ANTIOXIDANT EFFECTS OF *MUEHLENBECKIA HASTULATA* J. (POLYGONACEAE) EXTRACTSMARCO MELLADO^{1*}, ALEJANDRO MADRID¹, CARLOS JARA², LUIS ESPINOZA^{1*}¹Departamento de Química, Universidad Técnica Federico Santa María, Av. España N° 1680, Valparaíso, Chile.²Laboratorio de Radicales Libres, Universidad de Valparaíso, Gran Bretaña 1111, Valparaíso, Chile.

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ABSTRACT

The antioxidant properties of different extracts of *quilo* (*Muehlenbeckia hastulata* J., Polygonaceae) herb from Valparaíso region in Chile were evaluated. Antioxidant activity was assessed in three different model systems. Antioxidant models were examined by measuring the scavenging activity of extracts on 2,2-diphenyl-1-picrylhydrazil (DPPH•). Moreover extracts were evaluated by ferric reducing antioxidant power (FRAP) and the total peroxyl radical-trapping potential (TRAP). The amount of dried extract, the content of total phenolics, flavonoids, hydrolyzed sugar and anthraquinones were also determined. Extracts pertaining to stem and root of the plants were the significant activities DPPH radical scavenging (IC₅₀ 0.74 mg/mL to 1.33 mg/mL the more active extracts). The activities and TRAP and FRAP high values in extracts of EtOAc (1.423 and 1.540 mM TEAC) and ethanol (245.26 and 309.04 mM TEAC) stem and root of the plant are higher. The observed differences in antioxidant activity among the different parts of *quilo* could be partially explained by the levels of phenolics compounds present in the root extract of EtOAc (0.023 mg GAE/g dry extract), flavonoids in the leaves of EtOAc extract (99.12 mg QE/g dry extract), hydrolyzed sugar in the ethanol extract (21.87, 14.85 and 14.32 mg GES/ g dry extract of leaf, stem and roots, respectively) and anthraquinones in the EtOAc extracts (2.50, 1.43 and 0.84 mg EE/ g dry extract of leaf, stem and roots, respectively).

Keywords: *Muehlenbeckia hastulata*, Polygonaceae, Antioxidant activity, DPPH radical, FRAP, TRAP, Total phenolics, Flavonoids, Hydrolyzed sugar and Anthraquinones.

1. INTRODUCTION

Since ancient times, plants have been used as novel therapeutic agents and their uses have been passed from generation to generation, orally or in writing. The importance of natural product in medicine, is not based only on their pharmacological or chemotherapeutic effects, but they offer the possibility to develop the new drugs structures^{1,3}. Thus, phytochemicals and biochemistry of medicinal plant are of particular interest to many scientists, since they are widely used as nutrients, and the role they can play in the fight against diseases of the modern age (malignant tumors, cardiovascular disease, diabetes, rheumatism, Alzheimer's disease) and aging processes, are often connected with oxidative stress, reactive oxygen species (ROS) and lipid peroxidation (LP)²⁻⁸.

A reactive oxygen species is effectively eliminated with the help of antioxidant compounds within which polyphenols are included¹⁰. Therefore, attention is focused on the use of antioxidants to protect cells from the biological damage due to free radicals and oxidants species. Antioxidants have been widely used as food additives (such as tertbutyl-hydroxytoluene – BHT–, tertbutyl-4-hydroxyanisole – BHA – and propyl gallate – PG) to provide protection against oxidative degradation of foods and to measure its lifetime^{3-6, 8, 11}, but these compounds have been reported to be carcinogenic and hazardous for the human health. Hence, several attempts to replace synthetic antioxidants with natural antioxidants have been developed and investigated obtaining them from natural sources such as vegetables, fruits, leaves, bark, roots of plants, among others⁹.

Because the chemical diversity of the antioxidant compounds present in foods, complete databases on food antioxidant content are not yet available. Furthermore, levels of antioxidants in food alone does not necessarily reflect the total antioxidant capacity (TAC), which also depends on synergistic and redox interactions among different molecules present in food⁴.

Muehlenbeckia hastulata popularly known as *quilo*, is used by ancient cultures as diuretic and hypotensive, besides being used as compresses to relieve burns. The chemical composition of aerial part indicates the presence of tannins and flavonoids as routine and epicatechin. Moreover in the roots there are anthraquinones: chrysophanic acid, emodin, rhein, hypericin and protohypericin¹²⁻¹⁶.

