Comparative Analysis of the Physicochemical Properties and Antioxidant Capacity of a Morphotype of Oregano (*O. Vulgare* L.) Cultivated in Two Locations of the Ecuadorian Sierra

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Oregano (O.vulgare L.) is a species introduced in the Ecuadorian flora. Because of its properties it is highly appreciated and consumed, and it is important to determine the nature of its benefits. The chemical composition and the content of functional compounds responsible for the antioxidant activity of the plant identified by the INIAP Germplasm Bank as ECU-20229 were evaluated. It was cultivated in two areas of the provinces of Imbabura (Italquí) and Pichincha (Tumbaco). The whole plant and its parts (root, stem and leaves) were analyzed in order to determine where the highest content was found. Proximal analysis, minerals, polyphenols and total flavonoids were analyzed, as well as the antioxidant capacity. The analyses carried out on the plant parts showed significant differences, determining the influence of the environment where it was grown. It was possible to determine the presence of nutrients such as protein, fat and carbohydrates, which is higher in the leaves; macro elements are abundant in the stem and leaves and micro elements are found in greater quantity when it was cultivated in Italquí, as well as the content of polyphenols and total flavonoids; the antioxidant capacity is higher in the environmental conditions of Tumbaco. The properties described for this plant have been proven to benefit human and animal health, as well as adding value to its wasted parts.

Keywords: environment, chemical quality, functional compounds, Origanum vulgare

INTRODUCTION

Nowadays, mankind prefers to seek ways to incorporate healthy practices into their daily lives. Consequently, aromatic and medicinal plants, foods of vegetable origin have originated a growing interest in consumption, due to the various benefits for human and animal health. The oregano plant, when used in culinary recipes, is part of the human diet. Foods of vegetable origin are characterized by the presence of secondary metabolites, among which the presence of bioactive and functional compounds stands out. Oregano is a species that is attributed uses as a gastronomic condiment and is also appreciated for its medicinal function, where it is attributed the benefits as a tonic, soothing, appetite stimulant, among others. (de la Torre, 2008). Several research studies have shown that it has beneficial effects on people's health (Koksal *et al*., 2010). Oregano is an aromatic plant native to Europe, cultivated in various regions of the world, with different varieties with their own characteristics. In Ecuador it is an introduced species that for its organoleptic characteristics is very appreciated and consumed. However, of all its structural parts (root, leaves, stems), the most used are the leaves, both fresh and dried. According the (INIAP, 2014), some medicinal and aromatic plants, including oregano, are cultivated in home gardens that supply the food and medicinal needs of the families that grow them, using ancestral knowledge, which is why not much research has been done in the country on the benefits of the oregano plant produced at the national level, in terms of its nutritional composition, the presence of minerals and antioxidant capacity.

Encouraged by the rediscovery of beneficial plants present in the Ecuadorian flora, the present work sought to evaluate the nutritional chemical composition and the content of bioactive and functional compounds responsible for the antioxidant activity of the oregano plant cultivated in two environmental zones of the provinces of Imbabura and Pichincha, as well as to provide an added value to the unused parts, which are the stem and the root.

MATERIALS AND METHODS

This study was carried out with oregano (O.vulgare L.) ECU-20229 plants, conserved in the INIAP Germplasm Bank, which were multiplied in two locations. In the province of Pichincha, canton Quito, parish Tumbaco, locality of the INIAP Tumbaco Experimental Farm, with an average temperature of 16.2 ºC; altitude 2,348 m.a.s.l.; coordinates of the collection: latitude 00º 13' 00"S, longitude 78º 24'01"W. In the province of Imbabura, Cotacachi canton, El Sagrario parish, Italqui locality, with an average temperature of 15.0 ºC; altitude 2,684 m.a.s.l.; coordinates of the collection: latitude 00º 18' 06'"S, longitude 78º 18'53"W.

