

Chemical Constituents and Antifungal Activity of Leaf Essential Oil from *Oreopanax ecuadorensis* Seem. (Pumamaki), Endemic Plant of Ecuador

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ABSTRACT

Background: *Oreopanax ecuadorensis* Seem. is a plant from Ecuador, that is found in the Andean region of the country. The plant is commonly used in protective rituals, and as an anti-flu, analgesic and disinfectant medicine. **Aim:** The research aims to extract and analyze the chemical composition and evaluation of its antifungal potential of the essential oil extracted from its leaves. **Methods:** steam distillation was used for the extraction of essential oil, the evaluation of its components was performed by GC/MS, and the antifungal evaluation by the disc diffusion method. **Results:** The essential oil was obtained with a yield of 0.05%. 33 compounds were detected of which 30 were identified, corresponding to 99.28%; the most abundant molecules were: thujene α- (36.63%), followed by bicyclogermacrene with (8.76%), pinene β- with (8.32%) and limonene with 5.15%. Three of the four strains evaluated were affected by the oil at concentrations of 1.25%, inhibiting its growth. The strains were: *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporum canis*. **Conclusion:** The essential oil shows good antifungal activity, which could be less than 1.25%. In this way, this medicinal plant is valued by verifying ancestral knowledge in the use of medicinal plants by the Andean people of Ecuador.

Key words: *Oreopanax ecuadorensis*, Puma Maki, Antifungal activity, GC/MS.

INTRODUCTION

"Pumamaky", *Oreopanax ecuadorensis* Seem., is an endemic medicinal species from Ecuador.¹ It is characteristic of the Andean forests, and is generally found between 2500 and 3600 meters above sea level,²⁻⁴ it is common to find it in most of the provinces of the Ecuadorian highlands.¹

O. ecuadorensis belongs to the *Araliaceae* family, it is a tree or shrub that reaches 10 meters high and 50 cm in diameter.⁵ Its leaves have a characteristic shape similar to that of an animal paw, which gives rise to the traditional Quichua name of the species, which in Spanish means puma hand.

Pumamaky is considered by the Quichua people as a sacred plant,⁶ among its most prominent medicinal uses we have: protective and purifying baths,^{6,7} postpartum baths,^{7,8} colds⁷ and headaches.⁹ Traditional uses not described in the scientific literature show its ability to disinfect wounds caused by pathogenic fungi of the skin, for this reason a study is proposed that can verify the antifungal potential against dermatophytes, using the essential oils extracted from its leaves, because several species of the *Araliaceae* family, which contain essential oils, have proven effective as antifungal.¹⁰⁻¹²

Despite being a medicinal plant frequently used by the ancestral peoples of Ecuador, there are no studies on chemistry and biological activity in the species, therefore this research is an important starting point and opens the possibility of

knowing more on the pharmaceutical potential of *O. ecuadorensis*.

MATERIALS AND METHODS

Plant materials

The plant material was collected in the Quero canton, province of Tungurahua, at the following coordinates: Latitude of 13 ° 30'00 "S, longitude of 78 ° 37'00" W and altitude of 2760 m.a.s.l. The botanical identification of the species was in charge of botanist Alvaro Pérez of the herbarium of the Pontificia Universidad Católica de Ecuador. 60 kilograms of *O. ecuadorensis* leaves were collected. Subsequently, the oil was extracted from the fresh leaves in a 64-liter stainless steel distiller installed in the Life Sciences Laboratories of the Universidad Politécnica Salesiana, which operates through the water and water vapor mechanism.¹³ Time of distillation was three hours.

Gas chromatography-mass spectroscopy analysis (GC-MS)

Essential oils were analyzed using GC-MS. The analysis was done with a Varian 3900 gas chromatograph equipped with a Factor Four column VF-5ms 5%-phenyl-95%-dimethylpolysiloxane (internal diameter of 30 m x 0,25 mm, 0,25 µm film thickness) and directly interfaced to a Varian Saturn 2100 mass spectrometer. The carrier gas was helio (1 mL/min) with a split ratio of 1:50. The oven temperature was initially 45 °C and then raised to

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100 °C at a rate of 1 °C/min, then raised to 250 °C at a rate of 5 °C/min, and finally held at that temperature for 15 min. The MS conditions were as follows: ionization voltage, 70 eV; emission current, 10 µAmp; scan rate, 1scan/min; mass range, 35-400 Da; trap temperature, 220 °C; transfer line temperature, 260 °C.

