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Research Article

Pharmacognostic Study and Evaluation of the Antioxidant Activity of *Chuquiraga jussieui* J.F. Gmel (*Asteraceae*)

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Abstract

Chuquiraga jussieui J.F. Gmel has traditionally been used for its therapeutic virtues, nevertheless, scientific studies are scarce. The pharmacognostic, phytochemical evaluation and antioxidant activity of flowers, leaves, stems and roots of the species are presented. Some physicochemical parameters were determined for the powdered drugs and the hydroalcoholic extracts (30% ethanol), phytochemical screening was performed, and phenols were quantified by Folin-Ciocalteu and flavonoids by the aluminum-chloride method. Antioxidant activity was evaluated by the FRAP, DPPH and ABTS tests. Significant differences were found in the physicochemical parameters notably substances soluble in 30% ethanol, total solids, phenols and flavonoids were higher for leaves and flowers. All the extracts showed ferro-reducing activity, although greater in leaves and flowers. All extracts demonstrated radical-scavenging antioxidant activity by the DPPH and ABTS assays, highlighting the extracts of leaves (IC_{50} 40.57 µg/mL, DPPH and 242.2 µg/mL, ABTS) and flowers (IC_{50} 40.60 µg/mL, DPPH and 286.0 µg/mL, ABTS) for having more activity. The pharmacognostic study can contribute to the development of quality control standards of the species and the antioxidant potential demonstrated in the extracts is associated with the active substances present in them.

Keywords: *Chuquiragua*; Phenolic Compound; Flavonoids, Physicochemical Parameters Antioxidant Activity

Introduction

Ecuador has great diversity of medicinal plants which are used in traditional medicine. The species of the *Chuquiraga* genus are distributed in the Andes, from the southwest of Colombia to the center of Chile and throughout the Argentine Patagonia; where the greatest number of these species is found and it is mainly diversified in the deserts and semi-deserts of South America. Two species are represented in Ecuador: *Chuquiraga arcuata* Harling and *Chuquiraga jussieui* J.F. Gmel, both are always found over 3000 m above sea level [1].

C. jussieui has been used since ancient times by the native population of Ecuador and other countries in the region, where it is known by its common names: flower of the walker, flower of the

Andes, chuquiraga and chuquiraguac. It is a botanical species with flowers of the Asteraceae family (Figure 1) and is considered the "National Flower of Ecuador" [2].

Figure 1: Photographs of *Chuquiragajussieui* J.F. Gmel taken by the authors during the collection (April 2019, the Ecuadorian Andes, The Iris, Canton Colta, Chimborazo).

Traditionally, the leaves and stems kept in ethanol are used to treat rheumatism, fever and inflammation. The resin is used as poultice in wounds and pain relief caused by dislocations and fractures. Infusion or decoction of aerial parts are also used to treat several diseases such as those of the prostate, stomach, burns, superficial wounds, ulcers and as it is also used as an antipyretic. Effects such as: antioxidant, anti-inflammatory and antibiotic, among others, have also been reported in experimental studies, suggesting its possible use for a wide variety of pathologies [3-6].

The methanolic extracts of *Chuquiraga straminea* Sand with, subfamily *Barnadesioideae* (*Asteraceae*) showed the presence of quercetin-3-O-glucoside, quercetin-3-O-rutinoside, kaempferol-3-O-glucoside and kaempferol-3-O-rutinoside, and its total extracts demonstrated antioxidant activity by the DPPH and ABTS methods (IC_{50} 14.5 to 34.9 mg/mL), a significant positive correlation was observed between antioxidant activity and total phenols [7].

Due to scattering studies on the species and thus the lack of chemical information in the present study the study of the pharmacognostic, chemical parameters and the antioxidant activity of the different organs of the species is performed.

Materials and Methods

Plant material

The complete *Chuquiraga jussieui* JF Gmeil plant was collected on April 27th, 2019, in the Ecuadorian Andes, in El Lirio, Canton Colta, Chimborazo at an average altitude of 3.212 m.a.s.l, with the following coordinates 1°42'S 78 °45'O.