It has been described that the extract of the plant acts as an antiviral against influenza which has the chloroform extract of aerial part (0.125 mg/mL). Also, it has an oxitoxic effect (secretion of oxytocin promoter) of hexane root extract (62% effectiveness of phenoterol and papaverin). Besides, it has a pain killer effect and a poor microbacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella aviatum*, *Salmonella aeruginosa*, *Staphylococcus aureus*, *Micrococcus flavus* y *Bacillus subtilis* (hexane, dichloromethane and ethanol extracts of aerial parts)^{12, 17}.

In the present work we have made some phytochemical screens of hexane, CH₂Cl₂, EtOAc and ethanol crude of different parts (leaf, stem and root) extracts

of *Muehlenbeckia hastulata*, total phenolics, flavonoids, hydrolyzed sugar and total anthraquinones content were quantified and the antioxidant activity of the extracts was determined in vitro by the neutralization of 2,2-diphenyl-1-picrylhydrazil (DPPH•) radical, as well as by ferric reducing antioxidant power (FRAP) and the total peroxyl radical-trapping potential (TRAP).

2. EXPERIMENTAL PROCEDURES

2.1 Plant material

The plant was collected in the geographical position 33° 05' 45" S – 71° 35' 17" W at 460 meters of sea level in April 2011. Voucher specimen is kept in the Herbarium of the Natural Products Laboratory, "Dr. Herbert Appel A", UTFSM, Valparaíso, Chile (MHJ-2011). The plant was recognized by Rodrigo Villaseñor, professor of Biology and expert in botany, considering different morphological properties of the material.

2.2 Extraction Procedure

The plant material (leaves, stems and roots) was dried at room temperature and then was subjected to successive extractions with solvents of increasing polarity: hexane, dichloromethane, ethyl acetate and ethanol to total exhaustion. All extracts and fractions obtained were concentrated in a rotary evaporator at 40° C and then were taken to dry at room temperature in the dark.

2.3 Determination of total phenolic content

The amount of total phenolic compounds in extracts was determined spectrophotometrically using Folin-Ciocalteu (FC) as reagent according to the method used by Waterman¹⁸ with small modifications Köhler¹⁹. Each extract sample (5 mg) was diluted to 5 mL with ethanol. This solution (0.5 mL) was then mixed with 2.5 mL of 0.2 N FC reagent for 5 min and 2 mL 7.5% sodium carbonate solution was then added. The mixture was incubated in the dark at room temperature for 2 h. The absorbance of the resulting solution was measured at 700 nm on a UV-Vis spectrophotometer (Jenway 6320D, Bibby Scientific Limited, Beacon Road, Stone, Staffordshire ST15 0SA, UK) using ethanol as blank. The concentration of total phenolic compounds was expressed in mg of gallic acid equivalents (GAE) per g dried extract (d.e.), using a standard curve of gallic acid (0-200 mg/L) treated the same way as samples. All measurements were replicated three times.

2.4 Estimation of total flavonoid content

The total flavonoid content was determined using the Dowd method as adapted by Arvouet-Grand, Vennat, Pourrat, and Legret²⁰. Briefly, 5 mL of 2% aluminium trichloride (AlCl₃) in ethanol was mixed with the same volume of extract solution in ethanol (1.0 mg/mL). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without AlCl₃. The total flavonoid content was determined using a standard curve with quercetin (0-70 mg/L) as standard. The mean of

three readings was used and expressed as mg of quercetin equivalents (QE)/per g dry extract.

2.5 Estimation of total hydrolyzed sugar content

The total hydrolyzed sugar content was determined using the method by Bruneton²¹ and Dominguez²² with modifications²³. Briefly, 3 mL of ethanolic extracts were mixed with 4 mL 6M HCl and 3 mL of distilled water. After incubation at room temperature for 4 days, this mixture was extracted with 5 mL of EtOAc, two times, then discard organic layer. The aqueous solution was dilute to 20 mL. Then 1 mL extract were mixed with 1 mL 0.5% anthrone in ethanol and 5 mL of concentrate H₂SO₄. The mixture was cooled at room temperature for 10 min. The absorbance of the resulting solution was measured at 621 nm using distilled water as the blank. The total hydrolyzed sugar content was determined using a standard curve with glucose anhydrous (1 to 140 mg/L) as standard. The mean of three readings was used and expressed in mg glucose equivalents (GES) per g extract.

