Academic Journal of Science,
CD-ROM. ISSN: 2165-6282 :: 05(01):301–312 (2016)

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL OF *PIPER AURITUM* KUNTH RELATED TO THE TYPE OF SOIL AT VERACRUZ, MEXICO

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In order to know the variation of the principal components in the essential oil of *P. auritum* Kunth and their antioxidant properties, we collected leaves samples from this plant growing in three different type of soil in Veracruz State, Mexico.

We used five samples from each type of soil analyzed: *andosol*, *phaeozem* and *vertisol*; also geographic and weather variables were considered for each region studied. The essential oil was obtained from the aerial parts of the plant by hydrodistillation in Clevenger distillation apparatus. Yield, refraction index, free radical scavenging activity (DPPH test), total content of polyphenols and chemical composition (GS-MS) were determined for characterization of the oil. Data obtained from the chemical test were analyzed through the one-way ANOVA test for independent groups, and Tukey *post hoc* test was applied when the differences reached $p \leq 0.05$. Statistic analysis has shown that annual average temperature oscillating between 22-24 °C, altitude above the sea level and the phaeozem soil allow to the plant to produce a higher yield of essential oil. Variation in chemical composition of the essential oil was observed in all samples but in the case of vertisol soil, we found the highest content of phenolic compounds. In DPPH test significant free radical scavenger activity was observed (63% to 81%) with higher levels showed by samples from andosol soil. The analysis of the variables presented in this work could serve as a guide for selecting specific locations to cultivate this plant and maximize the production their essential oil.

Keywords: Soil, Essential oil, DPPH, Piper auritum, Vertisol, Andosol, Phaeozem.

Introduction

Piper auritum Kunth is a common plant in several countries in America, it grows from Mexico to Colombia from altitudes between sea level and 2000 meters of altitude. This plant could be cultivated and grow wild too. It is known as acuyo or sacred leaf (Atlas de las Plantas de la Medicina Tradicional Mexicana). Nowadays Piper auritum is used in traditional medicine to treat skin rash, digestive and respiratory diseases (Blanco-Hernández *et al*., 2010). According to some reports antimicrobial, antiparasitic, insecticide, citotoxic and carcinogenic activities have been described for *P. auritum* (Flores *et al*., 2001). The essential oil from *P. auritum* is constituted for a great variety of secondary metabolites, and safrole is the main component; this molecule has a low toxicity and some authors have been investigating for the synthesis of safrole derivatives with commercial value. In 2010, nine compounds were synthesized from this molecule and two of them showed antiproliferative activity in cells of breast cancer (MFC-7) (Espinoza-Catalán *et al*., 2010).

The amount and kind of compounds present in essential oils vary according to multiple factors, a plant with enough resources to grow could produce an oil with different characteristics to a plant growing in an environment with few nutrients; quality in soils can influence considerably the development of plants. Altitude and season of the year also could influence the yield of essential oil; however, there is not enough information about the effect of type of soil on yield, physical and chemical characteristics of essential oil obtained from *Piper auritum.*

In this study, we described the relation between the type of soil, yield, and physical and chemical characteristics from the essential oil of *Piper auritum* that grow in Veracruz, Mexico. At the same time, we have measured antioxidant activity by DPPH method and phenolic compound content in samples of essential oils obtained. Identification of optimal conditions to cultivate this plant could optimize the production of secondary metabolite precursors of molecules with important biological activity.

Methodology

Plant Material

A review of soils in Veracruz was realized in order to select those with the broad distribution across the region and places where *P. auritum* grows wild. Andosol, phaeozem and vertisol soils were chosen and leaves of plants from five places of each soil were collected (Table 1). Plants collected were keep in refrigeration until extraction was carried out.

