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Research Article

Essential oil composition of leaves, tertiary, and secondary branches of *Ocotea aciphylla* (Nees & Mart.) Mez (Lauraceae) from Ecuador

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Abstract

Ocotea aciphylla (Nees & Mart.) Mez (Lauraceae) is one of the least studied species of its genus. Essential oil obtained through steam distillation of *O. aciphylla* was examined to establish the chemical profile of samples (n = 9) from leaves, tertiary, and secondary branches. The resulting essential oils were analyzed by GC/FID and GC/MS. The chemical profile of the essential oil revealed decreasing trends in α-thujene (avg. 6.8 to 2.8 %), α-pinene (avg. 21.2 to 7.1 %), β-pinene (avg. 4.2 to 1.6 %), myrcene (avg. 2.5 to 0.5 %), δ-3-carene (avg. 3.3 to 1.2%), o-cymene (avg. 14.9 to 7.4 %), limonene (avg. 6.1 to 2.9%), 1,8-cineole (avg. 5.9 to 1.2%), γ-terpinene (avg 9.3 to 3.9 %), and α-terpineol (avg. 1.8 to 1.0%). In the same plant parts, increasing trends were observed for the compounds terpinen-4-ol (avg. 1.6 to 3.1 %), (E)-cinnamaldehyde (avg. 0.1 to 19 %), α-cubebene (avg. 0.1 to 3.7 %), (E)-methyl cinnamate (avg. not detected to 16.5 %) and δ-cadinene (avg. 0.1 to 1.9 %). This study elucidates compound differences in the essential oil from *O. aciphylla* based on the part of the plant used for the distillation and provides fundamental data for substantiating ethnobotanical applications.

1. Introduction

Ocotea is the most representative genera of the Lauraceae family, with about four hundred species [1]. It is widely distributed in South America and Africa, generally in Amazonian tropical forests and dry forests [2, 3]. Ethnobotanical uses of this genus have been reported to treat diseases such as headaches and urinary tract infections [4]. *Ocotea* species also have been used for antifungal, antibacterial, disinfectant, and anesthetic purposes [5, 6].

One of the least studied species of this genus is *Ocotea aciphylla*. It is native to tropical and subtropical America, including Bolivia, Brazil, Colombia, Ecuador, Peru and Venezuela [7, 8]. *O. aciphylla* is commonly known by different names, including *moena amarilla, alcanfor moena, canela moena, canelon, plata moena, roble Amarillo,* and *tinchi* [9]. This species grows to 35 m in height, has a trunk diameter of 60 to 80 cm, and bark with a similar aroma to cinnamon [9]. A member of the Shuar indigenous group (located in Taisha, Morona Santiago province) explained that he and his ancestors used *O. aciphylla* to make infusions to treat diabetes and in purifying alcoholic beverages [10]. This indigenous group eats the fruit mesocarp, and the flowers are prepared in preserves [11]. *O. aciphylla* has been used by indigenous groups to treat snake bites, as a stomachic, tonic, antirheumatic, and depurative [7,12,13]. The decoction or alcoholic macerate of stem bark has been used against dental caries, abdominal disorders, and bloody diarrhea [14]. An *in vitro* study of the leaves showed that effective acaricidal activity was likely attributed to the presen-

ce of secondary metabolites [15].

Essential oil of many species from the *Ocotea* genera have been studied, containing as prominent compounds trans-cinnamaldehyde, methyl cinnamate [16], α-copaene, δ-cadinene, spathulenol, globulol, β-caryophyllene [17], α-pinene, β-pinene and germacrene D [18]. *O. aciphylla* has been the subject of study in the identification of neolignans and phenylpropanoids from petrol extraction of its trunk powder [19, 20], but to the author's knowledge, the steam-distilled essential oil has not been fully analyzed. Essential oil from different genera and species have shown chemical profiles and yield variations based on the part of the plant distilled [21-23]. This study aims to determine and compare the essential oil yield and essential oil profile of *O. aciphylla* leaves, tertiary, and secondary branches from Ecuador, providing fundamental data for continual substantiation of ethnobotanical applications based on the compounds in the essential oil.

