Chemical Composition and Biological Activities of the Essential Oil Extracted from the Stem of *Olea europaea sub* spp. Africana (Mill)

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Abstract

This study aimed to extract and analyze the chemical composition of the stem essential oil of *Olea erupaea* Mill and extraction of the essential oil was carried out using dry distillation while gas chromatography/mass spectrometry (GC/MS) was used for the characterization of its composition. A mean oil yield of 5.19% v/w was obtained and GC/MS analysis identified 128 compounds corresponding to 89.4% of the total oil containing methyl ester hexa decanoic acid (4.1%), 2, 4-dimethoxyphenol (4.05%), 2-methoxy phenol (3.25%), 3,5-dimethoxy-4-hydroxytolune (3.2%), 2-methoxy-5-methylphenol (3.19 as major components. The antimicrobial activity of the essential oil was evaluated by disc diffusion method revealing moderate to highest susceptible against *C. albican, S. aureus, E. coli,* and *Salmonellae enterica.* This study confirms that the essential oil of *Olea erupaea* demonstrates significant antimicrobial properties. The antioxidant activity of the Essential oil was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay exhibiting the highest percentage of inhibition with 95.03% at a concentration of 0.1 µg/mL and its IC50 and AAI was 19.9 µg/mL and 5.5 µg/mL, respectively. These results confirmed the great potential of olive stem essential oil and its use in traditional medicine in the locality area.

Keywords: Antimicrobial activity • Antioxidant activity • Dry distillation • Essential oil • *Olea Erupaea* L.

Introduction

Nature has been a source of medicinal agents for thousands of years, and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population; especially in the developing world [1]. Essential oil is among the most interesting components of herbals and plants. Essential oils are rich in hydrocarbon compounds that have oxygenated, hydrogenated, and dehydrogenated functional groups. Most of these chemicals are monoterpenoid or sesquiterpenoids and phenols. They are odorous principles, which are found in various plants parts and evaporate at ordinary temperature [2, 3].

They are used as therapeutic agents in ethno, conventional, and complementary alternative medicines particularly as analgesics, anti-inflammatory, antispasmodic, local anesthetic, anthelmintic, antipruritic, and antiseptic as well as many other therapeutic uses [4-7]. Nowadays, essential oils are used broadly in medicine and cosmeceutical and pharmaceutical industries and as flavouring agents and preservatives in food industry and design [8, 9]. In addition, it is scientifically accepted that natural antioxidants exert health promoting effects and their consumption as food or as food additives or as nutraceuticals and supplements have been greatly promoted worldwide. A complex mixture of antioxidants may account for the improvement of cardiovascular health and decreased incidence of cancer in individuals who consume more of these substances [10-12].

In elderly subjects, a higher intake of antioxidants is linked with improved health compared to subjects consuming less fruits and vegetables in their diet [13-15]. On the other hand, many food industries utilize synthetic chemical preservatives to extend the shelf life of the produced commercial food products. Unfortunately, the chronic intake of these chemicals may cause carcinogenic or toxic effects to consumers. Accordingly, a growing interest in the discovery of new natural antimicrobial and antioxidant agent has been observed in order to avoid or minimize the undesirable consequences and side effects related to the consumption of chemical preservatives [16-18]. In fact, the so-called hurdle technology, combining antioxidant and antimicrobial agents, has gained wide acceptance within the food and pharmaceutical industries [19]. Combining two or more compounds could be more effective for improving antioxidant and antimicrobial activity and could offer a synergistic potential.

Olea erupaea commonly known as wild olive is found throughout the Mediterranean, Europe, Africa, Iran, Asia and Ethiopia is thought to have a cultivation history of several 1000 years [20]. It holds historic importance in the context of religion, and it is quoted in the Christian and Hebrew Bibles and the Koran [20, 21].

