cleome spinosa* Jacq. and *Croton rivinifolius* Kunth, were determined by the DPPH radical scavenging assay. This activity was expressed as % inhibition and IC50 values (Table 2). *P. elegans* and *C. jussieui* showed the highest scavenging activity (56.4% and 58.2%, respectively), with IC50 values ranging between 41.0 µg/ml to 161.8 µg/ml. Similar percentage of inhibition (57.41%) was shown

by Trolox 1mM at 15 µg ml. However, *G. pubescens*, *A. alba*, *C. spinosa*, P. acuminatus, and *Croton rivinifolius* only exhibited a low radical scavenging activity with values ranging between 9.46 to 12.19 % at same concentrations (2.5 mg/mL) (Figure 1). DPPH is a stable free radical that may be able to accept an electron or hydrogen radical to become a stable diamagnetic molecule. Interaction of an antioxidant molecule with DPPH lead to the transfer of an electron or hydrogen atom to it, thus neutralizing its free radical character and convert it to 1-1,diphenyl-2-picryl hydrazine. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidant molecules and it is visually noticeable as a change in color from purple to yellow [43]. In this type of assays the number of DPPH molecules that are reduced seems to be related to the number of available hydroxyl groups [44]. Thus, ethanol extracts composed by polar molecules with abundant free hydroxyl groups such as flavonoids, phenols, tannins, among others may exhibit a strong scavenge activity. These kind of secondary metabolites were observed in all extracts analyzed in present investigation and might be related to the results obtained for DPPH free-radical assay. Since those extracts rich in radical scavengers, such as flavonoids, phenols or polyphenols are known to possess antioxidant activity, extracts analyzed in this study may also present such activity.

Total phenol and flavonoids content

In total phenols content the highest values were determined in extracts from *P. elegans* and *C. juieui*, which also showed higher antioxidant activity (Figure 1). However, the rest of the extracts with total phenol concentrations significantly lower, the radical scavenging antioxidant activity exhibited similar values, no showing correlation to the phenol content. According to the references consulted the radical scavenging antioxidant activity and total phenol concentrations do not necessarily correlate to each other [45] The molecular antioxidant response of phenolic compounds in methyl linoleate varies remarkably depending on their chemical structure [46]. Thus, the AC (Antioxidant Capacity) of an extract may not be predicted on the basis of its phenolic content, but also requires proper characterization of individual phenolic compounds. However, there are several reasons to explain the ambiguous relationship between AC (Antioxidant Capacity) and TPC (Total Phenol Ciocalteu) found in published studies. Some explanations for this variation may be due to a high content of reducing agents such as ascorbic acid, minerals and carotenoids in the fruits [47], high protein content or genetic, agronomic and environmental influences [48]. On the other hand, flavonoids content found in the extracts in present study were determined by the quercetin regression equation as standard calibration (y = 1,1053x + 0.0036) r2 = 0.9957. Results (Figure 2) exhibited a similar profile to the findings on antioxidant activity and total phenolics. *P. elegans and C. jussieui* species reached the highest concentration of flavonoids while in extracts of less concentration (< 110mg EQct / 100g d.w) and the antioxidant activity were low and similar, ranged among 12,19 and 9,46 % of inhibition (Figure 2).

In this study the results obtained proved a relationship between the antioxidant activity, total phenol and flavonoids content (Figure 1 and 2) of the extracts analyzed. Those samples with higher phenolic concentration showed higher antioxidant activity as well. This behavior is similar to previous reports on this type of assays.

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Table 1. Phytochemical screening of alcoholic extracts achieved from seven Ecuatorian Plants.

Sample	WR	MR	DR	BR	SR	NaOH 10 %	$FeCl_3$ 5%	Foam-1	Foam-2	L-BR	SR*	BR*	NH_4OH 10 %	H_2SO_4
Pseudognaphalium elegans	_	_	_	_	+	+	+++	+++	+	++	+	_	+	_
Chuquiraga jussieui	_	+	+	_	+	+++	+++	+++	+	++	++	_	+	_
Gustavia pubescens	_	_	_	_	+	+++	+++	+ +	++	++	++	_	_	_
Phyllanthus acuminatus	_	_	_	_	+	+++	+	_	_	+	_	_	_	_
Aegiphila alba	++	++	++	++	+	+++	+	_	_	+	_	_	_	_
Cleome spinosa	+	++	+	+	+	+++	+++	_	_	+++	+	++	++	_
Croton rivinifolius	_	_	_	_	+	+++	+	_	_	+	_	_	_	_

Key:-Absence, + Poor, ++ Moderate, +++ Abundant. **WR**: Wagner reactive; **MR:** Mayer reactive; **DR:** Dragendorff reactive; **BR:** Bouchardat reactive; **SR:** Shinoda reactive; **SR*:** Salkowski reactive; **Foam-1:** Foam (out sodium bicarbonate); **Foam-2**: (whit sodium bicarbonate); **L-BR:** Liebermann-Burchard reactive; **BR*:** Bornatrager reactive.

Table 2. Determinations of DPPH scavenging activity, total phenol content (TP) and total flavonoids content (TF).

Samples	DPPH % Inhibition	DPPH IC_{50} ugE Trolox/mL	TP mg E Cat / 100g dry weight	TF EQuerct/100g dry weight
Pseudognaphalium elegans	56,38	41,85 ± 0,07	1362,08 ± 2,10	130,69 ± 5,70
Chuquiraga jussieui	58,14	41,02 ± 0,05	1979,07 ± 3,10	244,18 ± 9,80
Gustavia pubescens	9,46	167,20± 0,03	348,19 ± 0,37	79,73 ± 2,40
Phyllanthus acuminatu	10,00	168,72± 0,04	1340,43 ± 2,02	104,63 ± 3,90
Aegiphila alba	9,80	161,79± 0,04	1145,59 ± 1,80	108,10 ± 4,00
Cleome spinosa	12,19	131,59 ±0,03	1084,25 ± 0,95	51,65 ± 1,60
Croton rivinifolius	11,43	141,11± 0,04	755,91 ± 0,64	74,52 ± 2,30

Values are means (n = 3) ± SD. The results were found to be statistically significant P<0.05.