Table 1. Data from soils of sampling

Type of soil	Place of sampling	Label	Geographic location	Altitude (meter)
Andosol (AN)	Xalapa	XAL	19°32'42.63"N 96°54'26.28"O	1424
	Xico	XIC	19°25´18.01"N 97°00´18.01"O	1305
	Coatepec	COA	19°27´15.60"N 96°58´43.33"O	1246
	Teocelo	TEO	19°22´54.93"N 96°58´49.39"O	1203
	Las Trancas	LTR	19°30´11.10"N 96°51´49.34"O	1218
Phaeozem (PH)	Actopan 1	AC1	19°30´14.49"N 96°37´14.62"O	248
	Actopan 2	AC2	19°30'18.17"N 96°37'16.10"O	254
	El Lencero	LEC	19°29´27.51"N 96°48´53.28"O	1012
	Alto Lucero	ALT	19°37'26.22"N 96°44'05.98"O	1120
	El Castillo	CST	19°32'57.27"N 96°51'37.81"O	1172
Vertisol (VR)	Tierra Blanca 1	TI1	18°26'20.45"N 96°22'00.67"O	68

	Tierra Blanca 2	TI2	18°27'00.16"N 96°21'58.17"O	73
	Palo Gacho	PAG	19°23'30.15"N 96°38'19.09"O	348
	Vega de Alatorre	VEG	19°58'25.73"N 96°36'34.19"O	57
	Naranjos	NAR	21°19'56.74"N 97°40'30.70"O	78

Essential oil extraction

Leaves collected were cut in small pieces and its essential oil was extracted by hydrodistillation with a modified Clevenger apparatus. 200 mL of water for each 50 g of plant material were added to distillation flask and extraction was carried out during 1 hour. The essential oil obtained was dried by anhydrous sodium sulfate and filtered. Yield was calculated using following formula:

$$\text{Yield (\%)} = \frac{W_{ac}\ X\ 100}{W_{mv}}$$

where: W_{ac} = weight of essential oil; W_{mv} = weight of plant material.

Refraction Index

Refraction Index was determined in each essential oil sample using an Atago refractometer (T1 model) at 20ºC.

Chemical composition

Analysis of the chemical composition of essential oil was carried out by Gas Chromatography-Mass Spectrometry Analysis using an Agilent Technologies model 6890N coupled to Mass spectrometer model 5975 inert XL. Column: DB-5 column, 5% phenylmethylpolysiloxane, 60 m x 0.25 mm ID x 0.25 µm. The electron ionization energy was 70 eV. He flow 1.2 mL/min, inject 5 µL (split ratio 1:83) at 250°C injector temperature was employed, ion-source temperature 280°C. The oven temperature was programmed as follows: from 50 °C (2 min hold.) raised at 4 °C/min to 200 °C, 10 °C/min to 300 °C (10 min hold). The identification of compounds was performed by comparing mass spectra with data from NIST05 MS database 2.0 version.

Free radical scavenging activity of essential oil by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method

The DPPH radical scavenging capacity of each extract was determined according to Dominguez-Ortiz and coworkers (2009). DPPH radicals have an absorption maximum at 517 nm, which disappears with reduction by an antioxidant compound. The DPPH radical solution in methanol (9.1 x 10^{-3} mM) was freshly prepared, and 2.95 mL of this solution was mixed with 50 µL of methanolic solutions of essential oil (16,600 ppm), the analyses were carried out by triplicate. The samples were incubated for 30 min at room temperature, and decreasing in absorbance at 517 nm was measured (A_E). A blank sample containing 100 µL of methanol in the DPPH radical solution was prepared daily, and its absorbance was measured (A_B). Absorbance was measured using a spectrophotometer UV-Visible Cary 100 and radical scavenging activity was calculated using the following formula:

$$\%Inhibition = \left[\frac{A_B - A_E}{A_B} \right] x100$$

Determination of total phenolic content

The total phenolic concentration was determined using the Folin-Ciocalteu´s reagent according to the Spanos and Wrosltad, 1990. To 50 μL of each sample (1 mg/mL, three replicates), 2.5 mL 1/10 dilution of Folin-Ciocalteu´s reagent and 2 mL of Na_2CO_3 (7.5 %, w/v) were added and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a UV-Vis spectrophotometer. Results were expressed as gallic acid equivalent (μg EAG/g) by using a standard of gallic acid.