The taxonomic characterization was carried out by the MSc Xavier Cornejo from the herbarium of the Faculty of Natural Sciences of the University of Guayaquil, where the voucher assigned was: L. Allauca s.n. The different plant organs were separated, washed with abundant running water and dried in a recirculating oven at 50°C, until constant weight.

Physicochemical parameters of the powder drugs from *C. jussieui*

The quality parameters of the raw drug were determined according to procedures reported by Miranda and Cuellar [8]: residual humidity (gravimetric method), content of soluble substances (water and hydroalcoholic mixtures at 30, 50 and 80%), total ash, ash soluble in water, insoluble ashes in 10% hydrochloric acid.

Obtaining extracts and quality physicochemical parameters

Extracts were prepared from the plant material, at the rate of 20 g of drug/100 mL of solvent, by the method of maceration with sporadic agitation over a period of seven days at a temperature of 30°C ± 2°C, using as a solvent a 30% hydroalcoholic mixture (for being the one with the greatest extractive power). The procedure described by Miranda and Cuellar [8] and by the Cuban standard [9] was followed. The quality determinations were carried out by the procedure described by these standards; three replicates were made for each experiment, the following parameters being evaluated: organoleptic requirements, pH, total solids, relative density, and refractive index.

Qualitative chemical analysis was also developed according to the procedure described by Miranda and Cuellar [8].

Determination of total phenols and flavonoids

Total phenol content was determined by the Folin-Ciocalteu method [10-12]. The hydroalcoholic extracts of chuquiragua flowers, leaves, stems and roots were used and gallic acid (Sigma-Aldrich) was used as the reference substance.

The flavonoid content was carried out by the colorimetric method of aluminum trichloride [10,13]. The hydroalcoholic extracts of chuquiragua and quercetin (Sigma-Aldrich) flowers, leaves, stems and roots were used as the reference substance. For both methods, the determinations were made on a Rayleigh UV-1601 spectrophotometer, China.

Antioxidant activity

Ferric Reducing Antioxidant Power (FRAP) assay

The reducing capacity of hydroalcoholic extracts was measured according to the procedure described by Benzie and Strain [14]. The determinations were of a spectrophotometric nature, a Rayleigh UV-1601 UV-visible spectrophotometer, China, was used at an absorbance of 593 nm.

All reagents used were from Merck (2,4,6-tripyridyl-s-triazine (TPTZ), Sodium Acetate Anhydrous, Acetic Acid (99.7%), Hydrochloric Acid (37%), $FeCl_3$) and reference substances ascorbic acid (99% purity) and $FeSO_4$ x 7 H_2O from Sigma Aldrich.

The hydroalcoholic extracts of *C. jussieui* flowers, leaves, stems and roots were evaluated at concentrations of 20, 30, 40, 50 and 60 µg/mL. The results were expressed as µmol equivalents of ascorbic acid (EAA) and as µmol equivalents of $FeSO_4$, from the calculation by interpolating the optical density (OD) of the samples in the calibration curves of both reference substances at concentrations of 100, 200, 400, 800 and 1000 µM. The readings were made in triplicate.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

For the quantitative determination the DPPH free radical method (2,2-diphenyl-1-picrilhydracil radical) was used. A Rayleigh UV-1601 UV-visible spectrophotometer, China, was used and the determinations were measured at 517 nm after 30 min [15,16]. The extracts of flowers, leaves, stems and roots of *C. jussieui* were tested at concentrations of 20, 30, 40, 50 and 60 µg/mL as well as the reference substances Vitamin C and trolox. The percentage of inhibition of the DPPH radical was calculated according to the following formula:

% inhibition of the DPPH = [(Abs control - Abs sample)/Abs control] x 100.

The mean inhibitory concentration (IC_{50}) was determined with the help of the Graph prism 5.0 statistical program.