2. Materials and methods

O. aciphylla leaves, secondary, and tertiary branches were collected in January 2022 from wildcrafted populations in Morona Santiago, Ecuador (2°10'42.9"S 77°39'30.3"W). The trees observed were evergreen with a round crown. To obtain the secondary branches, cuts were made at 0.80 m from the apex to the part of the insertion of the axial buds. Once the secondary branches were cut, the tertiary branches were extracted at the apex of the secondary branch with an orientation perpendicular to it. From these tertiary branches, we found the leaves which were separated to be part of the study (Fig. 1). Secondary branches averaged 2.1 cm in diameter and 92 cm long; the tertiary branches averaged 0.4 cm in diameter and 19 cm long; the leaves averaged 4 cm wide and 12 cm long. Representative voucher is held in the herbarium Herbario Politecnica Chimborazo (CHEP) (Ofc.No.002.CHEP.2023).

The leaves, secondary, and tertiary branches of *O. aciphylla* were dried under ambient conditions and shade for 48 hours. Before the distillation process, the leaves were chopped, and the secondary and tertiary branches were crushed with a JF 10D triturator from JF Máquinas Agrícolas-Brazil. Sufficient raw material was obtained to carry out three laboratory-scale steam distillations for each plant part, resulting in a total of

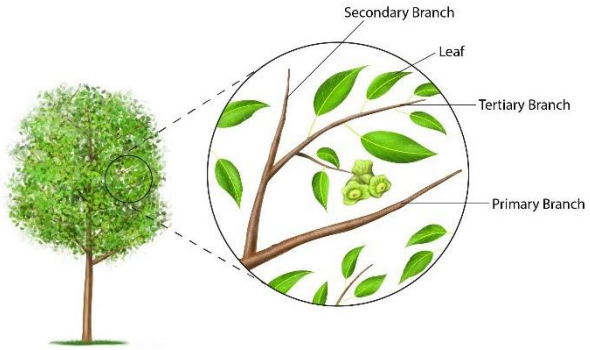

Figure 1. Botanical illustration of *Ocotea aciphylla* tree and plant parts used in the study, namely, leaves, tertiary branches, and secondary branches. Illustrated by Rick Simonson, Science Lab Studios, Inc. (Kearney, NE, USA).

9 essential oil samples (n = 9). All distillations were carried out in a 250 L distillation chamber (Albrigi Luigi S.R.L., Italy). Each plant part was weighed before placing it in the chamber. Distillation was carried out for 4 hours by steam distillation, separating the essential oil by a cooled condenser and Florentine flask. The essential oils were collected, filtered, and stored in sealed amber vials at room temperature (25 °C) until analysis. The essential oil yield for each plant part was calculated as the ratio of the essential oil volume (mL) to the plant material mass (kg) before the distillation process.

The essential oil compounds were analyzed and identified by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 60 m × 0.25 mm, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in ethanol), 150:1 split ratio, initial oven temperature of 40 °C with an initial hold time of 5 min, oven ramp rate of 4.5 °C per minute to 310 °C with a hold time of 5 min, helium carrier gas. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library [24] using Chemstation library search in conjunction with retention indices. Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B and Agilent J&W DB-5, 60 m × 0.25 mm, 0.25 µm film thickness, fused silica capillary column. Operating conditions: 0.1 µL of sample (20% soln. for essential oils in ethanol, 1% for reference compounds

in ethanol, 0.1% soln. for C7–C30 alkanes in hexane), 25:1 split injection, initial oven temperature at 40 °C with an initial hold time of 2 min, oven ramp rate of 3.0 °C per minute to 250 °C with a hold time of 3 min, helium carrier gas. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 1 for all compounds). Compounds were assigned using retention indices coupled with the retention time data of reference compounds (MilliporeSigma, Sigma-Aldrich, St. Louis, MO, USA). For simplicity and consistency, each essential oil sample is referred to by a letter (A-I).

3. Results and discussion

The averaged chemical profile of the essential oil (n = 3 for each part of the plant) of the leaves (samples A-C), tertiary (samples D-F), and secondary (samples G-I) branches of *Ocotea aciphylla* is detailed in Table 1.

Table 1. Chemical profile of *O. aciphylla* essential oil from the leaves, tertiary, and secondary branches of three samples for each part of the plant.

