



Analysis of the Chemical Composition of Essential Oil of Stem of *Olea europaea* sub spp. *Africana* (Mill) Grown in Ethiopia

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Abstract

The essential oil in dry stems of *Olea europaea*, a local aromatic plant, obtained by dry distillation were identified by a pipeline of gas chromatography (GC) techniques coupled with mass spectrometry (MS), flame ionization detector (FID). Compounds with a good match and high probability values were detected using this technique. One hundred twenty eight compounds comprising 89% of the oils were identified. The major components for the dry distillation 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-methylphenol (3.19%), 1,2,3-trimethoxy-5-methyl benzene (2.93%), 2-methoxy-4-vinylphenol (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 the essential oil was dominated by phenolic compounds.

Keywords: *Olea europaea*; Essential oil; Dry distillation; Chemical composition; GC/MS

1. Introduction

Traditional medicine has been practiced virtually in all cultures, and it has expanded globally and gained popularity (Galen, 2014). In Ethiopia, the knowledge of traditional medicine has been transferred from one generation to another, and about 80% of the Ethiopian population is still dependent on traditional medicine, especially on medicinal plants (Giday *et al.*, 2007; Kassaye *et al.*, 2006). Essential oils are complex mixtures of

volatile substances generally present at low concentrations, and they are important components used for their flavor and fragrances in food, pharmaceutical and perfumery industries (Maffei *et al.*, 2011).

Olea europaea commonly known as wild olive is found throughout the Mediterranean, Europe, Africa, Iran, Asia and Ethiopia and thought to have a cultivation history of several 1000 years (Masoko and Makgapeetja, 2015). It holds

historic importance in the context of religion, and it is quoted in the Christian and Hebrew Bibles and the Koran (Masoko and Makgapeetja, 2015; Muthee *et al.*, 2011). The olive shrub is rarely consumed as a natural fruit due to its bitter taste but used as oil or table olive and its wild as well as cultivated forms are considered as a significant botanical research subject (Masoko and Makgapeetja, 2015). The therapeutic utilities of *O. europaea* have been indicated in traditional medicine. It has been known to reduce blood sugar, cholesterol, and uric acid. It has also been used to treat diabetes, hypertension, inflammation, diarrhea, respiratory and urinary tract infections, stomach and intestinal diseases, asthma, hemorrhoids, rheumatism, laxative, mouth cleanser, and as a vasodilator. Many phenolic compounds, especially secoiridoids and iridoids (Berdini *et al.*, 2007), and their pharmacological activities have been the focus of attraction for scientists in the last decade (Ryan *et al.*, 1988, Ghisalberti *et al.*, 1998). However, the essential oil of *Olea europaea* grown in Ethiopia has not been investigated before. Therefore, the aim of this study is to determine the chemical composition of the essential oil of *Olea europaea* growing in Ethiopia by GC/MS analyses, and to make the comparison with the literature.

2. Experimental

2.1 Description of the study area

Woreilu is one of the 24 administrative districts in 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, 571km from Bahir Dar, capital city of Amhara Region, as well as 91km from Dessie, West of Zonal town. As of 2007 Ethiopia census, Woreilu town had a Population of 14,817 and 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 82% while the remaining 18% is “*Woina Dega*”. From the total number of 23 kebeles administrations 20 are rural. In the Woreda, most Kebeles produce crops in “*Meher*”

season, six kebeles in both seasons and only one kebele in “*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 1250mm. which is usually inadequate (short in duration), poorly distributed and highly variable in inter and intra seasons.