Determination of essential oil composition

The identification of compounds was achieved by comparing the mass spectra against commercial NIST 2001 MS library. The experimental calculation of retention indexes was determined in relation to retention times from a series of n-alkanes (C10-C30); and the theoretical retention indexes was compared to Adams databases for aromatic molecules.¹⁴

Antifungal activity

The technique employed for the determination of antifungal activity was that of disc diffusion, which has been used in a large number of tests with essential oils.¹⁵⁻¹⁷

Due to the fact that the plant is used in dermal pathologies, the potential of the essential oil in strains related to skin diseases was evaluated: *Trichophyton mentagrophytes* ATCC 9533, *Trichophyton rubrum* ATCC 28188, *Microsporum canis* ATCC 36299 and *Candida albicans* ATCC 10231. Various concentrations of the essential oil dissolved in

dimethylsulfoxide (DMSO) were evaluated, ranging from 5 to 1.25%. Clotrimazole was used as a positive control.

RESULTS AND DISCUSSION

Essential oil

The yield of the essential oil was 0.5 mL per kilogram of fresh plant, with a yield of 0.05%. A total of 33 compounds were detected, of which 30 were identified, representing 99.28%. The most abundant molecule was *thujene* <α-> with 36.63%, followed by *bicyclogermacrene* with 8.76%, *pinene* <β-> with 8.32% and *limonene* with 5.15%. Table 1 shows the composition of the essential oil of *O. ecuadorensis*.

We found monoterpene hydrocarbons and sesquiterpene hydrocarbons in greater quantity representing 96.93%, the amount of oxygenated compounds is very low.

Antifungal activity

With the exception of *Candida albicans*, the essential oil was active in the concentration ranges between 5% and 1.25%. Inhibition halos were measured in millimeters, with positive results of activity being those whose halos are greater than 6mm. The results of this test can be seen in full in Table 2.

Table 1: Chemical composition of the essential oil from the leaves of *O. ecuadorensis*.

Compounds	Theoretical AI	Experimental AI	Percent composition	Formula
artemisia triene	923	921	3,14 ± 0,07	C10H16
thujene <α->	924	929	36,63 ± 2,11	C10H16
camphene	946	944	2,16 ± 0,08	C10H16
sabinene	969	968	1,13 ± 0,06	C10H16
pinene <β->	974	973	8,32 ± 0,09	C10H16
myrcene	988	987	1,78 ± 0,10	C10H16
limonene	1024	1025	5,15 ± 0,13	C10H16
perillene	1102	1112	0,67 ± 0,10	C10H14O
elemene <δ->	1335	1333	4,63 ± 0,04	C15H24
copaene <α->	1374	1376	0,97 ± 0,05	C15H24
cubebene <β->	1387	1387	0,22 ± 0,04	C15H24
elemene <β->	1389	1388	0,44 ± 0,05	C15H24
gurjunene <α->	1409	1398	1,42 ± 0,06	C15H24
caryophyllene <(E)->	1417	1411	6,56 ± 0,05	C15H24
copaene <β->	1430	1424	1,31 ± 0,05	C15H24
aromadendrene	1439	1433	1,04 ± 0,05	C15H24
humulene <α->	1452	1451	2,72 ± 0,07	C15H24
farnesene <(E)-β->	1454	1456	1,46 ± 0,10	C15H24
unidentified		1463	0,35 ± 0,10	
cadina-1(6), 4-diene <trans->	1475	1473	0,79 ± 0,13	C15H24
muurolene <γ->	1478	1477	4,56 ± 0,42	C15H24
selinene <δ->	1492	1484	0,18 ± 0,01	C15H24
viridiflorene	1496	1487	0,56 ± 0,09	C15H24
bicyclogermacrene	1500	1491	8,76 ± 0,17	C15H24
muurolene <α->	1500	1495	0,14 ± 0,01	C15H24
bisabolene <(Z)-γ->	1514	1506	0,37 ± 0,13	C15H24
cadinene <δ->	1522	1509	0,30 ± 0,01	C15H24
zonarene	1528	1516	2,19 ± 0,24	C15H24
cadinene ether <cis->	1552	1546	0,22 ± 0,02	C15H24O
nerolidol <(E)->	1561	1564	0,36 ± 0,05	C15H26O
spathulenol	1577	1578	0,57 ± 0,07	C15H24O
unidentified		1638	0,23 ± 0,01	
unidentified		1725	0,14 ± 0,01	
eicosene <1->	1987	1990	0,17 ± 0,02	C20H40
Total				

Monoterpene hydrocarbons	58,31
Oxygenated monoterpenes	0,67
Sesquiterpene hydrocarbons	38,62
Oxygenated Sesquiterpenes	1,15
Others	0,17
Unidentified	0,72

Table 2: Growth of inhibition of various fungi exposed to the essential oil from *O. ecuadorensis*.