With the plants in a state of physiological maturity and medium size, random sampling was carried out. After cleaning to remove soil residues, the harvested plants were distributed as follows: for proximate and physicochemical analysis, 300 g of fresh whole plant and another 300 g from which the root, stem and leaves were separated. For proximate and mineral analysis, they were subjected to a drying process in an oven at 65 °C (LAB LINE Imperial V) for 12 hours, the dried samples were ground and stored in plastic jars until the time of analysis. For the analysis of functional compounds and antioxidant capacity, they were frozen at -18 °C (Elecrolux. EC306NBHW) and then freeze-dried for 72 hours at -30 °C and 0.1 Pa (Lab Kits FD-18-MR), the freeze-dried samples were ground (RETSCH ZM-200) and stored in plastic jars properly labeled.

Physicochemical Analysis

The proximate analysis was performed on the whole plant and its parts. The methods of analysis applied are described in the laboratory manual (ISAIA-INIAP, 2017). They included the following parameters: moisture obtained by evaporation of water by subjecting the sample to heat in a forced air oven (Lab-Line IMPERIAL V) (MO-LSAIA-01.01); protein was analyzed by the kjeldahl method (FOSS Tecator) (MO-LSAIA-01. 04); fiber obtained in the analyzer (LABCONCO) is the residue of an acid and alkaline digestion (MO-LSAIA-01.05); fat content was extracted with hexane in an analyzer (FOSS SoxtecTM 2043) (MO-LSAIA-01.03); ash was obtained by incineration in a muffle (Thermolyne 48000 Furnace) (MO-LSAIA-01.02); total carbohydrates were obtained by difference of 100 % of the fiber, fat, protein and ash content (MO-LSAIA-01.06). Phosphorus was determined by UV/VIS spectrophotometry (Shimadzu Uv-2600) (MO-LSAIA-03.01) and Ca, Mg, Na, K, Cu, Fe, Mn, Zn by atomic absorption spectrophotometry (Shimadzu AA-7000) (MO-LSAIA 03.02).

Analysis of Functional Compounds

This analysis was carried out with lyophilized samples, extraction was performed in a methanol/water/formic acid mixture in an ultrasound bath (Cole Parmer 8892-MHT), centrifuged (Damon/IEC) and once the supernatant was separated, the extraction was repeated five times. The supernatant was analyzed for functional compounds by UV/VIS spectrophotometry (Shimadzu Uv-2600). For total polyphenols, the absorbance of the extracts was measured at 760 nm according to the method of (Cross, Villenueve, & Vicent, 1982), using a gallic acid calibration curve (TraceCERT, Supelco). The total flavonoids were determined by the method (Zhishen, Mengcheng, & Jianming, 1999), absorbance was measured at 490 nm, with a catechin calibration curve (Sigma-Aldrich).

Analysis of Antioxidant Capacity

The antioxidant capacity in the lyophilized samples was determined by two methods: 2,2-azinobis-(3-ethylenebenzothiazoline-6-sulfonic acid radical cation) (ABTS+) suggested by (Re, Proteggente, Pannala, & Rice-Evans, 1998) and the reduction of ferric Fe2+ ions to ferrous Fe3+ ions (FRAP), using the methodology proposed by (Yen & Chen, 1995). For both methods, a trolox calibration curve (Sigma-Aldrich) was used as a standard in a concentration range from 0 to 600 µg/mL. The color change was measured in UV/VIS spectrophotometer (Shimadzu Uv-2600).

For the quantification of functional compounds and antioxidant capacity, the linearity, accuracy and precision of the methods were previously determined, with the parameters of detection limit, recovery percentage and repeatability.

Statistical Analysis

For each experimental response, a statistical analysis of the results obtained for each treatment was performed: Factor A (root, stem and leaves) and Factor B (two locations), with an ANOVA analysis of variance, in the significant treatments a separation of means was performed with Tukey’s test at 95 %.

RESULTS

Proximal Analysis. The results for the whole plant and its parts are presented in Table 1. In the whole plant, the values of all parameters were similar for the two locations, except for protein content, which was higher in Pichincha (13,560.09) compared to oregano from Imbabura (8,250.09).

