December 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.3 • 161-169

ANTIOXIDANT POTENTIAL OF FIVE ESSENTIAL OILS FROM KUTUKÚ BIOLOGICAL STATION

Noriega Rivera, P. F.*; Larenas Uría, C. F.; Chasipanta Ronquillo, E. C.; Chicaiza Galarza, T. S; Ibarra Martínez, M. V.

Centro de Investigación y Valoración de la Biodiversidad, Universidad Politécnica Salesiana, Avenida 12 de Octubre N 2422y Wilson, Quito-Ecuador

*pnoriega@ups.edu.ec

Abstract

The present investigation evaluated the antioxidant potential of five essential oils from medicinal plants found in the Kutukú Biological Station of the Universidad Politécnica Salesiana, which is located in the western part of the Kutukú mountain range. The essential oils were extracted from the leaves of the following species: *Eugenia stipitata, Ocotea quixos, Psidium guajava, Piper auritum, Piper imperiale*. The results of electron scavenging capacity evaluated by the DPPH and ABTS methods show that the oils with the highest potential are: *P. auritum* IC_{50} DPPH 4.931 ± 0.005 µl / ml, IC_{50} ABTS 2.960 ± 0.000 µl / ml; *O. quixos* IC_{50} DPPH 11.291 ± 0.055 µl / ml, IC_{50} ABTS 7.877 ± 0.020 µl / ml and *E. Stipitata* IC_{50} DPPH 12.866 ± 0.047 µl / ml, IC_{50} ABTS 18.834 ± 0.036 µl / ml, the positive activity reference oil, *T. vulgaris* resulted in IC_{50} DPPH 0.609 ± 0.001 µl / ml, IC_{50} ABTS 0.317 ± 0.006 µl / ml. The antioxidant activity measured by the β-carotene bleaching method test highlights the action of the oils of *P. auritum* IC_{50} 0.032 ± 0.005 µl / ml, P. *guajava* IC_{50} 0.215 ± 0.004 µl / ml and *O. quixos* IC_{50} 0.428 ± 0.012 µl / ml, for *T. vulgaris* we have an IC_{50} of 0.080 ± 0.000 µl / ml. The antioxidant bioautography highlights the activity of the following molecules: safrole in *P. auritum*, cadinene in *E. stipitata* and caryophyllene-E, humulene α, copaene and caryophyllene oxide in *O. quixos*. The results show a promising activity of several essential oils in which *P. auritum* stands out.

Keywords: *Antioxidant bioautography, DPPH, ABTS, beta-carotene bleaching test, P. auritum.*

Introduction

The Kutukú mountain range is a system located between the eastern mountain range of the Andes and the Amazonian plain. The region is considered as an area with high flora and fauna biodiversity [1] and has considerable endemism [2]. Since 1990 the area was declared by the Ministry of the Environment as "protective forest" with an extension of 344,002 Ha, the mountain range rises to 3000 m.a.s.l. [3]. Universidad Politécnica Salesiana has a research station located in the heart of the Kutuku, in which more than one hundred medicinal plants have been identified [4], several of them of aromatic nature.

Among the species that contain essential oils, the following have been identified: *Eugenia stipitata*, known as "arazá", is a very attractive species because it contains pleasantly tasting fruits used for various food preparations [5]. The presence of esters such as ethyl ethanoate and ethyl dodecanoate [6] stand out in the fruits. Another study highlights the presence of germacrene D, α pinene and β pinene [7]. β -caryophillene, caryophillene oxide and β pinene [8] are found in the oil of the leaves. In the fruit, free radical scavenger activity was identified with an IC_{50} (DPPH) of 79 mg / ml [9]. The essential oil of the leaves possesses a significant antibacterial activity [8].