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 and cultivated forms are considered as a significant botanical research subject [20]. The traditional use of leaves includes treatment for fever, malaria, bacterial infections, diabetes, inflammatory disorders and hypertension [22]. The decoction of leaves is also used as a mouthwash to treat aphthous, gingivitis and glossitis [22]. Therefore, the current study aimed to identify the chemical composition of the stem essential oil of *Olea erupaea* growing wildly in Woreillu Woreda South Wollo, Ethiopia. Afterwards, screening and comparison of their antimicrobial and antioxidant activity were conducted.

Materials and Methods

Collection of the plant material

The plant material (dried olive stems) were randomly collected from local market, 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 Figure 1) which is not previously been published.

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

Essential oil extraction

The dry stems of *Olea europaera* were trimmed (cut) into small pieces (≈ 20 cm long), weighed and washed under tape 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 vapour 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 as a result of evaporation in the stew pot was isolated from dust charcoal by decantation and stored into 250 mL airtight glass vials. Lastly, the EO was put in a deep freeze until required for chemical analysis and bioassays. (The extraction of the oil was done in triplicate).

GC-MS analysis

The GC analysis of the stem EO of *Olea europaera* was performed on a Shimadzu GC-2010 gas chromatograph with Flame Ionization Detector (FID), fitted with a 25 m × 0.25 mm × 0.25 µm CBP5 capillary column, using helium as the carrier gas. The oven temperature was programmed from 60ºC (after 10 min) to 230ºC at 3ºC/min and the end temperature was held for 10 min. The GC/MS analysis of the stem EO of *Olea europaera* was carried out on an Agilent 5975N gas chromatograph-mass spectrometer with a 30 m × 0.25 mm × 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 [23].

Antimicrobial tests

In this study, the antimicrobial activity of the EO has been tested against *Candida albican* (dermatophytes fungus), *Staphylococcus aureus* (gram-positive bacterium) *Escherichia coli,* and *Salmonella enterica* (both gram-negative bacteria). The antibacterial, antifungal activities and minimal inhibitory of the Essential oil of the sample were screened by the disk diffusion test [24]. For both the fungi and bacteria, the sensitivity of individual microorganisms to the EO was classified based on the inhibition zone value expressed as millimeters (mm) as follows: not sensitive (-) for total zone diameters less than 8 mm, sensitive (+) for diameters between 8 mm -14 mm, very sensitive (++) for zone diameters between 15 mm-19 mm and extremely sensitive (+++) for zone diameters equal to or larger than 20 mm [25, 26]

Antioxidant activity

For this test, the ability of hydrogen donating or radical scavenging was measured using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) was determined. A volume of 1 mL of essential oil from *Olea erupaea* stem prepared at different concentrations was mixed with 2 mL of 4.2 µM DPPH methanol solution. The disappearance of the DPPH was measured at 517 nm after 30 min of incubation at room temperature. The inhibition percentage of the DPPH radical by the essential oil was estimated using the following equation:

$$\% \text{ Inhibition rate} = (A0 - A1)/A0 \times 100 \quad (1)$$

Where A0-absorbance of the blank (negative control) and A1-absorbance of the test sample. The resulting data was presented in a graph of percent inhibition rate against sample concentration. From the plotted graph, IC50 was calculated.

The Antioxidant Activity Index (AAI) with DPPH was determined by the method developed by Rodrigo Scherer and Helena Teixeira Godoy. According to these researchers the antioxidant activity was expressed as Antioxidant Activity Index (AAI), which is calculated as follows [27].

$$AAI = (\text{Final DPPH concentration } (\mu g/mL)/(IC50\ \mu g/mL)) \quad (2)$$

Thus, the AAI was calculated considering the mass of DPPH and the mass of the tested compound in the reaction, resulting in a constant for each compound, independent of the concentration of DPPH and sample used.