Statistical Analysis

Experimental data obtained from the chemical test were analyzed through the one-way ANOVA test for independent groups, and Tukey *post hoc* test was applied when the differences reached $p \leq 0.05$, using STATISTICA 7.0 version.

Results and Discussion

Physical Analysis

Data from physical analysis and geographic variables of this study are showed in Table 2.

Table 2. Data of physical analysis and geographic variables

Type of soil	Place of sampling	Label	Yield (%).	d[1]	Ref. Ind.[2]	Altittude[3]
Andosol (AN)	Xalapa	XAL	0.6636	0.995	1.5260	1424
	Xico	XIC	0.1161	0.94	1.5245	1305
	Coatepec	COA	0.3107	1.035	1.5249	1246
	Teocelo	TEO	0.1714	1.000	1.5149	1203
	Las Trancas	LTR	0.4039	0.98	1.5260	1218
Phaeozem (PH)	Actopan 1	AC1	0.3961	0.995	1.5160	248
	Actopan 2	AC2	0.5659	0.975	1.5235	254
	El Lencero	LEC	0.1516	0.935	1.5140	1012
	Alto Lucero	ALT	0.3564	0.935	1.5200	1120
	El Castillo	CST	0.6671	1.055	1.5215	1172
Vertisol (VR)	Tierra Blanca 1	TI1	0.3047	N/D[4]	1.5260	68
	Tierra Blanca 2	TI2	0.2916	0.955	1.5080	73
	Palo Gacho	PAG	0.5590	1.015	1.5260	348
	Vega de Alatorre	VEG	0.3572	0.860	1.5110	57
	Naranjos	NAR	0.2064	N/D[4]	1.5235	78

[1]Apparent average density; [2]Refraction index; [3]Meters above sea level; [4]Not determined.

From the obtained results, a big dispersion of data was observed for each type of soil analyzed. For this reason we proceeded to make a more descriptive analysis of each variable.

Essential oil yield

According to results reported by Zulueta-Martínez (1988) higher yield of essential oil is expected in plants that grow in locations at level sea, considering these information plants were organized in two groups: the first group from level sea to 750 meters altitude and second group since 751 to 1500 meters above level sea (Figure 1). Statistical analysis realized established not significant differences between two groups; however, the tendency of higher yield in the first group (0-750 msnm) was observed.

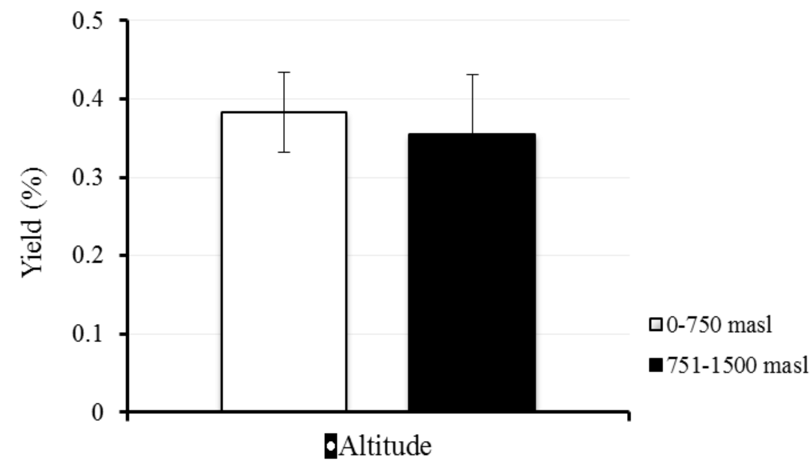

Figure 1. Relation between altitude and essential oil yield [$F_{(1,13)} = 0.085$, $p = 0.774$, NS].