The species *Ocotea quixos*, known as "ishpink, isphingo or American cinnamon", is a highly appreciated plant for its aromatic qualities similar to those of Asian cinnamon [10]. caryophyllene, humulene and various cinnamaldehyde derivatives stand out in the essential oil from its leaves [11-12]. Its antimicrobial and antifungal activity is appreciable [12-13] as is its potential for pharmaceutical and cosmetic use [14]. Medicinally it is used as antiarthritic, in cases of cramping [5] cooling [15] and as a eupeptic and local analgesic [16].

"Guayaba", *Psidium guajava*, is a species whose fruits serve as a food source in the American tropics, its highly aromatic leaves contain essential oil, highlighting the presence of limonene, caryophyllene, pinene, copaene, azulene and eucalyptol [17-19]. The best known traditional medicinal uses are as: antirheumatic, antidiarrheal, and for vaginal washes [5].

Piper auritum, traditionally known as "Sacha anis", is a plant with an aroma similar to anise. In its essential oil, the main component is safrole. Additionally, the presence of terpinolene, α and β pinene, α-terpinene, caryophyllene, and myristicin has been detected; as well as antimicrobial and antioxidant activity [20-21]. Traditionally the species is used for its analgesic, emollient, antirheumatic, diaphoretic, diuretic and stimulant properties [22].

For the essential oil of the species *Piper Imperiale* "ampar", no studies are found in its essential oil, the use of the plant is highlighted for its analgesic and antirheumatic properties [5].

Given that the oxidation mechanisms are harmful to the health and well-being of human beings, investigating natural sources is significant. Finding new compounds that stop or prevent oxidative processes at the cellular level; as well as at an industrial level, to be used in various food, pharmaceutical and cosmetic products, is of utmost importance.

Methods

Plant material and extraction

The plant material was collected at the Kutukú biological station, owned by the Universidad Politécnica Salesiana del Ecuador, which is located in the western foothills of the Kutukú mountain range, next to the Don Bosco church in the province of Morona Santiago, Latitude. : -2.31667, Length: -78.1.

To extract the essential oil, a steam distiller with a capacity of 100 litres belonging to the Life Science laboratory of the Universidad Politécnica Salesiana, was used. For the extraction, fresh leaves of each of the 5 species were used, the extraction time was 3 hours.

GC / MS Analysis.

From each of the oils, 10 µL of essential oil was taken and dissolved in 1 mL in dichloromethane, a volume of 1 µL was injected. The equipment used was composed of a Varian gas chromatograph, model 4000 and a Varian Saturn 2100 mass spectrometer. A VF5 column was used (5% -phenyl-95% dimethylpolysiloxane (5%-phenyl-95%-dimethylpolysiloxane (internal diameter of 30m [Símbolo] 0,25 mm, 0,25 µm film thickness), the carrier gas was helium with a constant flow of 1 mL/min and a split ratio of 1/50. The analysis started at 45 ° C and carried on until it reached 100 ° C at a speed of 1 ° C / min, the temperature is then raised

to 220 ° C at a rate of 5 ° C / min, remaining at that temperature for 15 minutes to allow for analysis time of 90 minutes. The ionization energy of the mass spectrometer was 70 eV and the mass range was 35-400 m / z.

For the identification of the compounds, the mass spectra were compared with the NIST 2001 database. Additionally, the theoretical retention indexes of the Adams 2009 database were compared [23].

Free radical scavenging activity

For the evaluation of the free radical scavenging capacity, the spectrophotometric techniques of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), were used, following the methodology proposed by Noriega 2016 [24].

In this methodology, various concentrations of the essential oil are prepared, which are combined with a DPPH radical solution 1 x 10-4M and a radicalized solution of ABTS 40mM with K2S2O8 until an absorbance of 0.700 ± 0.02 is obtained. Samples with DPPH were shaken for 30 minutes to read their absorbance at 517 nm on a UV / VIS Shimadzu UV 1240 mini spectrophotometer. For samples with the ABTS radical, the absorbance are measured after 1 min at 734 nm using a UV / VIS Shimadzu UV 1240 mini spectrophotometer.

With the obtained values, an essential oil concentration curve is plotted according to the absorbance, afterwards, the oxidation inhibition percentage is calculated and the percentage of 50% inhibition (IC50) is determined as described in several publications [25-26].