2.2 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. Dry pieces of *Olea europaea* stem (the author)

2.3 Isolation and characterization

The dry stems of *Olea europaea* were trimmed (cut) into small pieces (20cm long), weighed and washed under tap water to remove any foreign materials and dried on laboratory benches in a well-ventilated room before extraction of the EO. About 2.0 kg of the small pieces were loaded into the clay jar, then after, the jar was inverted on a stewpot (cooking pot) and hooked it up well (tightly secured) with mud in order not to release

any vapor out of it (outside). Finally, the packed jar was buried in a pit that has 50 cm by 50 cm size and lighted fire above it. The collection of the EO started after a heating time of about 30 min and continued for 1 hr until the clay jar becomes red hot. The hot jar was cooled for 10 min as it was in the pit and the volatile EO collected because of evaporation in the stew pot was isolated from dust charcoal by decantation and stored into 250 mL air tight glass vials. Lastly, the EO was put in a deep freeze until required for chemical analysis and bioassays.

The analysis of the stem EO of *Olea ertupaea* were performed on a Shimadzu GC-2010 gas chromatograph with flame ionization detector (FID), fitted with a 25 m x 0.25 mm x 0.25 µm CBP5 capillary column, using helium as the carrier gas. the oven temperature was programmed from 60 °C (after 10 min) to 230 °C at 3° C/min and the end temperature was held for 10min. the GC/MS analysis of the stem EO of *Olea ertupaea* were carried out on an Agilent5975N gas chromatograph-mass spectrometer with a 30 m x 0.25 mm x 0.25 µm film thickness HP5MS capillary column, using helium as a carrier gas. the oven temperature program was the same used in the gas chromatography (GC) analysis.

The chemical constituents of the Essential oils were recognized by comparing their MS with the reference spectra in the mass spectrometry data

center of the National Institute of Standards and technology (NIST) and by comparing their retention indices and Kovats indices in the literature. The quantitative data were obtained electronically from area percentages and integrated peaks without the use of correction factor (Lang and Buchbauer, 2011).

3. Results and Discussion

It was observed thatthe yield of from the local oil of *Olea europaea* was 315.5mL obtained from 6kg of plant materials from three distillation batches giving a yield of 5.19 ± 0.05 (% v/w) (table 1). The standard deviation of the three-extraction batches yield (% v/w) was found to be 0.05 which is equivalent to 0.96% of relative standard deviation (% RSD). The % of RSD was used as an indicator of the precision of the dry distillation process. The % of RSD for this study, which is less than 2%, showed the dry distillation process was more precise with minimal wastages (Caburian and Osi, 2006). Moreover, the method of distillation used in this study gave good results in comparable to the other distillation techniques in isolating higher molecular terpenes such as diterpenes and triterpenes which contradicts the study of Birhanu (2012), who argued thatditerpenes and higher terpenes cannot be detected by stem distillation method as these molecules are too heavy to allow for evaporation, so they are rarely found in distilled Essential oils.

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

One hundred twenty eight compounds comprising 89.4%ofthe essential oil were identified by GC and GC/MS. Its main compounds were as 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-vinylphenol, 2-hydroxy-3-methyl-2-cyclopenten-1-one, trans-Ioeugenol and (E)-2,6-dimethoxy-4-(prop-1-en-1-yl) phenol, respectively (Figure 2, table 2).

To my knowledge, this is the first report on the composition of the essential oil from Ethiopian *Olea europaea*. Phenolic compounds (35.49%),

non-terpenes (29.23%), terpenes (20.90%) and other miscellaneous compounds (6.37%) dominated the composition of the oil.

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 (%)	Class
1	2-hydroxy-3-methyl-2-cyclohex-1-one	C ₆ H ₈ O ₂	8.62	a	2.60	Ketone
2	2-methoxy phenol	C ₇ H ₈ O ₂	9.35	b	3.25	Phenol
3	2-methoxy-5-methylphenol	C ₈ H ₁₀ O ₂	10.85	c	3.19	Phenol
4	2-methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	12.76	d	2.70	Phenol
5	2,4-dimethoxyphenol	C ₈ H ₁₀ O ₃	13.50	e	4.05	Phenol
6	trans-isoeugenol	C ₁₀ H ₁₂ O ₂	14.48	f	2.45	Phenol
7	3,5-dimethoxy-4-hydroxytoluene	C ₉ H ₁₂ O ₃	14.65	g	3.20	Phenol
8	1,2,3-trimethoxy-5-methyl benzene	C ₁₀ H ₁₄ O ₃	15.54	h	2.93	Benzene
9	(e)-2,6-dimethoxy-4-(prop-1-en-1-yl) phenol	C ₁₁ H ₁₄ O ₃	17.02	i	2.25	Phenol
10	Methyl ester hexadecanoic acid	C ₁₇ H ₂₄ O ₂	18.45	j	4.10	Fatty acid