Results and Discussion

The percentage yield of *Olea erupaea* essential oil

About 315.5 mL of *Olea erupaea* EO was obtained from 6 kg 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 [28]. Moreover, the method of distillation used in this study gave good results in comparison to the other distillation techniques in isolating higher molecular terpenes such as diterpenes and triterpenes which contradicts the study of Kumar [29] who argued that diterpenes and higher terpenes cannot be detected by steam distillation method as these molecules are too heavy to allow for evaporation, so they are rarely found in distilled essential oils.

Physical properties

The result of this analysis indicated that the value of the specific gravity of the extracted EO is 0.82, which is under the accepted limit [30]. Other studies conducted by Rosier Davenne [31] and Kusuma, et al. [32] were also found the specific gravity of the EO of *Hypericum perforatum* and *Citrus aurantium* to be 0.83 and 0.84, respectively which are similar to the present investigation. The standard value of the refractive index for the extracted EO was recorded in the ranges of 1.420-1.510 (Table 2). Hence, the refractive index of the EO of *Olea erupaea* is between the accepted limits [30]. Hanafy also found the refractive indices of cumin and rosemary EOs were 1.502 and 1.47, respectively, which are in good agreement with this finding. The optical rotation of EO of *Olea erupaea* was 4 degrees which are within the acceptable limit as described in Table 2. In general, the EO obtained from the stem of *Olea erupaea* was less dense and insoluble in water. It was, however, soluble in methanol, DMSO at a level of 1:1 (v/v). The oil exhibited a dark yellow colour with a fragrance odour which is liquid at room temperature (25 ± 2°C) and maintained this state even in storage at -20°C.

The GC/MS chromatogram

A total of 128 compounds were identified from the extracted EO amounting to 89.4% of the total oil. Moreover, the EO of this stem showed a high content of phenolic compounds (35.49%), non-terpenes (29.23%), terpenes (20.90%) and other miscellaneous compounds (6.37%). Non-terpene compounds were also detected as the second most abundant group present in this oil containing fifty components: seventeen (9.16%) ketones, eight (6.83%) fatty acids, five (5.73%) oxygenated aromatic hydrocarbons, ten (3.00%) hydrocarbons, three (2.03%) aldehydes, four (1.60%) ethers, two (0.84%) alcohols and one (0.05%) esters. Terpenes were also recorded as the third major group identified constituting seventeen (13.97%) monoterpenes, seven (2.49%) sesquiterpenes, three (1.54%) diterpenes, six (0.63%) hemiterpenes, and one (0.35%) triterpene.

From the one hundred twenty eight compounds identified, the ten most abundant components were listed in the order 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-Isoeugenol and (E)-2,6-dimethoxy-4-(prop-1-en-1-yl)

Table 1: The percentage yield (% v/w) of *Olea erupaea* EO.

Sample	Brix (%) sucrose	Total sugar (g/100 g)	Sugar type
NTJ-2	14.0	10.92	Sucrose, glucose D-fructose
NRSS0012	23.0	10.94	Sucrose
NRSS0003	22.0	14.44	Galactose
Dan Sadau	22.0	9.61	Glucose, D-fructose, sucrose
503	22.0	13.1	Sucrose, D-fructose, sucrose
NRSS0005	22.0	13.1	Glucose, D-fructose, sucrose
501	19.0	11.46	Glucose, D-fructose, sucrose

phenol, respectively (Figure 2 and Table 3).

From Table 3, we can see that the EO of the stem of *Olea erupaea* was highly enriched in Phenolic compounds which are the major abundant secondary aromatic metabolites obtained from *Olea erupaea* EO consist of 35 compounds that have a strong antimicrobial and antioxidant effect, which act as free radical scavengers and are responsible for antioxidant activity in medicinal plants [33]. Some phenolic compounds and analogues also exhibit antibacterial, antifungal, antiviral, anti-mutagenic, and anti-inflammatory activities [34, 35].