Antioxidant Activity Beta-carotene bleaching test

The technique directly measures the antioxidant activity of the molecules that prevent the oxidation of the oleic acid unsaturation when subjected to peroxidation processes. The technique is frequently used in the evaluation of antioxidant capacity in essential oils [27-29]. In this work, the methodology used is proposed by Rossi et al. [30]

HPTLC-DPPH Bioautographic

The technique employs a mobile phase composed of toluene/petroleum ether/ethyl acetate (100/30/10) and Merk F60 silica gel plates. Of each essential oil, 15 µL of a solution of 30 mg of essential oil per millilitre of methanol was seeded. The plate was developed with a 1% DPPH solution. The regions where the developer turns yellow indicate the presence of antioxidant molecules. In a second separation, those regions that had activity were extracted and analyzed in gas chromatography equipment coupled to masses, under the same conditions as oil [26].

Statistical tests

In the spectrophotometric tests of ABTS and DPPH electron scavenger capacity, IC_{50} values were statistically compared, using the statistical method Kruskal-Wallis rank sum test, chi-square = 16.578, df = 5, p-value = 0.005372.

Results

Essential oil extraction.

By means of steam stripping, several quantities of essential oils were obtained in table 1, the yield values are appreciated.

Chemical Composition.

The studies made by GC / MS for each oil reveal its chemical composition in percentages over 90%. The molecules that make up each essential oil, as well as its percentage of abundance, can be seen in table 2.

Free radical scavenging activity

The activity of free radical scavenging is measured in terms of its concentration in µL of oil per mL and its percentage of oxidation inhibition. The reference value of the comparison is its IC_{50} (ability to inhibit 50% of oxidation), which was compared to a natural reference (the essential oil of *Thymus vulgaris*) a synthetic reference (butyl-hydroxy-anisole (BHA)).

The IC50 values can be appreciated in table 3.

The statistical treatment (cladogram) groups the essential oils depending on their activity, bringing together in the same group as the natural reference *T. vulgaris the oil of P. auritum, O. quixos and E stipitata*; in a second group we have *P. guajava* and in a third group *P. imp*eriale oil, which is the oil of less activity, this statistical grouping is observed in figure 1.

Antioxidant Activity Beta-carotene bleaching test

The results taken as values of the 50% antioxidant capacity, known as IC50, can be seen in table 4.

HPTLC-DPPH Bioautographic

The development with the 1% DPPH solution shows antioxidant activity in all those fractions where the violet colouration changes to yellow. The oils that show this change are *P. auritum* at an Rf of 0.96, *E. stipitata* at an Rf of 0.95 and *O. quixos* at an Rf of

0.96; coinciding with those oils that gave the best results in the activity tests, figure 2.

In *P. auritum* the molecule detected as active is safrole, in *E. stipitata* the responsible for the activity is the cadinene molecule, whose activity has already been analyzed [32]. Finally, in *O. quixos,* the activity is due to a mixture of several compounds such as caryophyllene-E, humulene α, copaene and caryophyllene oxide.

Discussion

The essential oil that showed the best results in the various tests of free radical scavenger activity is *P. auritum*, known as "Sacha anis". The bioautography revealed that the molecule responsible was safrole, which is consistent with other previous investigations with oils where this molecule is abundant [25]. *E. stipitata* also shows antioxidant activity, lower than that of *P. auritum*, the studies show that the active molecule is cadinene, which also has preliminary activity tests [31]. In *O. quixos,* whose activity is also significant, the activity is due to the action of 4 molecules, caryophyllene-E, humulene α, copaene and caryophyllene oxide. The essential oils of *P. imperiale* and *P. guajava*, have a very low activity, the bioautographic test for these oils does not show any fraction with activity, as no yellow colouration appears after the development with the DPPH solution.