2.6 Estimation of total anthraquinones content

This estimate is taken as reference the total flavonoids according to the points made by Arvouet-Grand, Vennat, Pourrat, and Legret²⁰ with modifications²³. Briefly, 5 mL of 2% aluminium trichloride (AlCl₃) in ethanol was mixed with the same volume of extract solution in ethanol (1.0 mg/mL). Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 5 mL extract solution with 5 mL of methanol without AlCl₃. The total anthraquinone content was determined using a standard curve with emodine (0–70 mg/L) as the standard. The mean of three readings was used and expressed as mg of emodin equivalents (EE)/per g dry extract.

2.7 Antioxidant activity

2.7.1 Reactive Oxygen Species scavenging capacity

Reactive Oxygen Species (ROS) scavenging capacity of the extracts was evaluated by measuring scavenging activity on DPPH•, FRAP and TRAP assays.

2.7.1 DPPH assay

The DPPH assay was performed as described previously by Brand-Williams²⁴ with modifications Lakić³. 0.1 mL the samples (from 0 to 4 mg/mL of the extracts) were mixed with 2.9 mL 50 µM DPPH• solution was freshly prepared in ethanol. 2.9 mL 50 µM DPPH• solution with 0.1 mL ethanol was used as a control. The absorbance of the resulting solutions, control and the blank (with the reagents only) were recorded after 15 min at room temperature. Each sample was replicated three times. The disappearance of DPPH• was detected spectrophotometrically at 517 nm. Percent radical scavenging capacity (RSC) was calculated by the following equation:

$$\text{RSC (\%)} = 100\% \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

From the obtained RSC (%) values the IC₅₀ value, which represent the concentrations of extracts that caused 50% neutralization, was determined by linear regression analysis.

2.7.2 Ferric Reducing Antioxidant Potential (FRAP) Assay

The ferric reducing power of plant extracts was measured as described Dudonné²⁵ with modifications. This method is based on the reduction, at low pH, of a colorless ferric complex (Fe³⁺-tripyridyltriazine) to a blue-colored ferrous complex (Fe²⁺-tripyridyltriazine) by the action of electron-donating antioxidants. The reduction is monitored by measuring the change of absorbance at 593 nm. The working FRAP reagent was prepared daily by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. A standard curve was prepared using various concentrations of Trolox™. All solutions were used on the day of preparation. One hundred microliters of sample solutions and 300 µL of deionized water were added to 3 mL of freshly prepared FRAP reagent. The reaction mixture was incubated for 30 min at 37 °C in a water bath. Then, the absorbance of the samples was measured at 593 nm. A sample blank reading using ethanol was also taken. The difference between sample absorbance and blank absorbance was calculated and used to calculate the FRAP value. In this assay, the reducing capacity of the plant extracts tested was calculated with reference to the reaction signal given by a Trolox™ solution. FRAP values were expressed as mM Trolox™. All measurements were done in triplicate.

2.7.3 Total Reactive Antioxidant Potential (TRAP) assay

The method developed by Romay²⁶ was slightly modified in this experiment. A 10 mM solution of ABAP (2,2'-azo-bis(2-amidino propane) was mixed with 150 µM solution of ABTS (2,2'-azinobi(3-ethylbenzothiazoline-6-sulfonic acid) in 100 mM solution of PBS (phosphate buffered saline) a pH 7.4. The mixture was incubated at 45 °C for 30 min. 10 µL of sample solution was added to 990 µL of the resulting blue-green ABTS radical solution. The decrease of absorbance of TRAP solutions and ABTS as blank were recorded after 30 s at room temperature. Then, the absorbance of the samples was measured at 734 nm. The total antioxidant capacity (TRAP) of extracts was expressed in mM Trolox™ equivalents (TEAC), using a standard curve of Trolox™ (0-120 mg/L). All measurements were replicated three times.

2.8 Statistical analysis

The data was reported as mean values ± standard deviation (SD). In the case that the distribution is parametric data, there will be a One-Way ANOVA test. If the distribution is non-parametric, then tests like Kruskal-Wallis ANOVA will be used with a confidence level of 95%. Values representing the concentrations of investigated extracts that cause 50% neutralization or inhibition (IC₅₀) were determined by linear regression analysis of RSC(%), FRAP and TRAP results (STATISTICA 7.0 program).

3. RESULTS AND DISCUSSION

3.1 Chemical composition

Air dried and ground plant material (0.5 kg for each of different organs) was extracted successively at room temperature with n-hexane (Hex), dichloromethane (CH₂Cl₂), ethyl acetate (EtOAc) and ethanol (EtOH). After removing of the solvents in vacuo, the dry extract was obtained for each organ from plant material (Table 1). All extracts were submitted to Antioxidant activity assays.