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelating potential [36]. The chemical compositions of the EO obtained from the current study were compared with the different plant parts (leaves, fruits, and stems) of the same species (or species belonging to the same family) found in Algeria [37], Tunisia [38] and South Africa [39]. As we observed from Table 4 and Table 5, 128 compounds with percent yield 5.19 and major compounds methyl ester hexadecanoic acid, 2,4-dimethoxyphenol, and 2-methoxy phenol were identified from the EO of the stem of *Olea erupaea* which are completely different from those reported by the authors mentioned in the above three countries [37, 39]. 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 [40].

Biological Activities of the EO of *Olea erupaea*

Antimicrobial activity

The results of the analysis showed that the pure EO of *Olea erupaea* has substantially antimicrobial activity against all the bacteria and yeast tested. The values of the microbial growth inhibition zone showed the highest to moderate sensitivity towards the EO. Accordingly, *Salmonella enterica*, *S. aureus,* and *E.coli* were classified as extremely sensitive (+++) to the oil, with the growth of zone of inhibition corresponding to 21.8 ± 0.29 mm, 20.3 ± 0.58 mm, and 20.0 ±1.00 mm, respectively, whereas, C.albican with a halo of inhibition of 15.7 mm ± 0.58 mm corresponds to a sensitivity classified as very sensitive (++) (Table 6).

The MIC is the lowest concentration of EO required to prevent visible growth of the tested microorganisms around the disc measured by assessing the zone of inhibition [41]. The experimental finding showed that the results of MIC are considered as strong when MIC ≤ 1000 µg/mL, moderate when MIC are between 1000 µg/mL-4900 µg/mL and weak when the MIC are ≥ 500 µg/mL [41]. The EO of *Olea erupaea* was found to exhibit strong activity (MIC ≤ 1000 µg/mL) against the tested microorganisms observed to have 30 µg/mL, 50 µg/mL, 100 µg/mL and

Table 2. The physical properties of Olea erupaea EOs extracted using dry distillation in comparison with standard values.

Physical properties	Results	Unit	Standard values	Physical properties	Results	Unit
Specific gravity	0.82	-	0.780-0.970	Specific gravity	0.82	-
Refractive index	1.501	-	1.420-1.510	Refractive index	1.501	-
Optical rotation	4.0	Degree	0°-10°	Optical rotation	4.0	Degree
Colour	Dark yellow	-	Colourless to dark	Colour	Dark yellow	-
Odour	Characteristic odour	-	Characteristic odour	Odour	Characteristic 6odour	-
Solubility in alcohol	Soluble	-	Soluble in alcohol	Solubility in alcohol	Soluble	-
Solubility in water	Insoluble	-	Insoluble in water	Solubility in water	Insoluble	-