In the natural pattern of activity, the essential oil of *T. vulgaris* is grouped statistically with *P. auritum*, *O. quixos* and *E. stipitata*, indicating a similar activity. The possibility of using these essential oils as natural antioxidants in food, cosmetics and pharmaceutical products is, therefore, highly likely.

References

1. Zapata-Ríos G, Araguillin E, Jorgenson JP. Caracterización de la comunidad de mamíferos no voladores en las estribaciones orientales de la cordillera del Kutukú, Amazonia ecuatoriana. Mastozoología neotropical 2006; 13(2): 227-238.

2. Brito M, Pozo-Zamora G.. Una nueva especie de rana terrestre del género Pristimantis (Amphibia: Craugastoridae), de la Cordillera de Kutukú, Ecuador. Papéis Avulsos de Zoologia (São Paulo) 2013; 53(24): 315-325.

3. Torres R, Hipólito N. Plan de Ordenamiento Forestal del Bosque Protector Kutukú–Shaimi 2012; (Bachelor's thesis, Escuela Superior Politécnica de Chimborazo).

4. Ballesteros JL, Bracco F, Cerna M, Vita Finzi P, Vidari G. Ethnobotanical Research at the Kutukú Scientific Station, Morona-Santiago, Ecuador. Biomed Res Int 2016.

5. De la Torre L, Navarrete H, Muriel P, Macía MJ, Balslev H. Enciclopedia de las Plantas Útiles del Ecuador. Herbario QCA de la Escuela de Ciencias Biológicas de la Pontificia Universidad Católica del Ecuador & Herbario AAU del Departamento de Ciencias Biológicas de la Universidad de Aarhus. 2008

6. Pino JA, Quijano CE. Volatile compounds of arazá fruit (*Eugenia stipitata* McVaught). Revista CENIC Ciencias Químicas 2007; 38(3).

7. Franco MRB, Shibamoto T. Volatile composition of some Brazilian fruits: umbu-caja (*Spondias citherea*), camu-camu (*Myrciaria dubia*), araça-boi (*Eugenia stipitata*), and cupuaçu (*Theobroma grandiflorum*). J Agr Food Chem 2000; 48(4): 1263-1265.

8. Medeiros JR, Medeiros N, Medeiros H, Davin LB, Lewis NG. Composition of the bioactive essential oils from the leaves of *Eugenia stipitata* McVaugh ssp. sororia from the Azores. J Essent Oil Res 2003; 15(4): 293-295.

9. Reynertson KA, Basile MJ, Kennelly EJ. Antioxidant potential of seven myrtaceous fruits. Ethnobotany Research and Applications 2005; (3): 25-36.

10. Noriega PF. Ishpink, *Ocotea quixos* (Lam.) Kosterm. History, Traditional Uses, Chemical, Pharmacological Properties and the Economic Potential of its Essentials Oils Present within this Amazonian Species, In: Peters M, eds. Essential Oils: Historical Significance, Chemical Composition and Medicinal Uses and Benefits. New York, Nova Science Publishers, 2016: 181-188.

11. Sacchetti G, Guerrini A, Noriega P, Bianchi A, Bruni R. Essential oil of wild *Ocotea quixos* (Lam.) Kosterm.(Lauraceae) leaves from Amazonian Ecuador. Flavour Frag J 2006; 21(4): 674-676.

12. Noriega P, Dacarro C. Aceite foliar de *Ocotea quixos* (Lam.) Kosterm.: actividad antimicrobiana y antifúngica. La granja 2008; 7(1): 3-8.

