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Chemical composition and biological activities of essential oil from *Ocotea quixos* (Lam.) Kosterm. leaves grown wild in Ecuador

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Abstract
The chemical composition of the essential oil from leaves of *Ocotea quixos* (Lam.) Kosterm. grown wild in Ecuador was studied by GC-FID and GC-MS. A total of 112 volatile compounds were identified in the essential oil, of which the most prominent were 1,8-cineol (21.4%) and *p*-cymene (12.6%). Furthermore, we assessed the antioxidant properties by using the DPPH and the ferric reducing antioxidant power assays and found that the essential oil had a moderate antioxidant activity. The essential oil has antimicrobial activity against Gram-positive and Gram- negative bacteria as well as fungi. The minimal inhibitory concentration were *Staphylococcus aureus*(0.5 µL/L), *Bacillus subtilis* (0.05 µL/L), *Escherichia coli* (5 µL/L),*Salmonella Enteritidis* (0.05 µL/L), *Aspergillus niger* (0.5 µL/L) and *Pennicilium citrinum* (0.5 µL/L).

Keywords: *Ocotea quixos*, essential oil, chemical composition, antioxidant activity, antimicrobial activity

1. Introduction
Ocotea quixos (Lam.) Kosterm. (Lauraceae) is amedium-sized tree native to Amazonian Ecuador and neighboring countries [1]. The plant produces biennial big and woody flower calices, locally called Ishpink or Ishpingo, which are traditionally used, eitherfresh or dried, by Amazonian indigenous people as a spice [2]. It has also been valued for its aromatic properties since the time of the Incas [3] and appreciated as an appetizer, eupeptic,antidiarrheal, disinfectant and local anaesthetic [2, 4].
The chemical composition of the essential oil of *Ocotea quixos* from flower calices [4-6] and leaves [7-9] has been reported; however neither of these works considering the antioxidant activity of the essential oilobtained from leaves. The present work is therefore aimed at determination of the chemical composition and biological activities of the essential oil of *Ocotea quixos* (Lam.) Kosterm. from leaves.

2. Materials and Methods
2.1 Materials
Leaves of *O. quixos* were collected by Fundacion Chankuap' (Macas, Ecuador)in January 2016 from wild trees on the outskirts of the Wasak'entsa reserve in eastern Ecuador and positively identified by the National Herbary of Pontificia Universidad Católica del Ecuador.

2.2 Isolation of essential oil
Fresh leaves were steam distilled for 6 h in a pilot-scale distiller. Essential oil yield was 0.3% v/m.

2.3 Gas chromatography for essential oil
Analyses of the essential oil was performed by gas chromatography with a flame ionization detector (GC-FID) on a Konik 4000A (Konik, Barcelona) equipped with a 30 m x 0.25 mm i.d. x 0.25 mm DB-5ms (J & W Scientific, Folsom, CA, USA) column. The analysis parameters were: oven temperature program, 60 ºC (2 min), 60–220 ºC (4 ºC/min) and 220 ºC (5 min); carrier gas Helium flow rate 1 mL/min; injector and detector temperatures 250 ºC, Samples (1 µL) were injected using split ratio 1:50, and previously diluted in *n*-pentane (1:6 v/v). The quantification of compounds was performed using relative percentage abundance and normalization method.

The essential oil was also examined by gas chromatography-mass spectrometry (GC-MS) using a QP-2010 Ultra (Shimadzu, Japan) with the same capillary column, temperature program and Helium carrier gas flow rate as in GC-FID. EIMS, electron energy, 70 eV; ion source and connecting parts temperature, 250 ºC. The acquisition was performed in scanning mode (mass range m/z 35–400 u). Compounds were identified using their retention indices and mass spectra. Linear retention indices, calculated using linear interpolation relative to retention times of C_8–C_{24} of *n*-alkanes, were compared with those standards and data from the literature [10]. Mass spectra were compared with corresponding reference standard data reported in the literature [10] and mass spectra from NIST 05, Wiley 6, NBS 75 k, and in-house Flavorlib libraries. In many cases, the essential oils were subject to co-chromatography with authentic compounds.

