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J P S P JOURNAL OF PLANT SCIENCE AND PHYTOPATHOLOGY
ISSN 2575-0135

Research Article

Chemical composition of olive stems essential oil from Ethiopia

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Abstract

In this article, the chemical compounds, antimicrobial and antioxidant activity of the volatile oil from leaves of *Olea Europaea* L. cultivar from Ethiopia has been studied. The essential oil was provided with a dry distillation apparatus and analyzed by GC-MS/FID. This analysis leads to the detection of 128 compounds representing 89.4% of the total oil. The major constituents were methyl ester hexadecanoic acid (4.10%), 2,4-dimethoxyphenolAa (4.05%), 2-methoxy-phenol (3.25%), 3,5-dimethoxy-4-hydroxytoluene (3.20%), 2-methoxy-5-methyl phenol (3.19%), 1,2,3-trimethoxy-5-methyl benzene (2.93%), 2-methoxy-4-vinyl phenol (2.70%), 2-hydroxy-3-methyl-2-cyclopenten-1-one (2.60%), trans-Isoeugenol (2.45%) and (E) -2,6-dimethoxy-4-(prop-1-en-1-yl) phenol (2.25%). The composition of essential oils was dominated by phenolic compounds.

More Information

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Submitted: May 17, 2022
Approved: June 13, 2022
Published: June 14, 2022

How to cite this article: Asfaw MD. Chemical composition of olive stems essential oil from Ethiopia. J Plant Sci Phytopathol. 2022; 6: 057-061.

DOI: 10.29328/journal.jpsp.1001075

Keywords: Olive; Essential oil; Dry distillation; Chemical composition; *Olea Europaea*

Introduction

Traditional medicine has been practiced in almost every culture, and it has spread worldwide and gained popularity [1]. In Ethiopia, knowledge of traditional medicine has been passed down from generation to generation, and about 80 percent of Ethiopians still rely on traditional medicine, especially for medicinal plants [2,3]. Essential oils are a complex mixture of variables commonly present in low concentrations and are essential components used for their flavor and aroma in the food, pharmaceutical, and perfume industries [4].

Olea Europaea commonly called wild olive is found throughout the Mediterranean, Europe, Africa, Iran, Asia, and Ethiopia and is thought to have a farming history of several 1000 years [5]. It holds historical significance in the religious context and is quoted in Christian and Hebrew Bibles and the Koran [5,6]. The olive tree is rarely eaten as a natural fruit because of its bitter taste but is used as oil or table olive and its wild and cultivated forms are considered an important subject of plant research [5]. *O. Europaea* has been shown in traditional medicine. It has been known to lower blood sugar, cholesterol, and uric acid. It is also used to treat diabetes, high blood pressure, inflammation, diarrhea, respiratory and urinary tract infections, gastrointestinal diseases, asthma, hemorrhoids, rheumatism, laxative, mouthwash, and vasodilator. Many phenolic compounds, especially secoiridoids and iridoids [7] and their pharmacological functions have been a major focus of scientists for the past decade [8,9]. However, the essential oil of *Olea Europaea* grown in Ethiopia has never been investigated before. Therefore, the purpose of this study was to determine the chemical composition of the essential oils *Olea Europaea* growing in Ethiopia through GC / MS analysis and to make comparisons with the literature.

Experimental

Description of the study area: Woreilu is one of the 24 administrative districts in the South Wollo Zone of Amhara Region, Ethiopia. It is located at 36° 26' 0" – 39° 43' 0" E longitude and 10° 34' 0" – 10° 60' 0 " N latitude and 492km far from Addis Ababa, Ethiopia, 571 km from Bahir Dar, the capital city of Amhara Region, as well as 91km from Dessie, West of Zonal town. As of the 2007 Ethiopia census, Woreilu town had a population of 14,817 and a 71013-hectare total area. According to the Agricultural and Rural Development office of the Woreda, agro-ecologically, the woreda is classified as "*Dega*" which accounts for 82% while the remaining 18% is "*Woina Dega*". Of the total number of 23 kebeles administrations, 20 are rural. In the Woreda, most Kebeles produce crops in the "Meher" season, six kebeles in both seasons, and only one kebele in the "Belg" seasons. The agro-climatic conditions of the Woreda ranged from moderate to high, with an average altitude of 2730m above sea level. Annual rainfall ranges from 766.2 to 1250 mm. which is usually inadequate (short in duration), poorly distributed, and highly variable in inter and intra seasons.

Plant material

The dried olive stems were randomly collected from the local market of Woreillu town, South Wollo district, Ethiopia, in May 2018. The authenticity of the plant material was done in the Department of Biology and Biodiversity Management, Wollo University. The extraction of the Essential oil was employed by a traditional method (dry distillation) which is not previously been published Figure 1.