Compound Name	KI	Leaves (A-C)	Tertiary Branches (D-F)	Secondary Branches (G-I)
Styrene	881*	nd	0.2	tr
Tricyclene	921	tr	tr	tr
α-Thujene	924	6.8	5.8	2.8
α-Pinene	932	21.2	17.3	7.1
Camphene	946	0.5	0.5	0.2
Benzaldehyde	952	tr	0.7	0.4
Thuja-2,4(10)-diene	953	0.2	tr	tr
Sabinene	969	5.2	1.3	1.8
β-Pinene	974	4.2	3.3	1.6
Myrcene	988	2.5	1.6	0.5
α-Phellandrene	1002	2.0	2.8	1.0
δ-3-Carene	1008	3.3	2.6	1.2
α-Terpinene	1014	5.2	10.5	4.0
p-Cymene	1020	0.3	0.3	0.1
o-Cymene	1022	14.9	13.4	7.4
Limonene	1024	6.1	5.4	2.9
1,8-Cineole	1026	5.9	3.9	1.2
trans-β-Ocimene	1044	0.1	0.1	tr
γ-Terpinene	1054	9.3	8.8	3.9
cis-Sabinene hydrate	1065	0.1	tr	tr
Terpinolene	1086	0.4	0.6	0.3
Linalool	1095	0.3	0.6	0.4
1,3,8-p-Menthatriene	1108	tr	nd	nd
endo-Fenchol	1114	nd	tr	nd
trans-p-Mentha-2,8-dien-1-ol	1119	tr	nd	nd
trans-Pinocarveol	1135	nd	tr	nd
Camphor	1141	tr	0.1	0.1
3-Phenylpropanal	1156*	nd	0.1	0.2
Benzyl Acetate	1157	nd	nd	nd
Borneol	1165	0.2	0.1	0.1
Terpinen-4-ol	1174	1.6	2.0	3.1
p-Cymen-8-ol	1179	0.1	tr	0.1
α-Terpineol	1186	1.8	1.4	1.0
Methyl chavicol	1195	nd	tr	tr
(*Z*)-Cinnamaldehyde	1217	nd	tr	0.1
o-Anisaldehyde	1239	nd	tr	0.1
(*E*)-Cinnamaldehyde	1267	0.1	5.3	19.0
Thymol	1289	tr	nd	nd
Carvacrol	1298	0.1	0.1	nd
(*E*)-Cinnamyl alcohol	1303	nd	0.1	0.1
Isoascaridole	1306*	1.0	nd	nd
α-Cubebene	1348	0.1	0.4	3.7
Eugenol	1356	0.3	0.1	tr
α-Copaene	1374	0.6	0.4	3.9
(*E*)-Methyl cinnamate	1376	nd	3.2	16.5
β-cubebene	1387	0.1	0.1	0.4
Methyl eugenol	1403	tr	0.1	tr
α-Cedrene	1410	tr	tr	nd
α-Cis-Bergamotene	1411	nd	nd	0.1
β-Maaliene	1411*	0.4	0.3	0.1
(*E*)-Caryophyllene	1417	0.9	0.5	1.5
α-*trans*-Bergamotene	1432	tr	tr	0.7
α-Guaiene	1437	tr	nd	nd
(Z)-β-Farnesene	1440	nd	nd	0.1
(*E*)-Cinnamyl acetate	1443	0.1	2.0	1.8
Epi-β-Santalene	1445	0.1	tr	nd
α-Humulene	1452	0.2	0.1	0.4
Allo-Aromadendrene	1458	nd	nd	tr
cis-Cadina-1(6),4-diene	1461	nd	nd	0.1
γ-Muurolene	1478	nd	nd	0.1
Ar-Curcumene	1479	0.1	nd	nd
Germacrene D	1480	0.1	0.1	0.5
β-Selinene	1489	0.2	0.1	0.2
δ-Selinene	1492	0.2	tr	0.2
trans-Muurola-4(14),5-diene	1493	nd	nd	0.1
α-Selinene	1498	nd	tr	0.3
Bicyclogermacrene	1500	0.2	0.1	nd
α-Muurolene	1500	nd	nd	0.1

Table 1. *(Continued)*

Compound Name	KI	Leaves (A-C)	Tertiary Branches (D-F)	Secondary Branches (G-I)
Unknown	1503*	nd	0.9	1.5
β-Bisaolene	1505	tr	nd	nd
δ-Cadinene	1513	0.1	0.3	1.9
Cubebol	1514	nd	nd	0.1
(*E*)-γ-Bisabolene	1529	0.1	tr	nd
trans-Cadina-1,4-diene	1533	nd	nd	0.1
Elemicin	1555	nd	nd	0.1
Germacrene B	1559	nd	nd	tr
(*E*)-Nerolidol	1561	nd	nd	1.5
Spathulenol	1577	0.1	tr	tr
Caryophyllene oxide	1582	0.2	tr	0.1
Humulene epoxide II	1608	0.1	nd	nd
1,10-di-Epi-Cubenol	1618	nd	nd	0.1
Epi-*α*-Muurolol	1640	nd	nd	0.1
Cubenol	1645	nd	nd	0.1
α-Cadinol	1652	nd	nd	0.2
Benzyl benzoate	1759	nd	tr	tr
8S,14-Cedranediol	1889	tr	tr	nd
Compound Classes				
Monoterpene hydrocarbons		82.3	74.1	34.9
Oxygenated monoterpenes		10.4	8.3	6.0
Sesquiterpene hydrocarbons		3.3	2.4	14.5
Oxygenated sesquiterpenes		0.4	0.0	2.2
Other compounds		1.3	12.5	39.8
Total identified		**97.7**	**97.3**	**97.4**