2.4Assay of 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The antioxidant activity of the essential oil was measured in terms of hydrogen-donating or radical scavenging ability, using the stable radical DPPH [11]. In the test tubes, 1.5 mL of DPPH (0.075 mg/mL) in ethanol was mixed with 750 µL of five concentrations of the essential oil to evaluate in a range of concentrations between 25-500 µg/mL. A control sample (absolute ethanol) and a reference sample (750 µL absolute ethanol and 1.5 mg/mL of DPPH solution) were also used. The decrease in the absorbance was determined at 515 nm, until the reaction plateau step was reached. Trolox was used as antioxidant standard. Three independent tests were performed for each sample. Then, the IC_{50} values (total antioxidant compound necessary to decrease the initial DPPH radical concentration by 50%) were determined.

2.5Ferric-reducing antioxidant power (FRAP) assay

The FRAPof the essential oil was measured by the method reported earlier [12]. Briefly, acetate buffer (300 mM, pH=3.6), TPTZ (2,4,6-tripyridyl-s-triazine; Sigma) 10 mM in 40 mM HCl and $FeCl_3 \bullet 6H_2O$ (20 mM) were mixed in the ratio of 10:1:1 to obtain the working FRAP reagent. The essential oil (20 µL) was mixed with 900 µL of FRAP reagent. A solution of ascorbic acid was used as standard and Trolox, a stable antioxidant was used as positive control. The mixtures were incubated at room temperature for 4 min and the absorbance was measured at 593 nm. The FRAP was expressed in units of ascorbic acid equivalent.

2.6Minimal Inhibitory Concentration

For determination of minimal inhibitory concentration (MIC) 5; 0,5; 0,05 y 0,005 µL/mL of the essential oil was placed in different test tubes and 1 mLof dimethyl sulfoxide added to each of them. One milliliter of peptone water (Mueller Hinton broth) was added followed by addition of 1 mL of 24h–broth culture of the microorganism. The test tubes were all sealed with sterile corks and subsequently incubated at 32 ºC for bacteria and 25 ºC for fungus during 48 h. After incubation the tubes were observed for clearance or turbidity. The tube with highest degree of clearance was taken as the MIC. Three independent tests were performed for each sample. This procedure was separately carried out for the six test microorganisms: *Bacillus subtilis* ATCC 6633 (G+), *Staphylococcus aureus* ATCC 25923 (G+), *Escherichia coli* ATCC 25922 (G-), *Salmonella Enteritidis* ATCC 13036(G-), *Aspergillus niger* ATCC 16404, *Pennicilium citrinum* ATCC 9849.

3. Results and Discussion

One hundred and twelve compounds were identified in the essential oil from *O. quixos* leaves (Table 1). As can be seen, monoterpene hydrocarbons were the most represented class of volatiles with 41.2%. Among them, *p*-cymene was the most abundant. Oxygenated monoterpenes were found as the second major chemical class (35.9%) with 1,8-cineol being the main component.

These results disagree with those reported in the literature in both quality and quantity. The essential oils previously analyzed from the same region were dominated by β-caryophyllene (15.1%), cinnamyl acetate (11.4%), sabinene (7.6%), geranial (5.6%) and *trans*-cinnamaldehyde (5.1%) [7] or β-caryophyllene (19.0%), humulene (14.3%) and eremophilene(11.4%) [8]. The essential oil from a similar edaphoclimatic Ecuadorian region showed *trans*-cinnamaldehyde (16.6%), (*E*)-methyl isoeugenol (11.9%), β-caryophyllene (10.6%) and α-pinene (9.4%) as major constituents [9]. The presence of some of these compounds was negligible, while others were not detected in the present study.The discrepancy in the compositional pattern of *O. quixos* essential oil can be attributed to several factors, including growth stages, climatic and drying conditions, adaptive metabolism of plant, distillation conditions, and the plant part being analyzed [13].