All reagents used were from Merck (DPPH (2,2 difenil-1-picrilhidracilo) and reference substances, ascorbic acid (99% purity) and trolox from Sigma Aldrich.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging activity

The test was carried out according to the methodologies of Re., *et al*. [17], Agudo [18] and Arnao., *et al*. [19]. The assay was based on the ability of different substances to sequester the cationic radical ABTS•+. A Rayleigh UV-1601 UV-visible spectrophotometer, China was used, and the measurements were measured at 734 nm. All reagents used were from Merck (ABTS (2,2'-azino-bis (3-ethylbenzothiazoline) -6-sulfonic acid), potassium persulfate, Ethanol 96%).

The extracts of flowers, leaves, stems and roots of *C. jussieui* and the reference substances vitamin C and trolox were tested at concentrations of 100, 200, 300, 500 and 700 µg/mL. The percentage of inhibition of the DPPH radical was calculated according to the following formula:

% inhibition ABTS = [Abs_{734} (ABTS) - Abs_{734} (antioxidant)]/Abs_{734} (ABTS) x 100

The mean inhibitory concentration (IC_{50}) was determined with the help of the Graphprism 5.0 statistical program.

Statistical analysis

The results corresponding to the physicochemical parameters of the drugs in powder and the extracts as well as those obtained in the quantification of total phenols and total flavonoids, were processed by the statistical program SPSS for Windows version 8.0. Experimental values were expressed as the mean/standard deviation (SD). A simple classification analysis of variance was carried out using ANOVA-1, for a confidence level of 95%, and the Duncan test was used to compare the means.

Data from the DPPH and ABTS trials were analyzed by single-way ANOVA, followed by a Tukey mean multiple comparison test with $p \leq 0.05$.

Results and Discussion

Results

Physicochemical parameters of the powder drugs from *C. jussieui*

Table 1 shows some parameters that were evaluated for *C. jussieui* powder drugs.

Preparation of the extracts, quality physicochemical parameters and phytochemical screening

Extracts at different alcoholic concentrations were made from the different organs, where the 30% hydroalcoholic extract was one of those that achieved the greatest extraction of metabolites in the determination of soluble substances. The physicochemical parameters determined for this extract, as well as its qualitative chemical composition are presented in table 2.

Paramters (%)	Results $\bar{X}$/SD			
	Flowers	Leaves	Stems	Roots
Moisture content	8.09/0.07[a]	7.79/0.03[b]	8.31/0.02[c]	8.07/0.03[a]
Water-soluble extractive	8.10/0.12[a]	17.20/0.05[b]	5.99/0.13[c]	6.29/0.1[d]
Alcohol-soluble extractive at 30%	9.48/0.07[e]	17.41/0.10[f]	6.48/0.08[g]	7.93/0.12[h]
Alcohol-soluble extractive at 50%	8.75/0.05[i]	15.24/0.05[j]	5.71/0.21[k]	7.20/0.18[l]
Alcohol-soluble extractive at 80%	7.91/0.13[m]	14.11/0.06[n]	6.36/0.12[o]	6.92/0.08[p]
Total ash content	3.37/0.19[a]	5.03/0.03[b]	3.15/0.04[c]	1.58/0.04[d]
Water-soluble ash	1.28/0.03[e]	1.45/0.04[f]	0.36/0.03[g]	0.80/0.04[h]
Acid-insoluble ash	1.93/0.03[i]	2.39/0.06[j]	2.42/0.06[k]	0.82/0.02[l]
Legend: $\bar{X}$/SD= Average value of determinations (n = 3)/standard deviation. Different letters in a row show significant differences ($p < 0.05$) according to Duncan test				

Table 1: Physicochemical parameters of the powder drugs from *C. jussieui.*

Physicochemical parameters of the extracts of different organs				
Parameters	**Results** $\bar{X}$/SD			
	Flowers	**Leaves**	**Stems**	**Roots**
pH	5.02/0.02[a]	4.99/0.005[a]	4.81/0.02[b]	4.74/0.01[c]
Total solids (%)	0.91/0.02[d]	1.85/0.03[e]	0.82/0.04[f]	0.84/0.03[df]
Refraction index	1.3570/0.005[g]	1.3545/0.0001[g]	1.3525/0.0002[g]	1.3524/0.0001[g]
Relative density (g/mL)	0.9187/0.0173[h]	0.9420/0.0001[i]	0.9366/0.0004[i]	0.9399/0.0005[i]
Phytochemical screening of the extracts				
Metabolites (Name of Test)	**Extracts**			
	Flowers	Leaves	Stems	Roots
Alcaloids (Dragendorff, Mayer and Wagner reagent test)	+	+	+	+