13. Scalvenzi L, Yaguache-Camacho B, Cabrera-Martínez P, Guerrini A. In vitro antifungal activity of

essential oils of *Ocotea quixos* (Lam.) Kosterm.and Piper aduncum L. Bioagro 2016; 28(1): 39-46.
14. Mosquera TDLÁ, Veloz Vera TM. Eficacia in-vitro de un colutorio elaborado con aceite esencial de la hoja de ishpingo *Ocotea quixos* (Lam.) Kostern. ex OC Schmidt y clavo de olor *Syzygium aromaticum* (L.) Merr. & LM Perry. LA GRANJA Revista de Ciencias de la Vida 2011; 13(1): 31-41.
15. Martínez CEC. Plantas medicinales de los Andes ecuatorianos. Plantas Medicinales de los Andes Ecuatorianos 2006; 285-296.
16. Naranjo P, Kijjoa A, Giesbrecht AM, Gottlieb OR. Ocotea quixos, American cinnamon. J Ethnopharmacol 1981; 4(2): 233-236.
17. Ogunwande IA, Olawore NO, Adeleke KA, Ekundayo O, Koenig WA. Chemical composition of the leaf volatile oil of *Psidium guajava* L. growing in Nigeria. Flavour Frag J 2003; 18(2): 136-138.
18. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 2005; 91(4): 621-632.
19. Li J, Chen F, Luo J. GC-MS analysis of essential oil from the leaves of *Psidium guajava*. Zhong yao cai Zhongyaocai. Journal of Chinese Medicinal Materials 1999; 22(2): 78-80.
20. Sánchez Y, Pino O, Correa TM, Naranjo E, Iglesia A. Estudio químico y microbiológico del aceite esencial de *Piper auritum* Kunth (caisimón de anís). Revista de Protección Vegetal 2009; 24(1): 39-46.
21. García Ríos A, Leyva MA, Martínez JR, Stashenko EE. Determinación de la composición química y actividad antioxidante in vitro del aceite esencial de *Piper auritum* Kunth (Piperaceae) difundida en la costa colombiana. Scientia et technica 2007; 13(33): 439-442.
22. Roig y Mesa JT. Plantas medicinales, aromáticas o venenosas de Cuba. Ciencia y Técnica. 1974
23. Adams RP. Identification of essential oil components by gas chromatography/massspectrometry. Allured publishing corporation. 2007.
24. Noriega PF, Paredes EA, Mosquera TD, Diaz EE, Lueckhoff A, Basantes JE, Trujillo, AL. Chemical Composition antimicrobial and free radical scavenging activity of essential oil from leaves of *Renealmia thyrsoidea* (Ruiz Pav.) Poepp. Endl. J Med Plants Res 2016; 10(33): 553-558.
25. Rivera PN, Mosquera T, Baldisserotto A, Abad J, Aillon C, Cabezas D, Manfredini, S. Chemical Composition and in-vitro biological activities of the essential oil from leaves of *Peperomia inaequalifolia* Ruiz &Pav. Am. J. Essent. Oils Nat. Prod 2015; 2: 29-31.
26. Noriega P, Mosquera T, Abad J, Cabezas D, Piedra S, Coronel I, Manfredini, S. Composición química, actividad antioxidante y antimicrobiana del aceite esencial proveniente de la hojas de *Piper pubinervulum* C. DC Piperaceae. La Granja 2016; 24(2): 111-123.
27. Smeriglio A, Denaro M, Barreca D, Calderaro A, Bisignano C, Ginestra G, Trombetta D. (2017). In Vitro Evaluation of the Antioxidant, Cytoprotective, and Antimicrobial Properties of Essential Oil from *Pistacia vera* L. Variety Bronte Hull. Int J Mol Sci 2017; *18*(6): 1212.
28. Snuossi M, Trabelsi N, Ben-Taleb S, Dehmeni A, Flamini G, De Feo V. Laurus nobilis, Zingiber officinale and Anethum graveolens Essential Oils: Composition, Antioxidant and Antibacterial Activities against Bacteria Isolated from Fish and Shellfish. Molecules 2016; 21(10): 1414.
29. Ebrahimabadi AH, Movahedpour MM, Batooli H, Ebrahimabadi EH, Mazoochi A, Qamsari MM. Volatile compounds analysis and antioxidant, antimicrobial and cytotoxic activities of *Mindium laevigatum*. Iran J Basic Med Sci 2016; 19(12): 1337.
30. Rossi, D, Guerrini A, Maietti S, Bruni R, Paganetto G, Poli, F, Sacchetti G. Chemical fingerprinting and bioactivity of Amazonian Ecuador *Croton lechleri* Müll. Arg.(Euphorbiaceae) stem bark essential oil: a new functional food ingredient?. Food Chem 2011; 126(3): 837-848.
31. Kundu A, Saha S, Walia S, Ahluwalia V, Kaur C. Antioxidant potential of essential oil and cadinene sesquiterpenes of *Eupatorium adenophorum*. Toxicol Environ Chem 2013; 95(1): 127-137.