Antioxidant properties of the essential oil were determined by two methods: radical-scavenging capacity of the oil (or DPPH bleaching) and ferric-reducing antioxidant power (FRAP) assay. Data on the antioxidant activity of the essential oil are shown in Table 2.For the first assay, solutions with essential oil concentrations of 0.5-80mg/mL were prepared to evaluate the DPPH radical-scavenging capacity. The respective scavenging capacities ranged from 8.3±1.6% to 79.9±2.4% with an IC_{50} value of 10.02 ± 1.71mg/mL for the essential oil. On the other hand, in the second test, the FRAP was between 304.6± 24.1to 482.3 ± 23.3µM of ascorbic acid equivalents.According to both methods, the *O. quixos* essential oil had a moderate antioxidant activity compared to Trolox. The major compounds present in the essential oil, 1,8-cineol and *p*-cymene, were previously tested and showed weak antioxidant activity [14-16]. The absence of phenolic compounds and the presence as major compounds of 1,8-cineol and *p*-cymene, previously reported as weak antioxidant [14-16], are responsible for such moderate activity.

O. quixos essential oil was noted to be active against all microbial strains but in different degrees (Table 3). *E. coli* ATCC 25922seems to be the least sensitive to wards the essential oil effect because these strains showed the higher value of MIC. Our findings about antifungal activity of *O. quixos* essential oil are in agreement with previous studies that reported the various degrees of growth inhibition effects of this essential oil with different compositions against *E. coli* (MIC 12.5 µL/mL) and other bacteria [8], and several phytopathogenic fungi [9].

Some of the compounds present in this essential oil have previously been reported to exhibit antioxidant activity. The antimicrobial activity of the 1,8-cineol alone had been demonstrated against numerous bacteria [17, 18], while several authors proven that *p*-cymene is less efficient as an antimicrobial agent than its derivative carvacrol, but *p*-cymene enhances the activity of other antimicrobial agents through synergism, antagonism and additive effects [19].

Table 1: Chemical composition (%) of the essential oil from *Ocotea quixos* leaves

Compound	LRI	%	Identity
(*Z*)-3-hexenol	859	tr	A
hexan-1-ol	871	tr	A
heptan-2-one	892	tr	A
n-nonane	900	tr	A
santolina triene	909	tr	B
tricyclene	927	tr	B
α-thujene	930	2.9	A
α-pinene	939	6.9	A
α-fenchene	951	tr	A
camphene	954	0.2	A
benzaldehyde	958	0.1	A
thuja-2,4(10)-diene	960	tr	B
sabinene	975	5.7	A
β-pinene	979	3.2	A
6-methyl-5-hepten-2-one	986	tr	A
myrcene	991	1.9	A
δ-2-carene	1000	tr	B
α-phellandrene	1005	0.9	A
δ-3-carene	1011	1.7	A
α-terpinene	1017	3.0	A
p-cymene	1025	12.6	A
limonene	1029	9.2	A
1,8-cineol	1032	21.4	A
salicylaldehyde	1045	tr	A
(*E*)-β-ocimene	1050	tr	A
γ-terpinene	1061	5.1	A
acetophenone	1065	tr	A
cis-sabinene hydrate	1070	0.1	B
p-cresol	1077	tr	A
trans-linalool oxide (furanoid)	1085	tr	A
terpinolene	1089	0.5	B
p-cymenene	1093	tr	A
linalool	1097	0.4	A
endo-fenchol	1117	tr	A
cis-*p*-2-menthen-1-ol	1121	0.2	B
α-campholenal	1126	tr	B
terpinen-1-ol	1134	tr	A
trans-pinocarveol	1139	0.2	B
sabina ketone	1159	tr	B
pinocarvone	1163	tr	A
δ-terpineol	1166	0.4	B
borneol	1169	0.2	A
terpinen-4-ol	1177	5.0	A
p-cymen-8-ol	1183	0.1	A
cryptone	1186	tr	B
α-terpineol	1189	6.8	A
methyl chavicol	1196	tr	A
myrtenol	1199	0.1	B
verbenone	1205	tr	A
trans-piperitol	1208	tr	A
linalyl formate	1214	tr	A
trans-carveol	1216	tr	A
nerol	1230	tr	A
(*Z*)-ascaridole	1237	1.0	B
carvone	1243	tr	A
cis-piperitone epoxide	1254	0.1	B
geranial	1265	tr	A
(*E*)-cinnamaldehyde	1270	0.7	A
thymol	1291	0.1	A
carvacrol	1297	0.2	A
iso-ascaridole	1302	tr	B
(*E*)-cinnamyl alcohol	1304	tr	A
α-cubebene	1351	tr	B
eugenol	1359	0.5	A
hydrocinnamyl acetate	1368	tr	A
α-ylangene	1373	tr	A
α-copaene	1377	0.3	A
methyl (*E*)-cinnamate	1379	0.8	A
β-bourbonene	1388	tr	B
β-elemene	1391	tr	B
methyl eugenol	1406	0.1	A
cis-α-bergamotene	1413	tr	B
(*E*)-caryophyllene	1419	1.1	A
coumarin	1434	tr	A
α-guaiene	1440	tr	B
(*E*)-cinnamyl acetate	1446	0.7	A
α-humulene	1455	0.4	A
cis-muurola-4(14),5-diene	1467	tr	B
drima-7,9(11)-diene	1473	0.3	B
ar-curcumene	1480	0.1	A
γ-himachalene	1483	tr	B
β-selinene	1490	0.8	B
(*E*)-methyl isoeugenol	1492	0.1	A
δ-selinene	1493	0.3	B
α-selinene	1498	0.3	B
β-bisabolene	1506	0.1	A
γ-cadinene	1512	0.4	A
β-curcumene	1516	tr	A
7-*epi*-α-selinene	1521	0.1	B
δ-cadinene	1524	0.1	A
(*E*)-*o*-methoxycinnamaldehyde	1527	tr	B
trans-calamenene	1529	0.1	B
(*E*)-γ-bisabolene	1531	0.1	B
α-cadinene	1539	tr	A
α-calacorene	1546	tr	B
elemol	1550	tr	A
elemicin	1557	tr	A
germacrene B	1560	tr	B
(*E*)-nerolidol	1563	tr	A
spathulenol	1578	0.2	A
caryophyllene oxide	1583	0.4	A
guaiol	1601	tr	A
methoxyeugenol	1604	tr	A
humulene epoxide II	1608	0.1	B
1,10-di-*epi*-cubenol	1619	0.2	B
epi-α-cadinol	1640	tr	B
caryophylla-4(14),8(15)-dien-5-.α-ol	1641	0.1	B
β-eudesmol	1652	tr	B
syringaldehyde	1657	tr	A
selin-11-en-4-α-ol	1660	0.1	B
α-bisabolol	1686	tr	A
benzyl benzoate	1760	0.1	A