Isolation and characterization

The dried stems of *Olea Europaera* were cut (cut) into small pieces (≈20 cm long), weighed, and washed under tap water to remove any foreign material and dried on laboratory benches in a well-ventilated room before EO. About 2.0 kg of small pieces were loaded into a clay pot, after which, the pot was turned into a cooking pot (cooking pot) and well mixed (tightly sealed) with mud so that it would not emit any steam from it (outside). Finally, the packed jar was buried in a hole 50 to 50 inches [50 × 50 cm] in diameter and set fire to it. EO collection started after a temperature of about 30 minutes and lasted for 1 hour until the clay pot became red hot. The hot pot was cooled for 10 minutes as it was in the oven and the flexible EO collected due to evaporation of the stew pot was separated from the charcoal by burning and stored in 250 ml solid glass containers. Finally, EO was refrigerated until it was needed for chemical analysis and bioassays testing.

Stem EO analysis of *Olea ertupaea* was performed on a Shimadzu GC-2010 gas chromatograph with flame ionization detector (FID), inserted 25 m x 0.25 mm x 0.25 µm CBP5 capillary column, and using helium as a carrier gas. the oven temperature was set from 60 ºC (after 10 minutes) to 230 ºC at 3 ºC / min and the final temperature was 10 min. GC/MS analysis of stem EO of *Olea ertupaea* was performed with Agilent 5975N gas chromatograph-mass spectrometer with 30 m x 0.25 mm x 0.25 µm film thickness capillary column of HP5MS, using helium as a carrier gas. The oven temperature system was similar to that used in gas chromatography (GC) analysis.

The chemical properties of Essential Oils have been identified by comparing their MS with the reference spectra at the National Institute of Standards and Technology (NIST) mass spectrometry data center and by comparing their storage indicators with Kovats' indications in the literature. Quantitative data were obtained electronically at a percentage of the area and peaks combined without the use of a corrective factor [10].

Figure 1: Dry pieces of Olea europaea stem (the author).

Results and discussion

It was noted that the local oil yield of *Olea europaea* was 315.5 mL obtained from 6kg of plant material in three abortion groups yielding a yield of 5.19 ± 0.05 (% v/w) (Table 1). The standard deviation of the three-group batch yield (% v / w) was found to be 0.05 equivalents to 0.96% of the standard deviation relative (% RSD). % Of RSD was used as an indicator of the accuracy of the dry distillation immersion process. The RSD% of this study, less than 2%, indicated that the dry distillation procedure was more accurate with less damage [11]. In addition, the distillation method used in this study yielded better results compared to other abortion techniques in classifying higher molecular terpenes such as diterpenes and triterpenes contradicting Birhanu's [12] study, which argued that diterpenes and higher terpenes cannot be detected by steam distillation method as these molecules are very heavy to allow evaporation, so they are rarely found in dissolved essential oils.

One hundred and twenty-eight compounds comprise 89.4% of the essential oils identified by GC and GC/MS. Its main compounds were methyl ester hexadecanoic acid, 2,4-dimethoxyphenol, 2-methoxy-phenol, 3,5-dimethoxy-4-hydroxytoluene,2-methoxy-5-methylphenol,1,2,3-trimethoxy -5-methyl benzene, 2-methoxy-4-vinyl phenol, 2-hydroxy-3-methyl-2-cyclopenten-1-one, trans-Isoeugenol and (E)-2, 6-dimethoxy-4- (prop-1- en- 1-yl) phenol, respectively (Figure 2, Table 2). To the best of my knowledge, this is the

Table 1: Percentage yield (% v/w) of *Olea europaea* EO.

Batch No.	Weight of plant material (kg)	Volume of Essential oil (mL)	Percentage yield (v/w)
1	2.0	105	5.25
2	2.0	103	5.15
3	2.0	103.5	5.18
Mean ± SD = 5.19 ± 0.05			

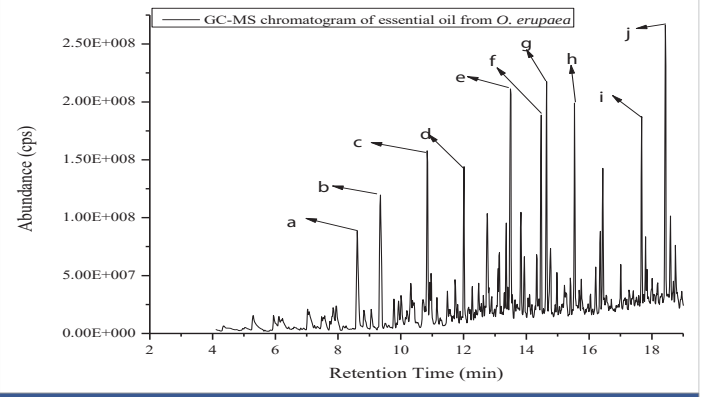

Figure 2: The representative ion chromatogram of the major compositions of the stem essential oil of *Olea europaea*.