Table 1. Performance of essential oils in mL / Kg

Plant	Performance in mL/kg
Eugenia stipitata	0.25 ± 0.02
Ocotea quixos	1.00 ± 0.03
Piper auritum	0.46 ± 0.02
Piper imperiale	0.59 ± 0.03
Psidium guajava	0,7 ± 0.02

Table 2. Chemical composition of the five essential oils from the medicinal plants of the Kutukú mountain range.

Compuesto	IK $_A$	*P. guajava*	*P. auritum*	*O. quixos*	*E. stipitata*	*P. imperiale*
thujene α	930	5,82±4,43	1,09±0,10	0,445±0,058		0,71±0,14
pinene α	939	**25,82±3,78**	2,00±0,22	3,437±0,497		
camphene	954			0,404±0,057		0,80±0,15
sabinene	975		1,46±0,08	1,023±0,144		
pinene β	979		0,64±0,18	2,682±0,359		
terpinene α	1017		**12,74±0,85**			
cymene (p)	1024		2,80±0,01	1,463±0,173		
limonene	1029	**28,911±2,18**		1,507±0,176		
cineole 1,8	1031	1,204±0,63		3,241±0,388		
ocimene (z-β)	1037		3,65±0,06			
ocimene (E-β)	1050		3,95±0,02			
terpinene γ	1059		**31,93±2,72**	1,246±0,150		
terpinolene	1088		**25,72±1,53**			
linalol	1096					2,76±0,50
safrol	1287		**25,72±1,54**			
elemene δ	1338				4,68±0,43	
cubebene α	1351				1,93±0,12	0,49±0,04
copaene α	1376	3,557±0,44	3,11±0,02	4,070±0,268	5,20±0,25	1,66±0,13
methyl cinnamate E	1378			**11,992±0,304**		
cubebene β	1388				0,75±0,16	1,68±0,12
elemene β	1390			1,043±0,020	1,46±0,03	0,38±0,03
cyperene	1398					0,39±0,04
caryophyllene Z	1408					**14,58±0,84**
gurjunene α	1409				0,53±0,11	
caryophyllene E	1419	**11,88±1,30**	4,67±0,07	**18,224±0,822**	**12,11±0,19**	
copaene β	1432				2,68±0,12	

elemene γ	1436				0,78±0,05	
guaiene α	1439				1,18±0,06	
aromadendrene	1441				0,53±0,05	
cinnamyl acetate E	1446			**7,001±0,716**		
muurola -3,5-diene<cis>	1450				0,66±0,06	2,14±0,10
humulene α	1454			**16,384±0,767**	2,19±0,04	1,35±0,03
aromadendrene <allo>	1460	0,476±0,04				
caryophyllene <9-epi-E>	1466				0,57±0,06	
muurola-4(14),5-diene <cis>	1466				0,54±0,11	
dauca-5,8-Diene	1472					0,68±0,04
cadina-1(6),4-diene <trans>	1476				0,68±0,04	
muurolene γ	1479	0,314±0,07	4,44±0,23	0,252±0,011	**22,34±1,19**	0,42±0,04
germacrene D	1481				5,04±0,19	
amorpha-4.7 (11)-diene	1481				0,54±0,03	
selinene β	1490	**7,042±0,90**		1,427±0,183		
methyl isoeugenol E <trans>	1492			2,368±0,471		
guaiene cis β	1493				1,88±0,46	
muurola-4 (14),5-diene <trans>	1493					1,51±0,03
valencene	1496					0,28±0,01
selinene α	1498	**7,084±1,19**				
muurolene α	1500	0,18±0,03				
bicyclogermacrene	1500			2,081±0,068		
himachalene β	1500			0,762±0,045		
muurolene α	1500				2,90±0,07	0,45±0,14
patchoulene γ	1502				0,84±0,03	
cadinene γ	1513	0,24±0,08			1,53±0,03	
calamenene <trans>	1522					**6,96±0,11**
cadinene δ	1523	1,63±0,23		2,609±0,189		0,89±0,24
calamenene <cis>	1529	0,99±0,18				
zonarene	1529				0,15±0,00	
bisabolene E-γ	1531			3,102±0,160		
cadina-1,4-diene <trans>	1534					0,29±0,05
cadinene α	1538				0,54±0,01	
calacorene α	1545			0,416±0,012		