TABLE 1
PROXIMATE ANALYSIS OF OREGANO ECU-20229 FROM THE LOCALITIES OF PICHINCHA AND IMBABURA, RESULTS ON A DRY BASIS

		Humidity (g 100g-^{1})	Protein (g 100g-^{1})	Fiber (g 100g-^{1})	Ethereal Ext. (g 100g-^{1})	Ash (g 100g-^{1})	Carbohydrates (g 100g-^{1})
Pichincha-Tumbaco	**PE**	77,91±0,02	13,56±0,09	27,88±0,48	1,55±0,02	9,93±0,08	47,09±0,33
	T_1	69,83±0,03	6,38±0,20	42,55±1,13	0,68±0,01	11,02±0,39	39,38±1,21
	T_2	74,10±0,04	7,93±0,22	45,95±1,31	0,73±0,02	10,63±0,47	34,76±0,78
	T_3	69,05±0,02	18,61±0,12	11,31±0,16	2,89±0,05	12,92±0,02	54,28±0,33
Imbabura-Italquí	**PE**	75,89±0,40	8,27±0,09	28,62±0,03	1,18±0,06	10,77±0,08	47,34±0,07
	T_1	68,81±0,33	6,41±0,29	63,41±0,19	0,86±0,06	10,18±0,35	18,53±0,22
	T_2	72,11±0,08	9,03±0,05	51,53±0,20	0,80±0,01	13,32±0,06	24,40±0,08
	T_3	74,10±0,14	19,58±0,36	10,21±0,16	1,93±0,06	12,06±0,17	50,49±0,19
		PE: whole plant**; T1:** root**; T2:** stem**; T3:** leaves **;** n = 3					

The highest protein content was obtained in the leaves with 18.61 % and 19.58 %, followed by the stem with 13.56 % and 8.27 % in oregano from Pichincha and Imbabura, respectively. The highest fiber content with 63.41 % was found in the root of oregano from Imbabura, followed by the stem with 51.53 %, the lowest content was obtained in the leaves for oregano from the two localities. For ashes, the highest content was found in the leaves with 12.92 % of oregano from Pichincha and in the stem 13.32 % of oregano from Imbabura. In total carbohydrates, the highest content was found in the leaves with 54.28 % in Pichincha and 50.49 % in those harvested in Imbabura; on the contrary, in the root oregano from Pichincha prevailed with 39.38 % and it was lower in oregano from Imbabura with 18.52 %. The highest content of ether extract (fat and pigments) was in the leaves with 2.89 % (Pichincha)and 1.93 % (Imbabura).