Note: Each essential oil sample was analyzed in triplicate to ensure repeatability (standard deviation < 1 for all values). Compounds detected in at least one but not all samples are denoted as not detected (nd). Values less than 0.1% are denoted as trace (tr). Unidentified compounds of less than 0.5% are not included. KI is the Kovat's Index previously calculated by Robert Adams using a linear calculation on a DB-5 column [24]. *KI not previously calculated [24] and manual calculation performed using alkane standards. Relative area percentage was determined by GC-FID.

The chemical profile of *O. aciphylla* in Table 1 reveals the highest number of detectable and identifiable compounds in the secondary branches (69 compounds), followed by tertiary branches (61 compounds), and leaves (57 compounds). The essential oil (EO) profile of *O. aciphylla* leaves (A-C) contained primarily *α*-pinene (avg. 21.2 %), o-cymene (avg. 14.9%), γ-terpinene (avg. 9.3%), *α*-thujene (6.8%) and limonene (avg. 6.1%). The presence of isoascaridole is unique to the leaves for this study (avg 1.0%). Major constituents in tertiary branches (D-F) include *α*-pinene (avg. 17.3 %), o-cymene (avg. 13.4%), *α*-terpinene (avg 10.5%), γ-terpinene (avg 8.8%), and limonene (avg. 5.4%). Principal compounds in secondary branches (G-I) include (E)-cinnamaldehyde (avg. 19%), (E)-methyl cinnamate (avg. 16.5%), o-cymene (avg 7.4%), *α*-pinene (avg. 7.1%), and γ-terpinene (avg. 3.9%).

The chemical composition of different plant parts of *O. aciphylla* in this study shows a decreasing percentage area trend, from leaves to tertiary branches to secondary branches of the following compounds: *α*-thujene (avg. 6.8 to 2.8 %), *α*-pinene (avg. 21.2 to 7.1 %), β-pinene (avg. 4.2 to 1.6 %), myrcene (avg. 2.5 to 0.5 %), δ-3-carene (avg. 3.3 to 1.2%), o-cymene (avg. 14.9 to 7.4 %), limonene (avg. 6.1 to 2.9%), 1,8-cineole (avg. 5.9 to 1.2%), γ-terpinene (avg 9.3 to 3.9 %), *α*-terpineol (avg. 1.8 to 1.0%). Compounds with an increasing percentage area trend, from leaves to tertiary branches to secondary branches are terpinen-4-ol (avg. 1.6 to 3.1 %), (E)-cinnamaldehyde (avg. 0.1 to 19 %), *α*-cubebene (avg. 0.1 to 3.7 %), (E)-methyl cinnamate (avg. not detected to 16.5 %) and δ-cadinene (avg. 0.1 to 1.9 %).

Results showed increasing and decreasing trends for particular compounds from different parts of the plant, which was analogous to studies carried out on other species and their parts [21-23]. If compared with other *Ocotea* species, it was found that *Ocotea aciphylla* differs in the major chemical compounds present in its essential oil [16,18], particularly for the presence of *α*-pinene, o-cymene, and γ-terpinene.

In plants, the synthesis and storage of volatile compounds can be unique to specific plant parts. A case in point is the disparity in *α*-pinene levels among various parts of the cinnamon plant (*Cinnamomum zeylanicum* Blum): the leaf contained 0.73%, while the fruit had 2.19%, and the bark and root had even higher amounts at 3.34% and 5.70% respectively [25]. Results from the current study on *O. aciphylla* essential oil showed increasing and decreasing trends for compounds from different parts of the plant, which was analogous to studies carried out on other species [21-23].