Coumarins/lactones (Baljet test)	+	+	+	+
Phenols/tannins (Ferric chloride test)	++ gd	++ gd	+ cg	+ cg
Saponins (Foam test)	+	+	+	+
Amino acids (Ninhydrin)	+	+	+	-
Flavonoids (Shinoda (Mg-HCl)	++ yd	++ yd	+ cy	+ cy
Anthocyanins (HCl conc./pentanol)	++	++	+	+
Reducing sugars (Fehling test)	+	++	+	+
Triterpenes/steroids (Liebermann-Burchard)	+ r	+ g	+r	+ g
Legend: $\overline{X}/SD$= Average value of determinations (n = 3)/standard deviation. Different letters in a row show significant differences ($p < 0.05$) according to Duncan test				
+: Positive, ++: Highly Positive, -: Negative, gd: Green Dark. cg: Clear Green. yd: Yellow Dark, cy: Clear Yellow, r: Red, g: Green.				

Table 2: Physicochemical parameters and phytochemical screening of the extracts from *C. jussieui.*

From the point of view of the organoleptic properties, the extracts were presented as slightly translucent liquids, with shades of color between yellowish and yellowish-brown, with the leaf extract being more intense, followed by the flower extract. The smell was characteristic for all extracts.

Total phenols and total flavonoids content

Phenols and flavonoids were quantified as part of the phytochemical analysis of the extracts, as they are metabolites widely distributed in the plant kingdom and considering the results obtained in the qualitative chemical analysis (Table 3).

In both quantifications, calibration curves (Figure 2) were obtained with a good correlation between the tested concentrations of the reference substances (gallic acid and quercetin) and the absorbances. The correlation coefficient (R^2) was ≥ 0.99, this is indicative of the good fit of the model equation to the experimental data.

Extracts	**Total phenols (mg/mL)** $\overline{X}/SD$	**Total flavonoid mg/mL)** $\overline{X}/SD$
Flowers	$1.33/0.01^{a}$	$0.46/0.01^{e}$
Leaves	$2.92/0.04^{b}$	$1.29/0.01^{f}$
Stems	$1.23/0.03^{c}$	$0.40/0.01^{g}$
Roots	$0.83/0.03^{d}$	$0.14/0.04^{h}$
Legend: $\overline{X}/SD$= Average value of determinations (n = 3)/standard deviation. Different letters in a column show significant differences ($p < 0.05$) according to Duncan test.		

Table 3: Total phenols and total flavonoids content of the extracts from *C. jussieui.*

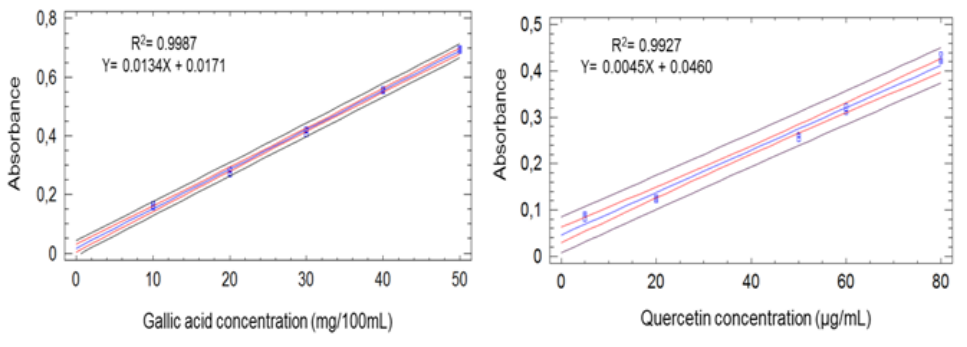

Figure 2: Calibration curves of the gallic acid and quercetin for the determination of total phenols and total flavonoids.