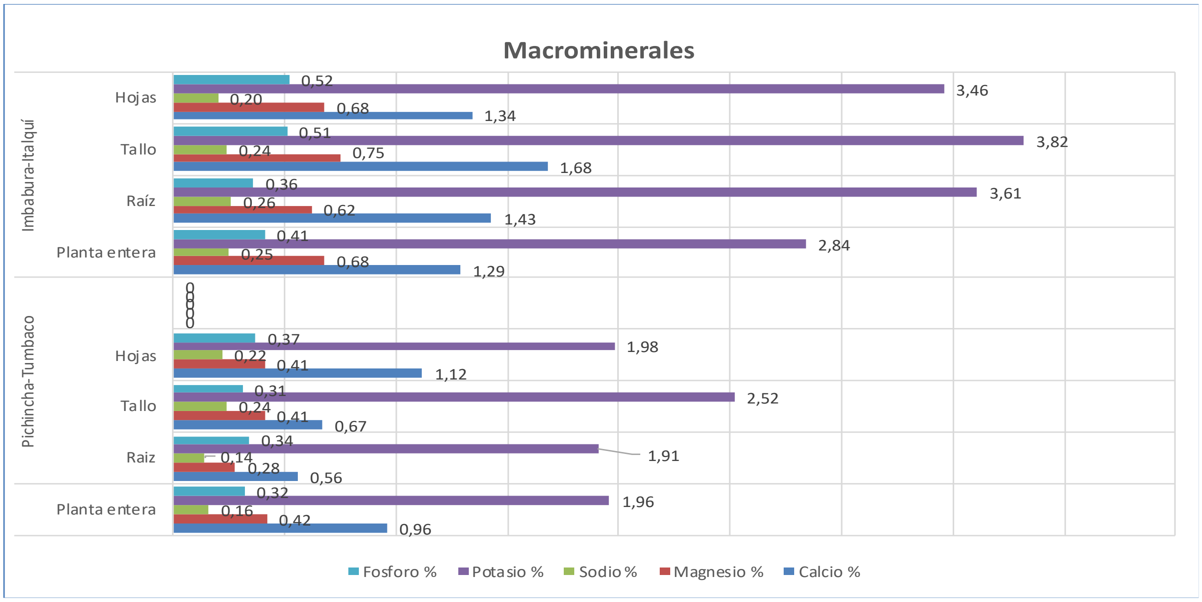
The ANOVA at 95 % confidence, indicated that in all cases, the experimental F value is greater than the tabulated F value, allowing to establish that there is a significant effect of Factor A (plant parts) on the nutritional composition of the samples planted in two different environments corresponding to Factor B.

Mineral Analysis

Macro Minerals

Figure 1 shows the results expressed as the mean and standard deviation (n=3) of the macro elements (calcium, magnesium, sodium, potassium and phosphorus) in the treatments of Factor A (plant parts) in the two locations corresponding to Factor B (plant parts).

FIGURE 1
MACRO MINERAL ANALYSIS OF OREGANO ECU-20229 FROM THE LOCALITIES OF PICHINCHA AND IMBABURA, RESULTS ON A DRY BASIS



According to the results presented in Figure 1, the values in percentage for the macro minerals of the samples were identified. In the whole plant, potassium (2.84%) and calcium (1.29%) are the minerals with the highest presence in Italquí compared to the whole plant from Tumbaco with 1.96%, followed by calcium and 0.96%, respectively. The same pattern is observed for magnesium, sodium and phosphorus, with the highest content in the Italquí oregano.

The part of the plant with the highest content of these nutrients was determined and analyzed separately in each of its organs, allowing us to identify that the element with the highest abundance is potassium and it is abundant in the stem with 2.52% in Tumbaco and 3.82% in Italquí. The nutrient with the lowest presence in the parts of oregano is sodium, with similar amounts in the stem and leaves with 0.24 and 0.22 % respectively in Tumbaco; 0.24 and 0.20 % in Italqui, it enters equally in all parts of the plant. Magnesium and phosphorus have higher values in oregano cultivated in Italqui in the province of Imbabura.

Microminerals

The analysis of microelements (copper, manganese, zinc and iron) was carried out in the whole oregano plant and in each of its parts. Manganese predominated both in the whole plant and in each of its parts, followed by zinc for oregano from Tumbaco, as shown in Figure 2, while for oregano from Italquí, manganese predominated in the whole plant, root and stem, and zinc predominated in the leaves.