By another side, statistical analysis realized about essential oil yield related to the type of soil, not show significant differences between three groups of soil (Figure 2). However, collected plants in Phaeozem soil provide a higher amount of essential oil than andosol and vertisol whose oil content was similar (0.33 and 0.34% respectively).

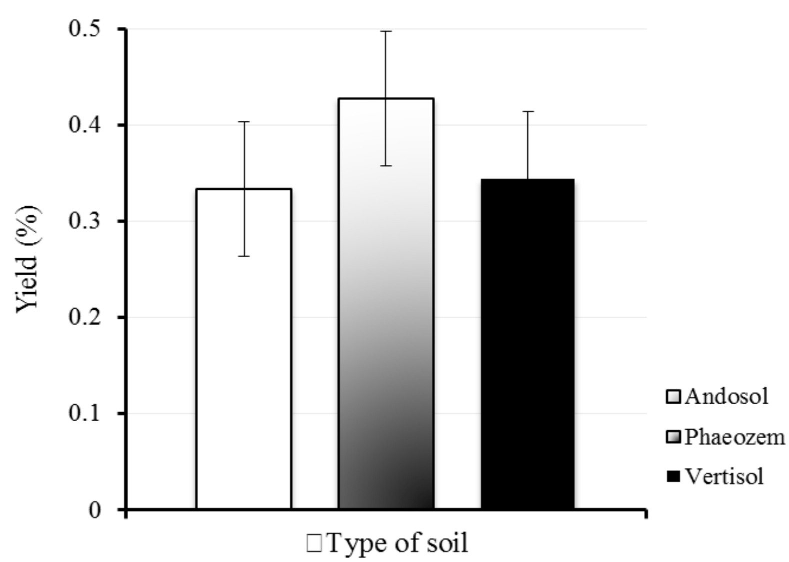

Figure 2. Relation between type of soil and essential oil yield [$F_{(2,13)} = 0.384$, $p = 0.689$, NS].

In this sense, the influence of soil structure on distribution, flow and retention of water, solved substances and gasses are known. The structural characteristics and stability are the main factors that impact root growth because they change the supply of elements needed for plant development (Osuna-Ceja et al., 2006). Some literature reports have described that andosol soil has a great amount of organic material (almost 8.61 %) (USDA, 1999); however, plants collected in andosol showed lower production of essential oil similar to vertisol type. Analysis of plants growing in Phaeozem soil had a better yield of essential oil, it could be explained because this soil has a stable structure, better water retention, aerated soil and 5% of the organic material.

Other analysis consisted in the association of yield with annual average temperature of each sampling place in order to establish differences, but the statistical analysis did not show significant differences between study groups. We could observe that higher yield is obtained in plants from places where the average temperature is 22 to 24 °C (Figure 3).