Antioxidant activity of the different organs of *C. jussieui*

The antioxidant activity of the extracts was evaluated using three different methods since it is known that antioxidants can act by multiple mechanisms depending on the reaction system and the radical or oxidant source.

Iron sulfate, vitamin C and trolox were used as reference substances. Iron sulfate ($FeSO_4$) was used as a standard, specifically in the FRAP trial. It is characterized by being soluble in water and by its high antioxidant activity. Vitamin C is a powerful water-soluble antioxidant that is associated with various beneficial effects on the immune system; in the aging process, in endothelial integrity and in lipoprotein metabolism. Trolox is a water-soluble antioxidant. It was synthesized as a derivative of vitamin E and has been used as a standard antioxidant for these antioxidant capacity tests. The results of these tests are shown in table 4.

A. Ferric Reducing Antioxidant Power (FRAP) Assay				
Concentrations (µg/mL)	**µM equivalents of Vitamin C/SD**			
	Extracts			
	Flowers	**Leaves**	**Stems**	**Roots**
20	310.3/11.63[a]	400.22/10.48[b]	228.12/15.13[c]	215.72/18.93[c]
30	415.72/12.80[d]	483.94/16.49[e]	314.95/8.38[f]	294.01/6.15[f]
40	672.31/12.81[g]	845.18/12.94[h]	468.43/10.65[i]	513.40/8.16[j]
50	739.75/10.48[k]	948.28/19.36[l]	659.90/10.48[m]	652.93/13.62[m]
60	923.47/6.71[n]	938.97/10.48[n]	737.42/12.80[o]	721.15/13.22[o]
Concentrations (µg/mL)	**µM equivalents of $FeSO_4$/SD**			
20	236.58/10.87[a]	320.64/9.79[b]	159.77/14.14[c]	148.18/17.70[c]
30	335.13/11.97[d]	398.90/15.42[e]	240.92/7.83[f]	221.41/5.80[f]
40	585.85/9.80[g]	736.58/12.10[h]	384.40/9.96[i]	426.43/7.63[j]
50	638.03/9.80[k]	832.96/18.10[l]	563.39/9.80[m]	553.24/17.43[m]
60	809.77/6.27[n]	824.26/9.80[n]	635.85/11.97[o]	620.64/12.36[o]

B. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity						
Concentrations (µg/mL)	**% inhibition of the DPPH/SD**					
	Extracts					
	Flowers	**Leaves**	**Stems**	**Roots**	**Vitamin C**	**Trolox**
20	53.87/0.43[a]	55.69/0.31[b]	50.81/0.65[c]	50.37/0.49[c]	78.43/0.76[d]	82.72/0.60[e]
30	60.07/0.54[f]	61.12/0.55[g]	55.84/0.57[h]	56.97/0.65[i]	84.58/0.39[j]	85.97/0.38[k]
40	66.91/0.50[l]	70.59/0.60[m]	58.71/0.27[n]	60.43/0.75[o]	86.29/0.49[p]	87.79/0.43[q]
50	76.78/0.34[r]	82.68/0.73[s]	68.12/0.93[t]	66.55/0.60[u]	88.92/0.25[v]	88.63/0.50[v]
60	84.07/0.72[w]	89.10/0.38[x]	72.12/0.65[y]	74.78/2.03[z]	89.76/0.51[x]	89.39/0.49[x]
IC_{50} µg/mL	40.60/5.99	40.57/4.37	40.94/8.63	40.75/10.42	31.07/8.70	31.45/8.32
C. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Activity						
Concentrations (µg/mL)	**% inhibition of the** ABTS•+**/SD**					
	Extracts					
	Flowers	**Leaves**	**Stems**	**Roots**	**Vitamin C**	**Trolox**
100	54.87/0.77[a]	63.98/0.80[b]	50.23/0.92[c]	51.82/0.78[d]	56.14/0.49[a]	43.04/0.72[e]
200	61.31/0.70[f]	70.15/0.62[g]	53.00/0.84[h]	53.80/0.37[h]	72.67/0.64[i]	56.61/0.75[j]
300	72.34/0.49[k]	83.65/0.78[l]	57.41/0.45[m]	55.77/0.64[n]	90.70/0.74[o]	62.81/0.50[p]
500	85.48/0.51[q]	87.36/0.49[r]	67.13/0.63[s]	68.30/0.87[s]	91.87/0.58[t]	91.31/0.80[t]
700	87.64/1.06[u]	89.10/0.90[v]	72.43/0.56[w]	69.76/0.77[x]	94.92/0.64[y]	94.36/0.92
IC_{50} µg/mL	286,0/2,73	242.2/3.69	356.90/4.71	356.90/4.71	210.60/3.79	306.50/7.91
Average value of determinations (n = 3)/standard deviation (SD) Different letters in a row show significant differences ($p < 0.05$) according to Tukey test.						