The major values of total phenolics are present in the extract of EtOAc of root (0.47 mg GAE/g dry extract), while the extract of stem (EtOAc) has a greater presence of anthraquinones (4.86 mg EE/g dry extract). The flavonoids and hydrolyzed sugar are concentrated in the extracts EtOAc (99.12 mg QE/g dry extract) and EtOH (21.87 mg GES/g dry extract) (see table 1).

One would expect to find larger amounts of phenolic substances (anthraquinones and flavonoids) in all organs collected but these were found mainly in the roots of the *Muehlenbeckia hastulata*, due to the properties attributed to this plant. These results could be related to phenolic compounds, particularly anthraquinones, which have laxative or purgative function, the most active *O*-glycosides²¹.

In the case of the sugars we have found a big amounts present into the extracts of root (EtOAc), this could be explained because sugars generally move from leaves to roots and they are usually absorbed in the form of starch or are simply metabolized by the plant. Although they do not exert biological activity in organisms by themselves, but they are used as protective agents of the active ingredients in order to maintain the integrity of these^{21, 27}.

The distribution of phytoconstituents in solvents shows that significant differences ($p < 0.05$), phenols (or reducing substances) are mainly found in the EtOAc and EtOH extracts, as well as flavonoids. While the anthraquinones are distributed essentially in EtOAc extracts and hydrolyzed sugars in extracts of EtOH. This phenomenon can be explained since the phenolic compounds derived have a high polarity, distributed in extracts of higher polarity. In the particular case of hydrolyzed sugars, it is common that they are in the EtOH extracts, especially compounds forming part of *O*-glycosylated or *C*-glycosylated.

Performing the analysis in plant organs, we found that there are significant differences ($p < 0.05$), we saw that phenols are mainly located in the root of the plant, while anthraquinones are on the stem. Also, flavonoids are concentrated in the leaves. However, the hydrolyzed sugars are evenly distributed in the plant organ.

On the other hand, the determination of phenols or reducing substances, performing relationship between the amount of these compounds and the polarity of the solvent extraction shows there are significant differences ($p = 0.001$) distributed mainly in the extracts of EtOAc and EtOH. However, in making this relationship in terms of plant parts, significant differences were also found ($p = 0.000$), indicating that the roots of *quilo* have most of such compounds. This may be because the roots of this plant are in contact with the ground, which is a potential redox system. This could explain that the plant can generate defense mechanisms, for example, the property of resisting a long time of extreme dryness and humidity^{12, 13, 15}.

Table 1. Amounts of dried extracts, total phenolics, anthraquinones, flavonoids and hydrolyzed sugar ($\pm$ SD) of *Muehlenbeckia hastulata* extracts.

Part Plant	Extract	Dried extract (g)	Phenolics (GAE)	Flavonoids (QE)	Sugars (GES)	Anthraquinones (EE)
Leaf	Hexane	7.65	0.008 $\pm$ 0.003	10.84 $\pm$ 0.07	0.04 $\pm$ 0.00	0.54 $\pm$ 0.04
	CH ₂ Cl ₂	25.47	0.009 $\pm$ 0.000	51.92 $\pm$ 1.38	0.36 $\pm$ 0.00	0.85 $\pm$ 0.23
	EtOAc	10.59	0.013 $\pm$ 0.003	99.12 $\pm$ 2.04	8.99 $\pm$ 0.72	2.50 $\pm$ 0.04
	EtOH	72.46	0.014 $\pm$ 0.000	94.29 $\pm$ 1.15	21.87 $\pm$ 0.12	1.86 $\pm$ 0.06
Stem	Hexane	4.24	0.009 $\pm$ 0.000	6.48 $\pm$ 0.26	0.00 $\pm$ 0.02	1.22 $\pm$ 0.10
	CH ₂ Cl ₂	3.27	0.013 $\pm$ 0.000	39.21 $\pm$ 0.72	1.69 $\pm$ 0.05	1.58 $\pm$ 0.01
	EtOAc	3.84	0.020 $\pm$ 0.000	40.63 $\pm$ 0.41	13.03 $\pm$ 1.29	1.43 $\pm$ 0.04
	EtOH	35.14	0.020 $\pm$ 0.000	21.77 $\pm$ 0.46	14.85 $\pm$ 0.46	2.49 $\pm$ 0.10
Root	Hexane	1.55	0.016 $\pm$ 0.000	0.00 $\pm$ 0.00	0.63 $\pm$ 0.03	4.86 $\pm$ 0.12
	CH ₂ Cl ₂	1.43	0.017 $\pm$ 0.000	1.91 $\pm$ 0.33	0.71 $\pm$ 0.03	1.38 $\pm$ 0.05
	EtOAc	2.38	0.023 $\pm$ 0.000	12.07 $\pm$ 0.59	7.47 $\pm$ 0.02	0.84 $\pm$ 0.00
	EtOH	14.88	0.022 $\pm$ 0.000	25.57 $\pm$ 0.10	14.32 $\pm$ 0.88	0.93 $\pm$ 0.02