Table 2: The ten major compounds of the EO of the stem of *Olea europaea*.

No	Name of compounds	Chemical formula	Retention time	Peaks	Area (%)	LRI*	Class
1	2-hydroxy-3-methyl-2-cyclopen-1-one	$C_6H_8O_2$	8.62	a	2.60	8.6178	Ketone
2	2-methoxy phenol	$C_7H_8O_2$	9.35	b	3.25	9.3519	Phenol
3	2-methoxy-5-methylphenol	$C_8H_{10}O_2$	10.85	c	3.19	10.851	Phenol
4	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	12.76	d	2.70	12.763	Phenol
5	2,4-dimethoxyphenol	$C_8H_{10}O_3$	13.50	e	4.05	13.503	Phenol
6	trans-isoeugenol	$C_{10}H_{12}O_2$	14.48	f	2.45	14.477	Phenol
7	3,5-dimethoxy-4-hydroxytolune	$C_9H_{12}O_3$	14.65	g	3.20	9.3519	Phenol
8	1,2,3trimethoxy-5-methyl benzene	$C_{10}H_{14}O_3$	15.54	h	2.93	15.545	Benzene
9	(e)-2,6-dimethoxy-4-(prop-1-en-1-yl) phenol	$C_{11}H_{14}O_3$	17.02	i	2.25	17.681	Phenol
10	Methyl ester hexadecanoic acid	$C_{17}H_{24}O_2$	18.45	j	4.10	18.44	Fatty acid

*LRI: Linear Retention Indices.

Table 3: Chemical components of the stem oil of *Olea europaea*.