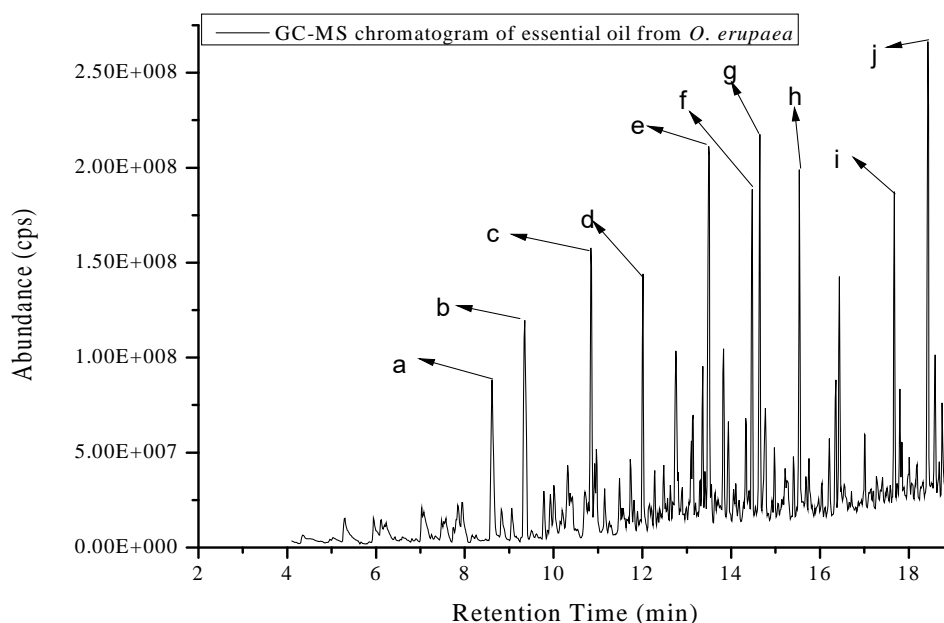


Figure 2. The representative ion chromatogram of the major compositions of the stem essential oil of *Olea europaea*

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 (Shahidi *et al.*; 1999, Silici *et al.*, 2007). This could well explain the importance of the *Olea europaea* in the traditional Ethiopian pharmacopeia

This investigation is different to those found in some oils from Algeria (from leaves) (Lograda et al., 2015) (Palmetic acid, Z-nerolidol, Octacosane), Tunisia (from fruits and stem) (Marzouk et al., 2010), (3-ethylpyridine, (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-

9- methylenebicyclo[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 (Viuda-Martos *et al.* 2011).

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

PK	Name of compounds	Rt	Area (%)
1	ethanedioic acid, bis(1-methylpropyl) 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

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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-Pyridinealoxime	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-methylbenzoic 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

4. Conclusions

The results presented in this study are the first given information's on the chemical composition of essential oil from the Ethiopian *Olea europaea* stem. It showed that phenolic compounds are the major fraction for the dry distillation extraction method (35.49%), and the *Olea europaea* stem essential oil can be a good source of methyl ester hexadecanoic acids.

The considerable reported biological activities of *Olea europaea* essential oil make it good candidates to develop natural derived therapeutics. It might be also, an alternative additive for foods and pharmaceuticals preparations. For future works, we will try to study some biological activities in order to find its applications.

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