To the best of author's knowledge, the complete chemical profile of any *Ocotea aciphylla* part has not

been previously reported. The essential oil of *Ocotea quixos* (Lam.) Kosterm. Leaves from Ecuador, a very close taxonomically related species to *O. aciphylla*, had β-Caryophyllene (15.1%), cinnamyl acetate (11.4%), sabinene (7.6%), 1,8-cineole (5.7%), and geranial (5.6%) as major components [26], which differ from the main compounds in the *O. aciphylla* leaves essential oil where α-pinene, o-cymene, and γ-terpinene are the main compounds. The results revealed the presence of a unique volatile compound, isoascaridole (Fig. 2), which was also found in *Ocotea quixos* leaves [27]. This uncommon volatile compound may be preserved within the *Ocotea* genus, however, additional research is needed.

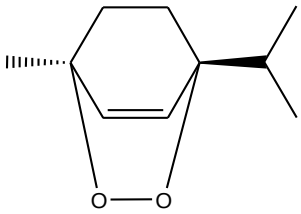

Figure 2. Isoascaridole chemical structure. Obtained from NIST [28]

In the current study, identical drying conditions and distillation method were employed, indicating that abiotic factors are unlikely to account for the differences in essential oil composition among distinct plant parts. Biotic factors encompassing living organisms may impact a plant's metabolite production. Indeed, secondary metabolites found in essential oils serve to protect plants from invaders, facilitate interactions with symbiotic organisms, and allure insects for pollination, among other functions [29]. These factors may constitute a primary influence on the varying chemical compositions of oils derived from different plant parts. Further exploration in this area is needed to identify the fundamental reasons for the observed variations in the essential oil compositions.

The principal chemical constituents of essential oils generally dictate their bioactivities [30]. Recent research has suggested that α-pinene and (E)-cinnamaldehyde have antimicrobial, gastroprotective and antiulcerogenic properties [31-33]. Antirheumatic usage is potentially linked to the prevalence of α-pinene, γ-terpinene, (E)-methyl cinnamate, and (E)-cinnamaldehyde in investigated parts of the plant, as these constituents have demonstrated anti-inflammatory activity [34-38]. (E)-methyl cinnamate and (E)-cinnamaldehyde have exhibited antibacterial properties [39, 40]. The prominent compounds identified in the essential oil of *O. aciphylla* could be related to the plant's ethnobotanical significance among indigenous populations. Further research on the essential oil bioactivities of this species is needed to validate traditional uses of the plant.

Table 2. Yield data, including the mass of plant material distilled (kg), essential oil yield (mL), and calculated yield (mL/kg).

Plant part	Sample	Mass Distilled (kg)	Essential Oil Yield (mL/kg)
Leaves	A	4.0	18.8
	B	3.0	20.0
	C	3.5	20.0
	Avg.	3.5	19.6
	Avg. RSD (*n*=3)		3.5
Tertiary Branches	D	3.0	3.3
	B	3.0	3.3
	C	4.0	4.5
	Avg.	3.3	3.7
	Avg. RSD (*n*=3)		18.7
Secondary Branches	A	7.0	4.3
	B	7.0	4.1
	C	6.8	4.4
	Avg.	6.9	4.3
	Avg. RSD (*n*=3)		3.6

Average calculated yields per distillation range from 3.7–19.6 mL/kg. The relative standard deviation (RSD) is provided for the essential oil yield of each part of the plant.

From the results in Table 2, the highest average essential oil yield was obtained from the leaves (19.6 mL/kg), followed by the secondary branches (4.3 mL/kg) and the tertiary branches (3.7 mL/kg). On average, the essential oil yield from the leaves of *O. aciphylla* was approximately 5.3 times greater than that from tertiary branches, and approximately 4.6 times greater than that from the secondary branches.

4. Conclusions

This study provides, for the first time to the author's best knowledge, the chemical composition of *Ocotea aciphylla* leaves, tertiary and secondary branches of essential oil, demonstrating how the quantity and quality of the oil are distributed within the different plant parts of the species. This information is relevant

for the identification and characterization of different plant species and for the evaluation of their therapeutic or pharmacological potential, as well as for the understanding of the chemical diversity within the genus *Ocotea* and may be used as a guide if a specific chemical profile is desired for industrial production.

Authors' contributions

Conceptualization, A.A. and C.P.; Methodology, A.A. and C.P.; Software, A.A., C.P. and T.M.W.; Validation, A.A.; Formal Analysis (GC/MS, GC/FID), A.A., C.P. and T.M.W.; Investigation, A.A. and C.P.; Resources, C.P., O.P.; Data Curation, A.A. and T.M.W.; Writing – Original Draft, A.A., C.P.; Writing – Review & Editing, A.A., C.P., T.M.W., R.E.C., and O.P.

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Conflicts of interest

The authors declare no conflict of interest. The funding entity had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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