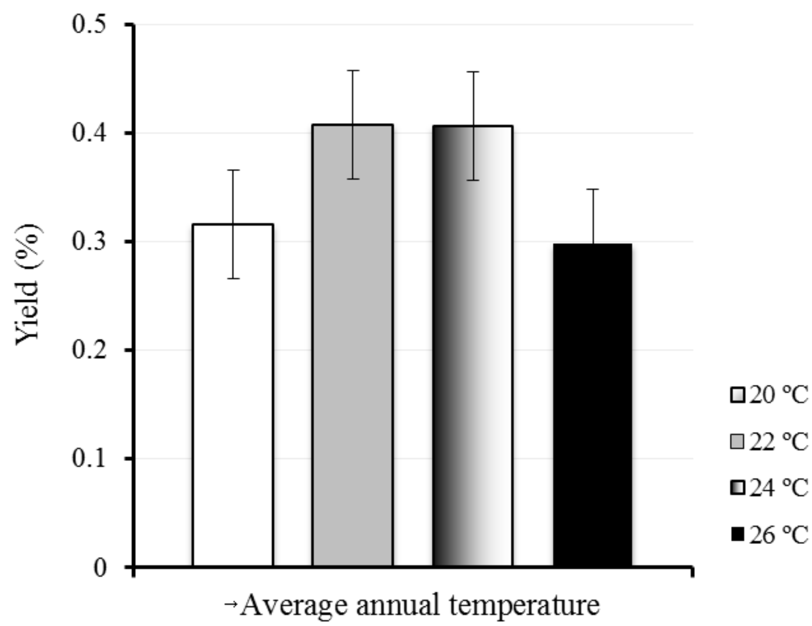

Figure 3. Relation between average annual temperature and essential oil yield [$F_{(3,13)} = 0.310$, $p = 0.817$, NS].

Refraction Index

The essential oil of *P. auritum* is colorless liquid slightly yellow with the characteristic odor (Sánchez *et al*., 2009) and a refraction index from 1.5012 to 1.5343 (Gupta y Arias, 1985; Zulueta-Martínez, 1988).

Refraction index is a physical characteristic related to the chemical composition in essential oil, for this reason in this study we analyzed refraction index variation related to three type of soil (Figure 4). Although significant differences were not observed, there is a slight tendency to a greater refraction index of the essential oil when *P. auritum* plants grow in andosol soil, in comparison with another type of soil.

In general, the physical properties in essential oils analyzed remain similar and there is not direct correlation between measured variables: refraction index and apparent density. There is slight tendency to obtain essential oil with higher density in phaeozem and more refractive in andosol soil.

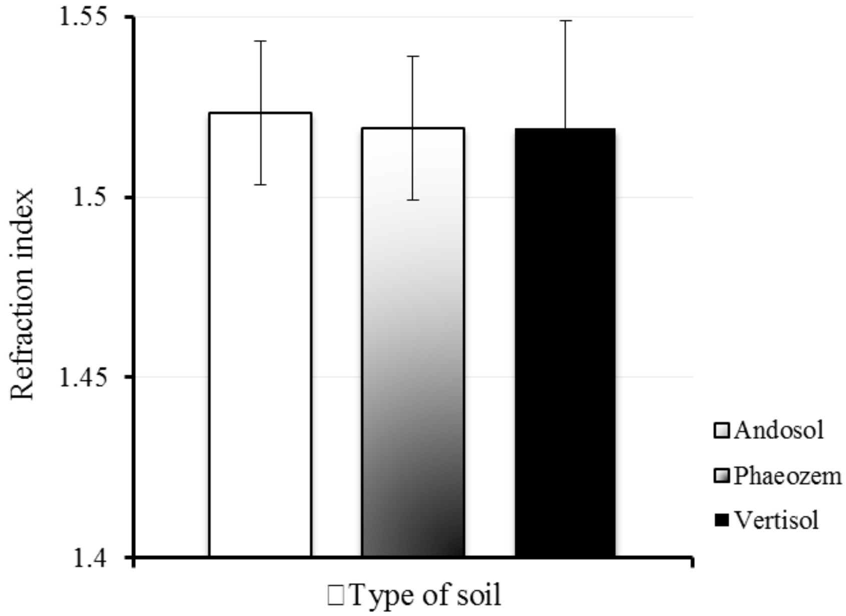

Figure 4. Relation between type of soil and refraction index [$F_{(2,13)} = 0.801$, $p = 0.464$, NS].

Chemical composition of essential oil of P. auritum

Some investigations have determined chemical composition in essential oil from *P.* auritum and the amount of each one. Gupta y Arias found around 40 compounds; most of them belong to terpene compounds and their isomers and safrole as the most abundant molecule (70%) (Gupta and Arias, 1985).