Identity A: identification based on the linear retention times (LRI) and mass spectra of pure compounds; B: identification based on LRIand mass spectra comparison with databases or literature data. tr: traces(< 0.1%).

Table 2: Antioxidant effectiveness of the essential oil from from *Ocotea quixos* leaves[1]

Sample	DPPH	FRAP	
	IC_{50} (mg/mL)	Concentration (mg/mL)	(µM of ascorbic acid equivalents)
Essential oil	10.0 ± 3.5	2	482.3 ± 23.3
		1	416.8 ± 30.9
		0.5	352.3 ± 29.1
		0.25	304.6 ± 24.1
Trolox	0.012 ± 0.004	0.025	295.7 ± 8.4

[1]Antioxidant effectiveness expressed as IC_{50} and FRAP (in units of ascorbic acid equivalent). Values represent an average of three determinations with standard deviation.

Table 3: Minimal inhibitory concentrations in essential oil from *Ocotea quixos* leaves

Microorganism	MIC (µL/mL)
*Bacillus subtilis*ATCC 6633	0.05
*Staphylococcus aureus*ATCC 25923	0.5
*Escherichia coli*ATCC 25922	5.0
*Salmonella Enteritidis*ATCC 13036	0.05
*Aspergillus niger*ATCC 16404	0.5
*Pennicilium citrinum*ATCC 9849	0.5

4. Conclusions

Essential oil composition of *Ocotea quixos* leaves show the presence of 102 volatile constituents, of which the most prominent were 1,8-cineol (21.4%) and *p*-cymene (12.6%). The essential oil had a moderate antioxidant activity by using the DPPH and the ferric reducing antioxidant power assays, while it oil has antimicrobial activity against Gram-positive and Gram-negative bacteria as well as fungi.

Conflict of interest

The authors declare no conflict of interest.

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