Essential oil concentration of <i>O. ecuadorensis</i>	Average halos of inhibition in millimeters			
	<i>C. albicans</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>M. canis</i>
5%	6.0 ± 0.0	13.23 ± 1.93	11.83 ± 1.58	13.77 ± 1.62
2.5%	6.0 ± 0.0	8.40 ± 0.93	10.82 ± 1.57	13.31 ± 1.23
1.25 %	6.0 ± 0.0	6.14 ± 0.09	8.84 ± 1.26	10.97 ± 0.57

The results observed for the strains, *T. rubrum* and *M. canis*, indicate that the activity would continue to occur at concentrations below 1.25%, which leads us to presume values of minimum inhibitory concentration, resulting in this value.

CONCLUSIONS

The most abundant molecule was thujene < α ->, a hydrocarbon monoterpene, followed by bicyclogermacrene, pinene < β -> and limonene. The most abundant compounds are hydrocarbon monoterpenes with formula $C_{10}H_{15}$. Some species rich in thujene < α ->, such as *Eucalyptus alba*, have been shown to have high antifungal activity.¹⁸ Similarly, both pinene < β -> and limonene are molecules whose antifungal potential is proven.¹⁹⁻²⁰

Through this study the antifungal potential of the species is confirmed, therefore the ancestral medicinal use of the species by the inhabitants of the Ecuadorian Andes is valued.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

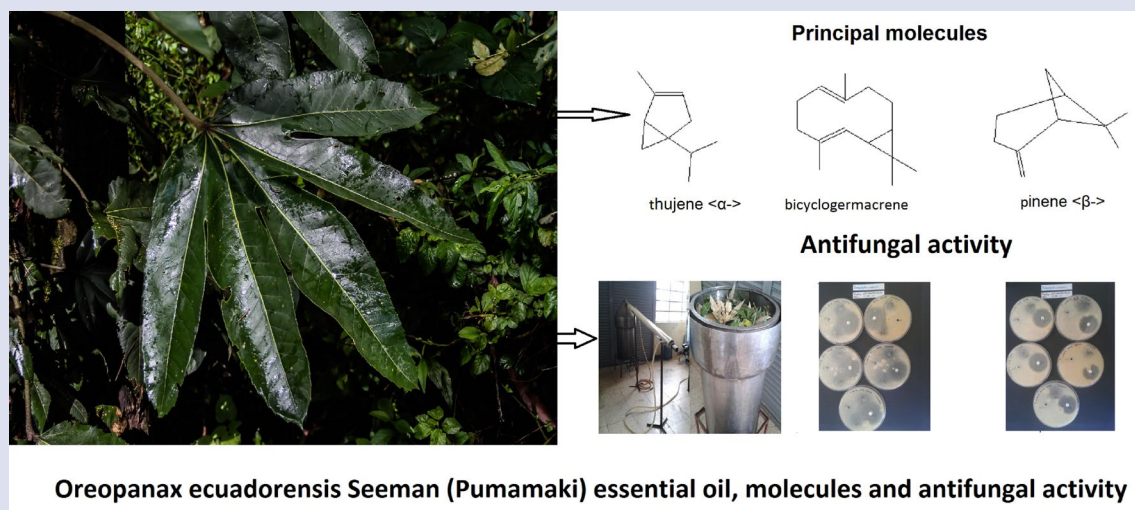
ABBREVIATIONS

GC/MS: Gas chromatography Mass Spectrometry; *O. ecuadorensis*: *Oreopanax ecuadorensis*; *C. albicans*: *Candida albicans*; *T. mentagrophytes*: *Trichophyton mentagrophytes*; *T. rubrum*: *Trichophyton rubrum*; *M. canis*: *Microsporum canis*.

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GRAPHICAL ABSTRACT



SUMMARY

- *O. ecuadorensis* is a sacred medicinal plant of the Andean peoples of Ecuador, used for the treatment of various ailments and diseases, including for the disinfection of wounds.
- The extracted essential oil had a yield of 0.05%. Using the GC/MS technique, 30 compounds in the essential oil were identified, corresponding to 99.28%. The most abundant component was thujene α-> with 36.63%.
- In the range of concentrations between 5 and 1.25%, the oil is active in 3 of the 4 evaluated strains. Activity is observed against: *T. mentagrophytes*, *T. rubrum* and *M. canis*.

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