Analysis by gas chromatography coupled mass spectrometry (GC-MS) permitted identification o 23 components in 15 samples of essential oil (Table 3), in all of them safrole was the most abundant compound (73-100%) and it was present in all samples analyzed. The second abundant compound was α-terpinolene (1.5-7.4%) although there was not present in all essential oils. The relative percentage of safrole determined in essential oils is similar to amounts described for this especie (Zulueta-Martínez, 1988) and in three of them (TI2, PAG y VEG) safrole was the only compound identified. From 23 identified compounds 19 molecules are agreed to the previous report for this specie (Gupta y Arias, 1985; García-Ríos et al., 2007; Bueno-Sánchez et al., 2009; Sánchez et al., 2009; Monzote et al., 2010) and other four compounds (2- y 4-carene, estragol and cubebene) have not been described in essential oil of *Piper auritum.*

In the first analysis of chemical constituents identified by GC-MS, the components were grouped according to the family of secondary metabolites which they belong (monoterpenes, sesquiterpenes and phenolic compounds) for the statistical analysis.

In the statistical analysis for monoterpene compounds, andosol and phaeozem have not shown significant differences between both groups; however, plants from vertisol soil has lower levels for this kind of compounds (Figure 5).

The vertisol soil showed higher values of phenolic compounds content and statistical analysis realized in the study groups showed there are not significant differences between andosol and phaeozem (Figure 6).

Table 3. Chemical constituents in samples of *Piper auritum* essential oil and their relative abundance

COMPOUND/PLACE	XAL	XIC	COA	TEO	LTR	AC1	AC2	LEC	ALT	CST	TI1	TI2	PAG	VEG	NAR
p-cymene		0.16		0.38			7.14								
α-pinene	0.59	0.18	0.65	1.74	0.78	0.65		1.41		0.96	0.73				
β-pinene	1.39	0.44	1.56	3.85	2.08	0.79		2.17		2.25	1.21				
α-tuyene						0.4									
Sabinene				0.32		0.51									
β-myrcene						4.65									
2-carene		0.68			0.76										1.24
4-carene	0.73	2.04	1.04												
Limonene				0.39											
α-ocimene		0.3													
trans-β-ocimene			0.4	0.42											1.9
cis-β-ocimene			0.43	0.47											1.87
α-terpinene				2.33				0.41			0.74				
γ-terpinene	1.89	2.3	2.65	6.33	2.11	10.58	6.41	1.8	1.1	3.74	2.13				1.64
α-terpinolene	1.58		2.16	5.29	1.77	7.43	4.83	1.15		2.99	1.64				1.34
Safrole	93.55	92.06	90.4	77.56	92.47	73.49	81.62	93.03	98.9	90.05	92.73	100	100	100	91.96
Estragole			0.23												
Linalool		0.31													
Methyl eugenol		0.27													
β-Cariophyllene	0.23	1.21	0.22								0.42				
Germacrene D				0.41		0.72					0.36				
β-cubebene			0.21	0.45											
Methyl *trans*-9-octadecanoate						0.74									

*Blank spaces denoted a not detected component.