selina-3,7 (11)-diene	1546			**9,29±0,13**
maalene	1562		0,21±0,01	
calacorene β	1565			4,23±0,17
isoelimicin	1570			**10,17±0,53**
bendrolasin	1571	0,479±0,042		
spathulenol	1578	0,344±0,082		0,21±0,02
caryophyllene Oxide	1583	3,566±0,413		5,21±0,07
guaiol	1600		0,53±0,12	
humulene epoxide II	1608	2,059±0,093		
dimethoxy-5-vinyl-1,2-benzodioxide(4,6)	1654			**14,71±0,72**
calamenen-10,ol <trans>	1669			0,50±0,07
tetradecanol	1672		0,99±0,53	
asarona	1676			**13,01±0,92**
heptadecane(n)	1700		0,24±0,07	

A: theoretical retention indexes

Table 3. Free radical scavenging activity of essential oils by the spectrophotometric methods of DPPH and ABTS.

Essential oil	DPPH IC_{50} µL/mL	ABTS IC_{50} µL/mL
E. stipitata	12.868	18.834
O. quixos	11.291	7.897
P. guajava	89.788	50.155
P. imperiale	313.724	179.383
P. auritum	4.931	2.961
T. vulgaris	0.608	0.315
BHA	0.006	0.00248

Table 4. Results of antioxidant activity of essential oils by the β-carotene bleaching method.

Essential oil	Antioxidant Activity Beta-carotene bleaching test IC50 µL/mL
E. stipitata	0.638 ± 0.270
O. quixos	0.428 ± 0.012
P. guajava	0.215 ± 0.004
P. imperiale	48.178 ± 6.303
P. auritum	0.032 ± 0.005
T. vulgaris	0.080 ± 0.000
BHA	7.000 $_{E}$-06 ± 4.800 $_{E}$-06

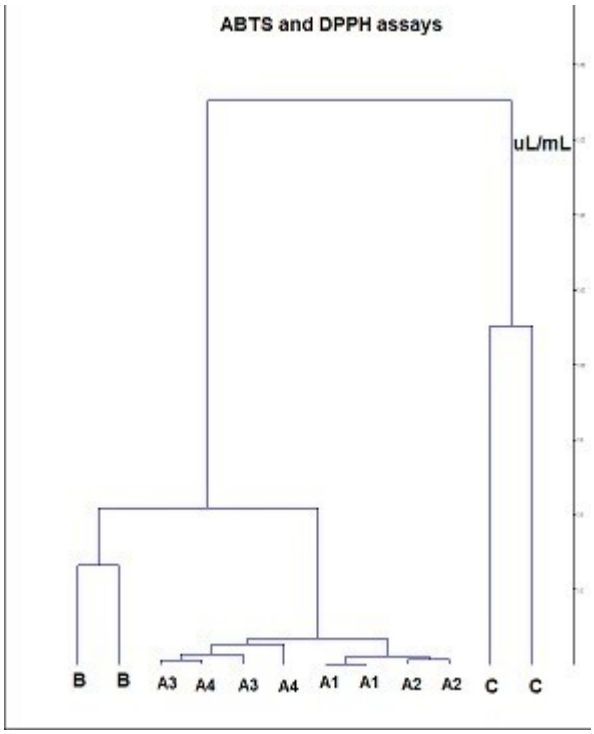

Figure 1. Graph of comparative free radical scavenging activity (cladogram) between essential oils. A1: *T. vulgaris*; A2: *P. auritum*; A3: *O. quixos*; A4: *E. stipitata*; B: *P. guajava* and C: *P. imperiale.*