For the flavonoids, the relationship between the amount of these compounds and the polarity of the solvent extraction indicates that there are significant differences ($p = 0.001$), distributing into the highest polarity extracts (EtOAc and EtOH). On the other hand, considering only the parts of the plant, these showed significant differences ($p = 0.001$), leading to the leaf of the plant with higher values in comparison to other organs. The previous result has a close relation to the effect of UV-B photoprotection, preventing from the decomposition of chlorophyll.

The hydrolyzed sugar, has a relationship between the amount of these compounds and the polarity of the solvent extraction showing that there are significant differences ($p = 0.000$), where EtOH extracts are those with the highest concentration of such metabolites. However, in performing the same comparison among *quilo* parts we can see that there are no significant differences ($p = 0.941$), distributing these structures equally in the plant. The correlations between antioxidant activities and the amount of metabolites present in each of the organs how that there is, in general, a dependence of phenolic compounds and flavonoids on the activities measured.

For the case of the determination of anthraquinones, in making the relationship between the amounts of such compounds and the polarity of the extracting solvents, it is appreciated that there are significant differences ($p = 0.003$), mainly distributed in EtOAc extracts. However, when making the relationship between the amounts of anthraquinone and different parts, it is shown that there are significant differences ($p = 0.039$), indicating metabolites are concentrated in the stem.

3.2 Antioxidant activity

The antioxidant activity of *Muehlenbeckia hastulata* extracts was evaluated in a series of *in vitro* tests. Each of these assays is based on one feature of antioxidant activity, which is the ability to scavenge or the ability to evaluate the reducing power. Multiple *in vitro* methods for the evaluation of the antioxidant activities of different plant products are recommended because the composition of plant extracts is complex^{28,29}. Thus, the antioxidant properties of the examined *Muehlenbeckia hastulata* extracts were evaluated as free radical scavenging capacity (RSC) as well as TRAP and FRAP. In the DPPH-test, the ability of examined extracts to act as donors for hydrogen atoms or electrons to reduce DPPH• into DPPH-H was measured spectrophotometrically. Assessed extracts, made from *Muehlenbeckia hastulata*, were able to reduce the stable radical DPPH• to the yellow coloured DPPH-H, the experiments confirmed that free radical scavenging activity of all leaves extracts reaching a 50% reduction with an IC₅₀ of 0.60 mg/mL to 43.42 mg/mL. (See table 2). The extracts were active against the DPPH solution, however extracts of EtOAc, EtOH and CH₂Cl₂ (only the stem extract) have an activity comparing with Trolox (IC₅₀ 10.52 mg/mL). This may be because the extracts have a complex mixture of compounds with potential antioxidant activity (phenols, flavonoids, anthraquinones, etc.), which may have synergistic effect, increasing the activity of the extract. Moreover, it has been reported that the plant *Muehlenbeckia*

platyclada (plant from Peru) possesses an antioxidant activity mainly in the extract of EtOAc and this extract, the most active of all, however, does not have any information about the content of phytochemicals³⁰.

The Total Antioxidant Capacity (TAC) of extracts from different parts of the *quilo* (leaf, stem and root) using two different assays was evaluated. TAC values of the extracts for each assay are shown in Table 2. In examining results of the FRAP assays, EtOAc and EtOH extract of leaves, stem and roots had the greatest antioxidant capacity, in the same manner as in the TRAP assay. The high antioxidant capacity of the EtOAc and EtOH extract of leaves is due to the large amount of free phenols present.

Table 2. IC₅₀ DPPH• and TEAC mM of FRAP and TRAP ($\pm$ SD) values of extracts of *Muehlenbeckia hastulata*.