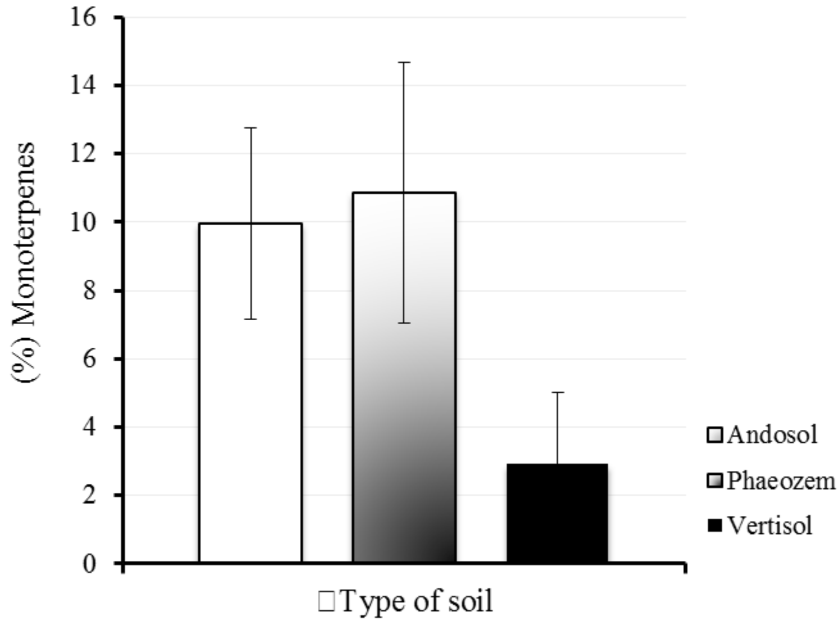

Figure 5. Content of monoterpenes in the essential oil related to the type of soil [$F_{(2,10)} = 2.110$, $p = 0.164$, NS]

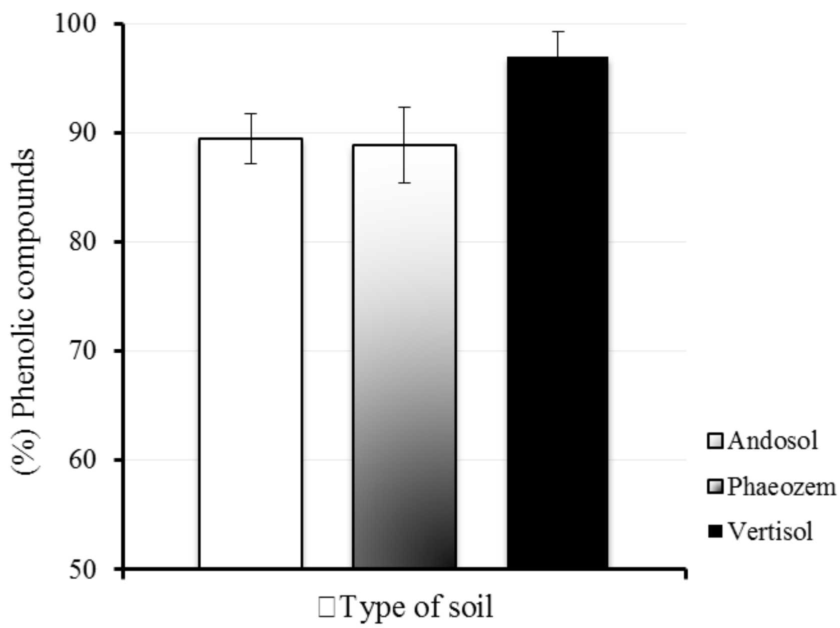

Figure 6. Content of total phenolic compounds in the essential oil related to the type of soil [$F_{(2,10)} = 2.054$, $p = 0.171$, NS]

From the data obtained we established that essential oil from plants growing in vertisol soil has a major amount of phenolic compounds and minor monoterpene compounds; this fact could be related to characteristics of vertisol soil and the adaptability of plants to weather conditions for biosynthesizing this kind of secondary metabolites.

Antioxidant activity in essential oils

In recent years, the study of the essential oils has gained importance specially the determination of their antioxidant compounds because the relation of chronic diseases and oxidative stress characterized for major levels of free radicals and reactive oxygen species; when antioxidants defense systems are not compensating this, damage and cell death could happen (Dorado-Martínez *et al*., 2003; Ramos-Ibarra *et al*., 2006; Pérez Gutiérrez, 2012).

Several studies have been carried out to observe free radical scavenging activity in essential oils (Guala *et al*., 2009). According to a review realized by Edris (2007), the main compounds responsible for antioxidant activity in essential oils are timol and carvacol, two phenolic compounds; however, its activity is due to phenolic compounds but monoterpenes oxygenated, ketone, aldehyde and esters contribute too.

The antioxidant activity in the essential oil of *P. auritum* was evaluated and the statistical analysis have not shown significant differences between collected samples from phaeozem and vertisol soil while plants from andosol are statistically different to other two groups (Figure 7).