Table 4: Antioxidant activity of different extracts from *C. jussieui.*

Discussion

Within a pharmacognostic study of a drug a set of determinations are performed to establish the physicochemical parameters, that are essential to establish quality and purity, which translates into its intrinsic value. Table 1 shows some of the parameters that were evaluated for the plant material.

Regarding the moisture content the values found for the different plant organs are between 7.7 and 8.3%. Different pharmacopoeias and regulations establish a residual moisture content of no more than 14%, depending on the plant organ [20-22]. In the study carried out, the humidity value was below the maximum limit allowed for medicinal plants, being much lower for the leaves; amongst flowers and roots there were no significant differences observed, but there were differences between these and the other plant organs.

The determination of extractable or soluble substances is one of the most important numerical indices to select the best solvents in the extraction process. Different menses were used, and the results revealed (Table 1) that a higher yield of extractable substances is obtained in general with the 30% hydroalcoholic mixture in all the organs evaluated. Statistical analysis of the results showed that there were significant differences, although the highest percentages were achieved for the leaves and flowers. In general, in all the solvents studied, the highest contents of soluble substances were found in leaves and flowers.

Ashes constitute a basis for judging the purity and identity of plant material, providing information regarding possible adulteration with inorganic materials or foreign bodies that it possesses, or the amount of these in its content. Some Pharmacopoeias propose a total ash index of up to 5% [20,21] and in others such as the

Chinese Pharmacopoeia, refers to up to 15% [23]. In the experience carried out, the percentage was below the maximum allowed limit. On the other hand, the acid insoluble and water-soluble ashes were also small, mostly less than 2.5%. Significant differences were found between the different plant organs, being in the leaves where the highest contents of total ash and soluble in water were found and in the stems that of insoluble ashes in hydrochloric acid.

Regarding the species, some widely scattered information has been found on the physicochemical parameters [5,7,24-26], but in most cases they carry out the analysis to the mixed aerial organs, or acquire the material from the markets, without specifying date of collection or origin, making it impossible to make a comparison with the results obtained in this work.

For the 30% hydroalcoholic extract obtained from the different plant organs, the quality parameters determined (Table 2) show that all present acidic pH values, although the highest were found in flowers and leaves, with no significant differences. These may be related to the presence of phenolic compounds, as observed in the qualitative chemical analysis in the tests for phenols and tannins, flavonoids and anthocyanidins.

The total solids content is related to the non-volatile solids present in an extract, the values obtained reaffirmed what was found in the plant material, a higher content in leaves and flowers with significant differences between the different organs.

Phenols and flavonoids were quantified as part of the chemical analysis of the extracts. The statistical analysis of the results revealed significant differences in the content of said metabolites, with the concentration in the leaf extract being higher, followed by the flower extract. The results are in correspondence with those obtained for soluble substances and total solids. Table 3 shows the results. It is highlighted that the percentage of total phenols is higher in all organs than that of flavonoids and that the lowest values of both phenolic and flavonoid compounds are present in the roots.