FIGURE 2
MICRO MINERAL ANALYSIS OF OREGANO ECU-20229 FROM PICHINCHA AND IMBABURA, RESULTS ON DRY BASIS

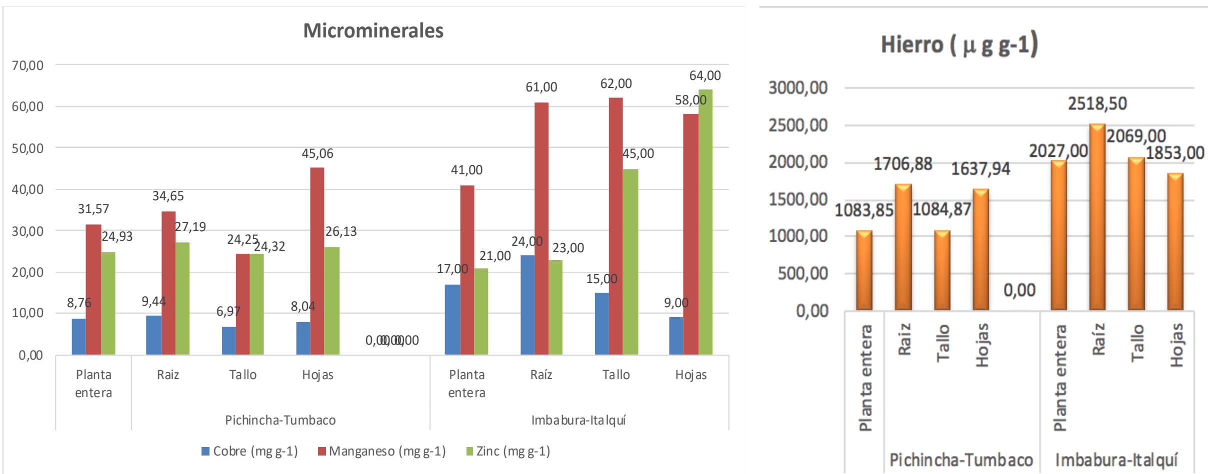


Figure 2 shows that the highest iron content was found in the root of oregano from Italquí with 2518 µg g-g-1 and from Tumbaco with 1706 µg g-g-1. The leaves presented values of 1637 µg g-g-1 in Tumbaco and 1853 µg g-g-1 in Italquí.

Analysis of Functional Compounds and Antioxidant Capacity.

In order to ensure that the results are reproducible and reliable, the method of analysis of total polyphenols, total flavonoids and antioxidant capacity (ABTS.$^+$ and FRAP) was adapted and studied for linearity, precision and accuracy. The calibration curve showed a high correlation coefficient for linearity (R^2>0.995). The accuracy with five extraction cycles showed a recovery of 98.47 % of total polyphenols and 95.54 % of total flavonoids. The precision ensured the repeatability of the methods, with a coefficient of variation of 1.09 % (polyphenols) and 1.33 % (flavonoids). For comparative purposes, the experimental data of flavonoids were transformed from mg catechin-g-^{1} to mg gallic acid-g-^{1} using a ratio of the molecular weights.

Table 2 shows the results of functional compounds and antioxidant capacity. It can be observed that oregano from Italquí has a higher content of polyphenols and flavonoids, both in the whole plant and in its parts, with the highest content in the leaves with 145.24 and 131.71 (mg-g-^{1}), the values obtained in Tumbaco were 93.56 and 83.00 (mg-g-^{1}), respectively. On the other hand, the lowest contents are in the stem and roots. The contribution in the values of the whole plant is due to the high content of the leaves.

TABLE 2
FUNCTIONAL COMPOUNDS AND ANTIOXIDANT CAPACITY OF OREGANO ECU-20229 FROM PICHINCHA AND IMBABURA, RESULTS ON A DRY BASIS

Location	Trat.	Total polyf. (mg gallic acid..g-^{1})	Flavon. Total (mg catechin..g-^{1})	C.A. FRAP (µmoltrolox-g-^{1})	C.A. ABTS (µmoltrolox·g^{-1})
Pichincha Tumbaco	**PE**	80,29 ± 1,20	75,24 ± 2,94	1144,76 ± 32,29	567,00 ± 19,32
	T_1	44,03 ± 0,85	38,37 ± 0,47	949,01 ± 3,17	384,15 ± 3,71
	T_2	37,55 ± 0,65	32,56 ± 1,28	728,14 ± 9,89	344,48 ± 8,73
	T_3	93,56 ± 1,51	86,00 ± 2,59	1327,86 ± 36,62	800,16 ± 39,01