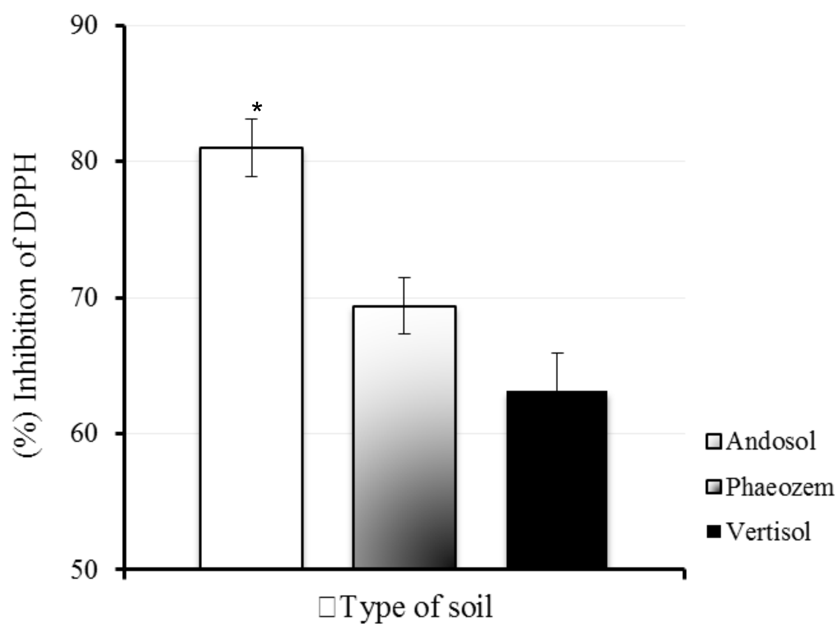

Figure 7. Percentage of inhibition of free radical DPPH with the essential oil related to the type of soil [$F_{(2,40)} = 9.450$, $p \leq 0.0004$]

In 2007 García-Rios and coworkers measured antioxidant activity in essential oil *P. auritum* collected in Colombia using DPPH method, they observed low values (EC_{50}= 348 ppm), less than vitamin E (EC_{50}= 0.27 ppm) at a concentration of 90µM of DPPH. According to this data, the antioxidant effect observed in that essential oil is due to the presence of terpene compounds.

Several reports have been established that phenolic compounds are responsible for antioxidant activity in most of the essential oils, like timol and carvacol; Ruberto y Baratta evaluated around 100 pure compounds present in several essential oils in two models for determination of antioxidant activity (Thiobarbituric acid and linoleic acid peroxidation model) and they determined that most of the chemical compounds analyzed have antioxidant activity. The terpenic compounds have this activity although their action mechanism remains unknown (Carhuapoma et al., 2005; Graßmann, 2005; Jaramillo *et al*., 2010).

From data of this work, the antioxidant activity of *P. auritum* could be related to amount, kind and percentage of each compound identified in essential oils. From obtained data, an abundance of terpenic species in essential oils obtained from plants growing in andosol soil, (See Table 4) is related with its higher capacity to inhibit DPPH radicals; that tendency is supported by low quantity of monoterpenes determined in vertisol samples and its low inhibition of DPPH radical.

Conclusions

According to results obtained in this study we could observe that higher yields of *Piper auritum* essential oil from plants that grow at level sea with an annual average temperature between 22 and 24 °C, and phaeozem soil. This type of soil seems to have ideal characteristics (organic material and porosity) for a better development of the plants and yield of essential oil. Refraction index remains similar in all essential oil obtained.

Variation in chemical composition of Piper auritum essential oil was observed in some places, type and relative percentage of each identified compound by GC-MS. The main component was safrole (73-100%). Four chemical constituents identified (2- y 4-Carene, estragol and cubebene) were not described in the literature for this plant.

The essential oil of *Piper auritum* obtained has a significant percentage of DPPH radical inhibition (63%-81%) at 16 600 ppm.

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