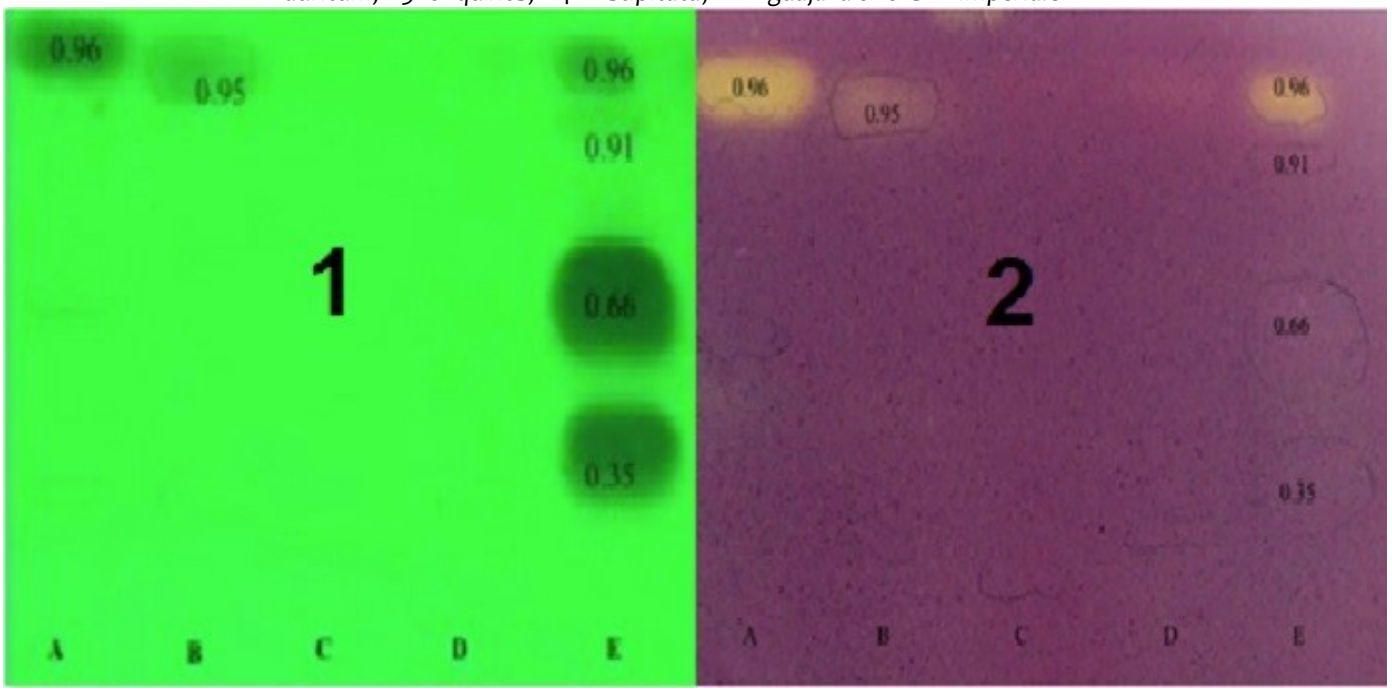

Figure 2. Molecules responsible for the antioxidant activity detected in essential oils by the HPTLC-DPPH Bioautographic method. Plate 1 revealed to 350nm, plate 2 revealed to DPPH 1%. (A)*Eugenia stipitata*; (B) *Piper auritum*; (C) *Piper imperiale*; (D) *Psidium guajava*; (E) *Ocotea quixos*.