Imbabura Italqui	**PE**	109,28 ± 4,23	95,66 ± 1,86	603,40 ± 2,06	497,32 ± 0,14
	T_1	57,66 ± 1,12	48,88 ± 6,31	357,73 ± 2,50	378,54 ± 0,39
	T_2	61,99 ± 1,69	55,21 ± 0,74	458,74 ± 7,43	381,76 ± 3,20
	T_3	145,24 ± 6,04	131,71 ± 2,03	898,41 ± 2,16	897,14 ± 11,02

PE: whole plant; T1: root; T2: stem; T3: leaves ; n = 6

Based on the results reported in Table 2, the values of the antioxidant capacity expressed in trolox concentration were established, the analysis of the samples was carried out using two quantification methods, the results in the whole plant for oregano from Tumbaco were 1144.76 and 567.00 µmol trolox-g-^{1} by the FRAP and ABTS.$^{+}$ methods, respectively; for oregano from Italquí the values decreased by 603.40 and 800.16 µmol trolox-g-^{1}. Based on these results, it is observed that the oregano plant has a higher antioxidant capacity in the environment of Tumbaco (Pichincha), obtaining the highest content in the leaves with 1327.86 µmol trolox-g-^{1}.

DISCUSSION

In his research (Antal *et al*., 2015) present an analysis of the macro mineral content of oregano plants from three sites in the southern part of Romania, (Zagula *et al*., 2016) analyzed the mineral content of different types of dried herbs. In both cases, the results obtained for oregano were similar to those found in the present study.

In the same study by (Antal et al., 2015) refers to the content of micro minerals in oregano plants; results that present similar values to those obtained in this research for copper, manganese and zinc; similarly the research conducted on medicinal plants and their extracts by (Kostic'et al., 2011) report a similarity in the experimental values that has been obtained in this article.

The research conducted by (Flores-Martínez et al., 2016) determined that the content of total polyphenols in oregano leaves, obtaining a result close to 101.0 mg gallic acid-g-1, which is similar to the values obtained for oregano leaves from Tumbaco, surpassed by its content in the leaves of the Italquí samples. Similarly, in the study (Shan et al., 2005) when evaluating the antioxidant capacity obtained 101.7 mg gallic acid-g-^{1} of total polyphenols for the leaves of O. vulgare L. (Mercado-Mercado et al., 2013) presented the results of phenolic compounds and the antioxidant capacity of some typical spices consumed in Mexico; in O. vulgare L., the results presented for total flavonoids in mg catechin-100g-^{1} exhibit similarity to the results obtained in the present investigation. In the study by (Zavala-Nigoa et al., 2010) of extracts from the leaves of oregano species (Lippia graveolens), they determined the total flavonoid content with 163.75 mg catechin g-^{1}; their results are higher than the values obtained in this research.

(Mercado-Mercado et al., 2013) in his research on the antioxidant capacity of typical Mexican spices using various methods of analysis (FRAP, ABTS-+, ORAC and DPPH) showed results for a sample of oregano, where the antioxidant capacity value obtained by the ABTS-+ method was 1007 mol trolox-g-1, which was higher than that obtained experimentally in this research
(Vallverdú-Queralt *et al*., 2014) carried out a study on the phenolic profile of the herbs and spices used, the antioxidant capacity was determined by the ABTS-$^{+}$ and DPPH methods, for the specific case of the oregano sample, the value of the antioxidant capacity obtained by ABTS-$^{+}$ was 1340 mol torolox-g-^{1}, a value higher than those obtained in the present research work.

CONCLUSION

This study allowed the comparison of an oregano morphotype from two areas of the country. Proximal analysis indicates that the presence of nutrients such as protein, fat and carbohydrates is higher in the leaves. Macro elements are abundant in the stem and leaves. Micro elements are found in greater quantity in the morphotype cultivated in Italquí, as well as the content of total polyphenols and total flavonoids. The antioxidant capacity prevails with higher content in the morphotype cultivated in the environmental conditions of Tumbaco.

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