Part Plant	Extract	IC ₅₀ DPPH• (mg/mL)	FRAP (mM Trolox _{TM})	TRAP (mM Trolox _{TM})
Leaf	Hexane	20.70 $\pm$ 0.08	0.13 $\pm$ 0.01	60.38 $\pm$ 3.34
	CH ₂ Cl ₂	11.23 $\pm$ 0.15	0.16 $\pm$ 0.02	102.77 $\pm$ 2.74
	EtOAc	5.79 $\pm$ 0.00	0.17 $\pm$ 0.02	206.91 $\pm$ 3.70
	MeOH	2.19 $\pm$ 0.01	0.51 $\pm$ 0.01	299.06 $\pm$ 27.41
Stem	Hexane	27.33 $\pm$ 0.80	0.05 $\pm$ 0.00	61.59 $\pm$ 5.00
	CH ₂ Cl ₂	5.80 $\pm$ 0.06	0.12 $\pm$ 0.00	75.76 $\pm$ 1.06
	EtOAc	1.33 $\pm$ 0.05	1.42 $\pm$ 0.00	245.26 $\pm$ 16.49
	MeOH	0.60 $\pm$ 0.48	1.44 $\pm$ 0.01	240.36 $\pm$ 7.51
Root	Hexane	43.42 $\pm$ 0.78	0.05 $\pm$ 0.00	80.66 $\pm$ 4.60
	CH ₂ Cl ₂	11.11 $\pm$ 0.93	0.59 $\pm$ 0.02	148.79 $\pm$ 7.54
	EtOAc	0.74 $\pm$ 0.02	1.54 $\pm$ 0.03	309.04 $\pm$ 15.45
	MeOH	0.75 $\pm$ 0.02	1.75 $\pm$ 0.02	308.49 $\pm$ 10.28

Observing all these values and relating then to the polarity of the solvents, we noticed significant differences ($p < 0.05$), distributed mainly in EtOAc and EtOH extracts. However, in making a comparison of this activity with the plant organ it is appreciated that there are no significant differences ($p > 0.05$), indicating an equal distribution of this activity in the plant.

For the DPPH activity, in all the organs there is a correlation between flavonoids and DPPH• RSC (between R² = 0.667 and 0.939), so this activity

depends on the concentration of these metabolites.

The FRAP activity, in the leaves, stem, possesses a dependency on the activity in the presence of phenolic compounds and hydrolyzed sugars ($R^2 =$ between 0.410 and 0.981, see table 3). Moreover, in the root of *quilo*, there is a dependency on the amount of flavonoids ($R^2 = 0.906$). This may be due to a flow through the plant metabolites, subject to transformation into other phenolic compounds.

Finally, TRAP activity, in the leaves, stem and root, possesses a dependency on the activity in the presence of phenolic compounds ($R^2 = 0.661, 0.993$ and 0.965 , respectively). Also, it can be seen a dependency on the amounts of sugar hydrolysed with this activity (between 0.766 and 0.984, see table 3) because the possibility of the osidic portion is donate $H^\bullet$ for reducing the concentration of TRAP solutions.

Table 3: R^2 values for the correlation of data between trials and amount of antioxidant metabolites.

Part Plant	Metabolite	DPPH	FRAP	TRAP
Leaf	Phenol	0.598	0.410	0.661
	Flavonoid	0.939	0.330	0.785
	Sugar	0.698	0.887	0.935
	Anthraquinones	0.668	0.158	0.616
Stem	Phenol	0.795	0.928	0.933
	Flavonoid	0.607	0.104	0.126
	Sugar	0.599	0.981	0.984
	Anthraquinones	0.210	0.171	0.198
Root	Phenol	0.677	0.924	0.965
	Flavonoid	0.676	0.906	0.831
	Sugar	0.471	0.824	0.766
	Anthraquinones	0.393	0.146	0.085

4. CONCLUSIONS

The measurement of antioxidant activity by DPPH RSC, FRAP and TRAP assay was used as a method for the evaluation of *quilo* extracts which have not been reported previously. The comparison of antioxidant activities (IC_{50} values) of investigated extracts (table 2) showed variable effects that depended on examined extracts and the model systems used for evaluation. Based, on the distribution of metabolites quantified in each of the organs, it can be seen that antioxidant activity (measured with DPPH, FRAP and TRAP assays) is scattered in all organs. However, we must emphasize that this activity is mainly focused on EtOAc and EtOH extracts, which have significant amounts of phenolic compounds, anthraquinones and flavonoids. In the future you can make a bioguided study for the discovery of new compounds with antioxidant activity in these plant extracts.

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