Some studies have reported the content of phenols and flavonoids for this species, but with similar problems to those detected in the analyzes of plant material, various methodologies, mixtures of plant organs, lack of information on the place and time of collection, which prevents make comparisons [7,27]. The antioxidant potential of plant samples is routinely assessed by three approaches: direct measurement of antioxidant enzyme activity, radical removal and reduction *in vitro*, and measurement of the protective response of plant samples against oxidants induced by chemical stress. However, each of these approaches has its own limitations on applicability [28].

The antioxidant effect of plant products has been determined to be mainly attributed to phenolic compounds such as flavonoids and phenolic acids, as well as to ascorbic acid, vitamin E and different carotenoids, among others. These natural antioxidants are very effective in preventing destructive processes caused by free radicals [29-32].

In the FRAP test, the results expressed the reducing capacity of the Fe^{3+} cation of the extract as µM equivalents of ascorbic acid and µM equivalents of $FeSO_4$ (reference substances used and recognized as having a high antioxidant value). Table 4a shows the ferro-reducing activity associated with the evaluated extracts. Antioxidant activity was evidenced in a concentration-dependent manner, achieving in all the concentrations tested of the extracts, higher values (in equivalents of ascorbic acid and $FeSO_4$) at the lowest concentration tested (100 µM) of each reference substance.

The results lead us to suggest that the hydroalcoholic extract of leaves and flowers have a high antioxidant activity, which translates into the high equivalent µM values expressed as a function of the reference substances tested, finding no significant differences at the 60 µg/mL concentration. The stem and root extracts also showed ferro-reducing activity with a similar behavior.

An analysis of the results with reports from the literature allowed us to suggest the good ferro-reducing power of the extracts when comparing them with plants such as *Achyranthes bidentata* (117.70 ± 18.29), *Allium macrostemon* (101.69 ± 4.85), *Angelica sinensis* (101.69 ± 22.06), *Cortex dictamni* (141.24 ± 51.78), among others, whose values in µM equivalents were lower than the lowest. Value obtained for one of the extracts tested (in the root extract the µM equivalents of Vitamin C = 215.72/18.93 and the µM equivalents of $FeSO_4$ = 148.18/17.70) at the lowest concentration (20 µg/mL) [33].

In the DPPH assay, antioxidant capacity to reduce 1,1-diphenyl-2-picrilhydracil radical is evaluated [34,35]. There is a tendency to increase the inhibitory capacity of said radical as the concentration

increases. At the maximum concentration tested (60 µg/mL), the leaf extract, vitamin C and Trolox had a comparable behavior without statistically significant differences.

As can be seen in table 4b, from the lowest concentration evaluated inhibition percentages of more than 50% were presented, highlighting the reference substances and the extracts of leaves and flowers for presenting the highest values. At maximum concentration, the leaf extract had a behavior similar to vitamin C and Trolox without significant differences with inhibition percentages greater than 89%, evidencing a high sequestering power of the DPPH radical.

An important aspect to consider is the determination of IC_{50} (concentration value at which 50% inhibition of the maximum effect of DPPH sequestration is reached). In this sense, the extracts showed good antiradical activity with similar values, although the highest activity was for the reference substances with the lowest IC_{50} value. The results are considered good if it is considered that the concentration of the metabolites responsible for the antioxidant action in the extracts would always be much lower than the referred concentration of the pure compounds (vitamin C and Trolox).

The DPPH assay is used in numerous studies evaluating the antioxidant activity of extracts obtained from various species of medicinal plants, in these comparisons are made with various antioxidant patterns and the results are variable in relation to the antioxidant capacity of the extracts. When comparing the IC_{50} results achieved for the four extracts with those of other medicinal plants reported in the consulted literature, it is highlighted that the obtained value is comparable to those obtained for different extracts of Datura alba (leaves) at concentrations of 30, 40 and 50 µg/mL, with which IC_{50} less than 50 µg/mL [36] was achieved, and others such as *Portulaca oleracea* (41.18 µg/mL), *Solanum nigrum* (42.89 µg/mL), *Ipomoea aquatica* (42.43 µg/mL) [37].