PK	Name of compounds	LRI	Area (%)
1	ethanedioic acid, bis(1-methyl propyl) ester	4.3603	0.3862
2	Silver butanoate	4.9696	0.0485
3	3-Piperidinol, 1,4-dimethyl-, trans-	5.0419	0.1902
4	Pyrazole, 1,4-dimethyl-	5.2979	0.7987
5	2-Furanmethanol	5.9539	0.4980
6	1,6:2,3-Dianhydro-4-O-acetyl-.beta.-d-mannopyranose	6.1198	0.3169
7	2,4-Pentanedione, 3-methyl-	6.234	0.6179
8	D-Limonene	6.6053	0.2173
9	1,3-Cyclopentanedione	7.0359	0.3313
10	[1,3,4]thiadiazol, 2-amino-5-(2-piperidin-1-ylethyl)-	7.0809	0.6856
11	2,5-Hexanedione	7.2579	0.0920
12	2-Furancarboxaldehyde, 5-methyl-	7.4851	0.2218
13	Piperidine-4-carbonitrile	7.5745	0.5887
14	2-Cyclopenten-1-one, 3-methyl-	7.7484	0.1425
15	tetrahydro[2,2']bifuranyl-5-one	7.8526	0.6810
16	2(5H)-Furanone	7.9479	0.7259
17	2(5H)-Furanone, 5-methyl-	8.169	0.1052
18	2H-Pyran, 3,4-dihydro-2-methoxy-	8.2595	0.1147
19	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	8.6178	2.5996
20	2-Furanone, 2,5-dihydro-3,5-dimethyl	8.8347	0.5642
21	Phenol	9.0642	0.4353
22	Phenol, 2-methoxy-	9.3519	3.2458
23	Methyl ethyl cyclopentene	9.5105	0.1684
24	Cyclohexane, (1-methylethylidene)-	9.6202	0.0984
25	Phenol, 2-methyl-	9.7876	0.4697
26	Cyclohexene, 1-methyl-4-(1-methylethyl)-, (R)-	9.873	0.0718
27	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	9.9341	0.4840
28	Maltol	10.016	0.7629
29	Naphthalene	10.205	0.5577
30	Phenol, 3-methyl-	10.326	0.9758
31	Phenol, 2-methoxy-3-methyl-	10.381	0.3767
32	Oxirane, 3-hydroxypropyl-	10.424	0.6297
33	Glycoluril	10.715	0.7149
34	2-Methoxy-5-methylphenol	10.851	3.1932
35	2H-Azepin-2-one, hexahydro-1-methyl-	10.931	0.5768
36	Phenol, 2,4-dimethyl-	10.972	0.8009
37	3,4-Dimethoxytoluene	11.157	0.4976
38	Phenol, 2,4,6-trimethyl-	11.262	0.1204
39	ethanone, 1-cyclohexyl-	11.298	0.0839
40	Phenol, 2-ethyl-	11.491	0.5437
41	Phenol, 4-ethyl-	11.55	0.4447
42	Benzene, 1-(2-butenyl)-2,3-dimethyl-	11.664	0.1810
43	4-Hydroxy-2,4,5-trimethyl-2,5-cyclohexadien-1-one	11.741	0.7174
44	2(3H)-Furanone, 5-acetyldihydro-	11.816	0.2873
45	Phenol, 2,4-dimethyl-	11.896	0.1728
46	2-Pyridinealdoxime	12.016	2.2458
47	2,4,6-Cycloheptatrien-1-one, 2-amino-	12.163	0.5262
48	Acetic acid,1-methyl-3-(1,3,3-trimethyl-bicyclo[4.1.0] hept-2-yl)-	12.282	0.5426
49	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-	12.353	0.1334
50	4-Hydroxy-3-methyl benzoic acid, methyl ester	12.402	0.2437
51	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	12.488	0.7493
52	Cyclopentane, 2-methyl-1-methylene-3-(1-methylethenyl)-	12.57	0.2661
53	2,4-Dimethylanisole	12.638	0.3567
54	2-Methoxy-4-vinylphenol	12.763	2.6978
55	Pentadecane	12.9	0.5852
56	4-ethylbenzoic acid, 2-(1-adamantyl)ethyl ester	12.994	0.2205
57	ethyl Vanillin	13.142	1.7299
58	Naphthalene, 2,6-dimethyl-	13.204	0.2171
59	Spirohexane-5-carboxylic acid, 1,1,2,2-tetramethyl-, methyl ester	13.25	0.1113
60	5-Hydroxymethylfurfural	13.304	0.4006
61	Catechol	13.361	1.2459
62	Naphthalene, 2,6-dimethyl-	13.419	0.3924
63	2,4-Dimethoxyphenol	13.503	4.0507
64	Benzene, 1,2,3-trimethoxy-5-methyl-	13.562	0.2827
65	ethanone, 1-(2,5-dimethoxyphenyl)-	13.642	0.3037
66	Aromandendrene	13.714	0.3760
67	Naphthalene, 1,2,3,4-tetrahydro-2,2,5,7-tetramethyl-	13.761	0.1581
68	1,2-Benzenediol, 4-methyl-	13.831	1.6478
69	Phenol, 3,4-dimethoxy-	13.941	0.9395
70	2(3H)-Furanone, 3-acetyldihydro-3-methyl-	14.062	0.3197
71	1,4-Benzenediol, 2,5-dimethyl-	14.109	0.3584
72	1,7-Octadien-3-one, 2-methyl-6-methylene-	14.181	0.2387
73	1,2-Benzenediol, 3-methyl-	14.339	1.1046
74	Citral	14.405	0.3183
75	trans-Isoeugenol	14.477	2.4466
76	Methyleugenol	14.553	0.1495
77	3,5-Dimethoxy-4-hydroxytoluene	14.649	3.2041
78	Benzaldehyde, 3-hydroxy-4-methoxy-	14.774	1.4052
79	m-ethylaminophenol	14.927	0.1873
80	ethanone, 1-(2,3,4-trihydroxyphenyl)-	14.98	0.5293
81	Benzene, 1-methyl-4-(methylsulfonyl)-	15.065	0.1777
82	1,3-Benzenediol, 4,5-dimethyl-	15.223	0.7862
83	Naphthalene, 1,4,6-trimethyl-	15.27	0.5307
84	3-Acetyl-2,5-dimethyl furan	15.414	0.4634
85	Benzene, 1,2,3-trimethoxy-5-methyl-	15.545	2.9307
86	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	15.693	0.4183
87	ethanone, 1-[4-(methylthio)phenyl]-	15.758	0.6582
88	5-Sec-butylpyrogallol	15.975	0.3256
89	Benzeneethanol, 4-hydroxy-	16.049	0.2884
90	Cyclohexanone, 2,5-dimethyl-2-(1-methylethenyl)-	16.119	0.0865
91	3-tert-Butyl-4-hydroxyanisole	16.217	0.6369