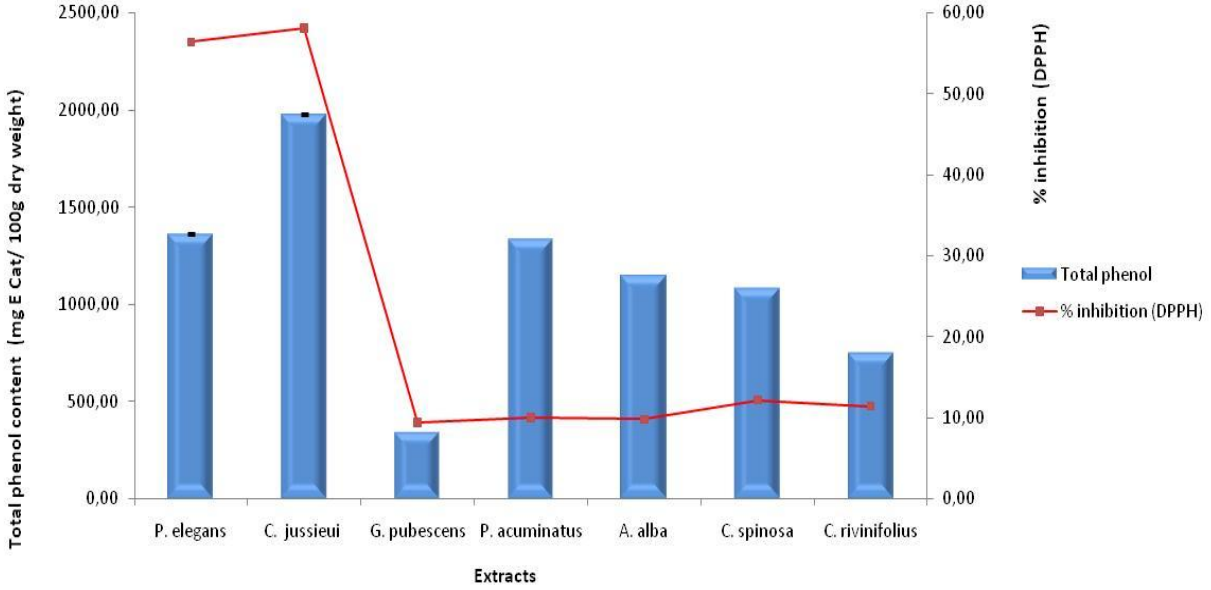

Figure 1. Total Phenols and % of inhibition of DPPH

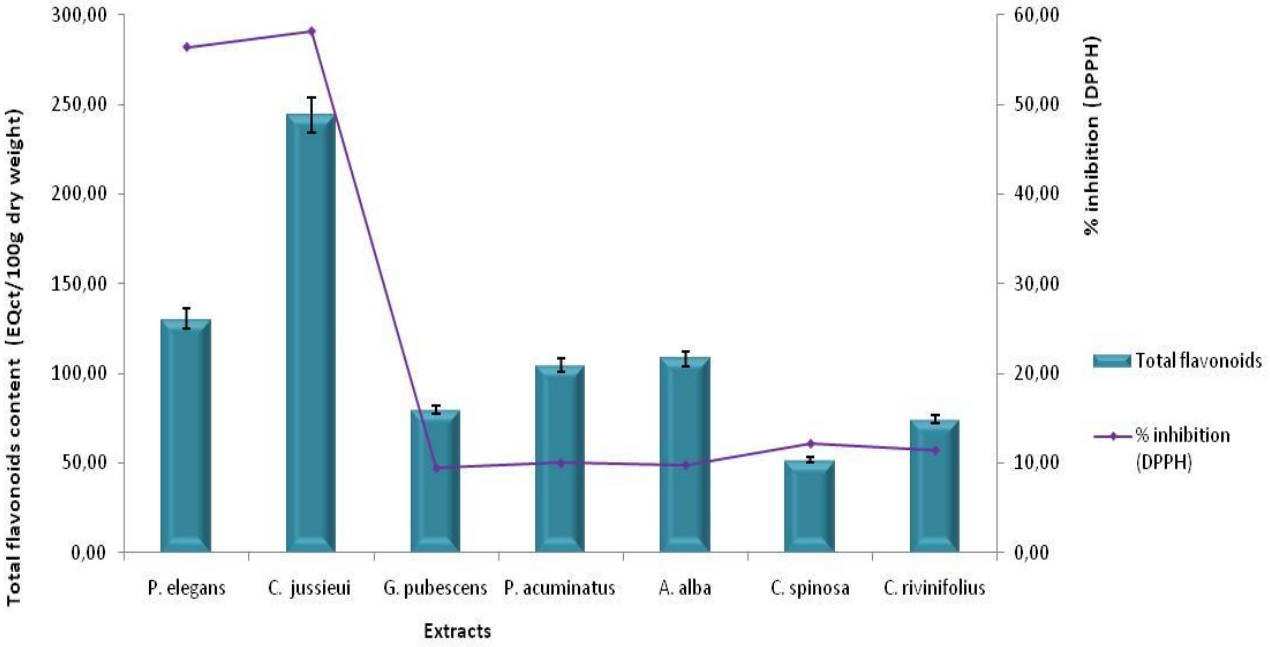

Figure 2. Total flavonoids and % of Inhibition of DPPH.