Other species recognized as antioxidants, but with IC_{50} much higher than those obtained for the *C. jussieui* extracts, we could mention the seeds of *Nigella sativa* (IC_{50} of 624.7 ± 12.77 µg/mL), *Nigella damascena* (IC_{50} of 177.6 ± 3.71 µg/mL) [38], seed and leaf extracts of *Allium ampeloprasum* subsp. *persicum* (IC_{50} of 315 to 792 µg/mL) [39]. All the above highlight the good sequestering power of the DPPH radical of the extracts evaluated.

During the development of the method using the 2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid radical (ABTS•+), a trend was observed to increase the inhibition capacity of said radical as that increased concentration.

All the extracts showed percentages of ABTS radical inhibition greater than 50% at the minimum concentration tested (100 µg/mL), even higher than Trolox, where the leaf extract also surpassed vitamin C. Additionally, at concentrations of 200 and 300 µg/mL the extracts of leaves and flowers surpassed Trolox in terms of percentage of sequestration. However, the remaining extracts also managed to inhibit the ABTS radical. Table 3c illustrates the results.

Of the samples evaluated, the one with the lowest IC_{50} and therefore the highest antioxidant activity was vitamin C, followed by leaf extracts and flowers. Stem and root extracts behaved similarly in terms of antiradical activity.

The synergism between the antioxidants of a mixture causes that the antioxidant activity depends not only on its concentration but on the interaction between them. The antioxidant capacity of an extract is not only given by the sum of the antioxidant capacities of each of its components, it also depends on the microenvironment in which they are found. The compounds interact with each other and synergistic or inhibitory effects can occur [40]. That is why, at present, the determination of the antioxidant activity of an extract must be verified by at least three or more methods.

For *C. jussieui*, several studies have reported antioxidant activity using different methods, different plant organs or mixtures of these, and different extraction solvents.

Padilla and Paucar [41] evaluated the antioxidant activity by the DPPH method of a methanolic extract from the flowers, reporting that it had no activity.

Dueñas [5] reported the antioxidant activity of a mixture of leaves and stems, by the method of hemolysis of red blood cells.

On the other hand, Ortíz [42], used the chemiluminescence method to determine the antioxidant activity of the mixture of leaves and flowers.

Other works report the antioxidant activity of mixtures of aerial parts of the species mainly by the methods of DPPH and ABTS

[25,26], in different solvents, but in none of the reported the FRAP method is evaluated for the evaluation, thus as the plant organs are not studied independently, nor is there any previous information on these determinations in the roots.

In the phytochemical study of the extracts, the presence of phenolic compounds (tannins, flavonoids, anthocyanidins, coumarins), triterpenoids, among other compounds, was detected. From this analysis, it could be suggested that the joint action of all these secondary metabolites favors the antioxidant effect observed in our study. However, future experiments must be carried out to study the structure-activity relationship of the metabolites present.

The antioxidant assays showed that the extracts of leaves and flowers were the most active of all the extracts tested, which is in correspondence with the highest content of total phenols and flavonoids. The results suggest that these compounds are very directly related to the activity evaluated.

Conclusion

Some physicochemical parameters of the powdered drugs of flowers, leaves, stems and roots and their hydroalcoholic extracts were determined, vital for the preparation of future Quality Control Standards for the species.

The phytochemical study suggested the notorious presence of phenolic compounds in general, with differences in the concentration of phenols and flavonoids according to the vegetative organ tested.

Taking into account the results of the three *in vitro* methods used, it was found that as the concentration of the extracts increased the reducing power (FRAP test) and the anti-radical activity (DPPH and ABTS tests) of the same increased, manifesting a high antioxidant activity.

Of the four extracts evaluated, those of leaves and flowers showed the highest antioxidant capacity by the three methods, with a behavior similar or superior to the reference substances in some tested concentrations.

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Conflict of Interest

The authors declare no conflict of interest.

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