92	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-	16.36	1.0852
93	5,7-Dimethyl-1,3-diazaadamantan-6-one Hydrazone	16.443	1.9776
94	1,4-Benzenediol, 2,3,5-trimethyl-	16.556	0.5361
95	1,6-Dimethyl-4-ethylnaphthalene (Norcadalene)	16.668	0.1184
96	N',N'''-Bis(6-nitro-4H-pyran-2-ylmethylene)-2,5-pyridinedicarbohydrazide	16.716	0.2386
97	Dithiocarbonic acid,O-ethyl ester, methylene-S(IV)-trifluoromethyl est	16.813	0.0660
98	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	16.872	0.1100
99	(e)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	17.017	0.9346
100	tyrosol, acetate	17.151	0.4435
101	1-Acenaphthylenol, 1,2-dihydro-1-methyl-	17.204	0.2000
102	1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,4,7-tetramethyl-,[1aR	17.286	0.4625
103	1,3-Oxathiolane, 2-(4-chlorophenyl)-2-methyl-	17.413	0.5472
104	5-Methyl-5,8-dihydro-1,4-naphthoquinone	17.518	0.4139
105	Ketone, methyl 2-methyl-1-cyclohexen-1-yl, semicarbazone	17.592	0.2294
106	(e)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	17.681	2.2473
107	Benzenepropanol, 4-hydroxy-3-methoxy-	17.745	0.2394
108	1,5,9-Undecatriene, 2,6,10-trimethyl-, (Z)-	17.808	0.8683
109	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	17.851	0.5235
110	beta.-D-Mannofuranoside, farnesyl-	17.895	0.2757
111	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	18.019	0.6421
112	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-,	18.076	0.3685
113	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-,	18.188	0.6376
114	tricyclo[4.3.0.0(7,9)]non-3-ene,2,2,5,5,8,8-hexamethyl-,	18.324	0.5180
115	Hexadecanoic acid, methyl ester	18.44	4.1051
116	5,6-Azulenedimethanol,1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-	18.52	0.3135
117	Naphthalene, 2,3-dimethoxy-	18.598	1.3904
118	Methyl 4-hydroxy-3,5-dimethoxybenzoate	18.693	0.4963
119	Benzaldehyde, 3,4,5-trimethoxy-	18.761	1.1728
120	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	18.96	0.4079
121	Hexadecanenitrile	19.082	0.5492
122	4-Hydroxy-2-methoxycinnamaldehyde	19.144	0.1379
123	Benzenepropanoic acid, 2,5-dimethoxy-	19.215	1.1188
124	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-,	19.3	0.5383
125	Oxirane,2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-henei	19.395	0.3530
126	7H-Furo[3,2-g][1]benzopyran-7-one, 4-hydroxy-	19.492	0.1530
127	.beta.-Humulene	19.543	0.3816
128	3-Amino-7-methyl-1,2,4-benzotriazine 1,4-dioxide	19.597	0.0695
	total		89.395

first report on the production of essential oils from Ethiopian *Olea europaea*. Phenolic compounds (35.49%), non-terpenes (29.23%), terpenes (20.90%), and other compounds (6.37%) dominated fat formation.

The essential oil of *Olea europaea* contains compounds of interesting biological properties. Some authors stated that phenolic compounds and their analogs have strong antibacterial, antifungal, antiviral, anti-mutagenic, anti-inflammatory, and antioxidant activities [13,14]. This could well explain the importance of the Olea europaea in the traditional Ethiopian pharmacopeia.

This investigation is different from those found in some oils from Algeria (from leaves) [15] (Palmetic acid, Z-nerolidol, Octacosane), Tunisia (from fruits and stem) [16], (3-ethyl pyridine, (E)-2-decanal, 2-ethylbenzaldehyde, and Nonanal, (E, E) -2,4-decenal, Benzyl alcohol respectively) and South Africa (from leaves) (Iweriebor, et al. 2012) (Nonanal, Phytol, 2-isopropyl-5-methyl-9methylenebicyclo[4.4.0]dec-1-ene. this variation in compositions and yield of the EO could be due to factors such as plant age, plant part, development stage, growing place, harvesting period, method of extraction, and principally by chemo-type since they influence the plant biosynthetic pathways and consequently the relative proportion of the main characteristic compounds [17] Table 3.

Conclusion

The major components of the essential oil of the examined *Olea europaea* dry stems are methyl ester hexadecanoic acid, 2,4-dimethoxyphenol, 2-methoxy-phenol, 3,5-dimethoxy-4-hydroxytoluene,2-methoxy-5-methylphenol,1,2,3-trimethoxy -5-methyl benzene, 2-methoxy-4-vinyl phenol. The essential oil of *Olea europaea* dry stems is a potential source of natural antioxidants and antibacterial compounds which are used for the treatment of various diseases caused by free radicals and microbes.

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