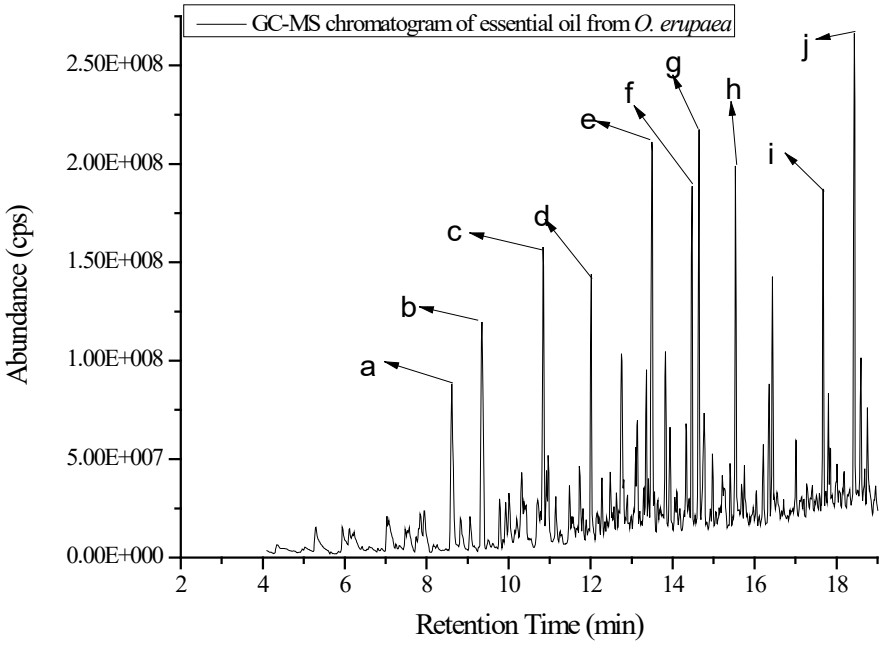

Figure 2. The representative ion chromatogram of *olea erupaea* stems essential oil.

Table 3. Ten major compounds of the EO of the stem of *Olea erupaea.*

No	Name of compounds	Chemical formula	Retention Time	Peaks	Area (%)
1	2-hydroxy-3-methyl-2-cyclopen-1-one	$C_6H_8O_2$	8.62	a	2.60
2	2-methoxy phenol	$C_7H_8O_2$	9.35	b	3.25
3	2-methoxy-5-methylphenol	$C_8H_{10}O_2$	10.85	c	3.19
4	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	12.76	d	2.70
5	2,4-dimethoxyphenol	$C_8H_{10}O_3$	13.50	e	4.05
6	trans-isoeugenol	$C_{10}H_{12}O_2$	14.48	f	2.45
7	3,5-dimethoxy-4-hydroxytolune	$C_9H_{12}O_3$	14.65	g	3.20

Table 4. Comparison of the chemical composition of EOs of the stem of *Olea erupaea* with other reported values of the same species.

Country	Plant parts used	Method of extraction	% yield of oil	No of compounds identified	Major compounds	Country	Plant parts used	Method of extraction	% yield of oil
Algeria (2015)	Leaves	Hydro distillation	0.01	38	Palmetic acid	Algeria (2015)	Leaves	Hydro distillation	0.01
					Z-nerolidol Octacosane				
Tunisia (2013)	Fruits	Hydro distillation	0.09	38	3-ethylpyridine	Tunisia (2013)	Fruits	Hydro distillation	0.09
					(E)-2-decanal				
					2-ethylbenzaldehyde				
Tunisia (2013)	Stems	Hydro distillation	0.92	38	Nonanal	Tunisia (2013)	Stems	Hydro distillation	0.92

Table 5. Chemical components of the stem oil of *Olea erupaea.*

PK	Name of compounds	RT	Area Pct
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
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-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	

Table 6. The antimicrobial activity of *Olea erupaea* essential oil.

Microorganisms	Gram-negative bacteria	Gram-positive bacteria	Fungus
	Salmonellae enterica	*E. coli*	*S. aureus*
Average zone of inhibition (mean ± SD)	21.8 ± 0.29	20.0 ± 1.00	20.3 ± 0.58

Table 7. The zones of inhibition and MIC of *Olea erupaea* essential oil.

Concentration (µg/mL)	***Salmonellae enterica***	***E. coli***
30	7.7 ± 0.58	6.7 ± 0.58
50	9.0 ± 1.00	7.7 ± 0.58
100	11.7 ± 0.58	11.0 ± 1.00
120	14.3 ± 1.15	12.8 ± 0.29

120 µg/mL for *Salmonella enterica, E. coli, S. aureus,* and *C. albican*, respectively (Table 7). Therefore, the MIC results of the EO of olive tree subspecies of Africana showed an appreciable spectrum of antibacterial activity against the entire selected gram-positive and gram-negative bacteria as well as the fungus species.

From the data presented in Table 6 below, the antimicrobial activity of EOs of *Olea erupaea* has an almost equal effect on both gram-positive and gram-negative bacteria, which is against the fact previously observed with EOs of other plants species. Most studies investigating the action of whole EOs against food spoilage organisms and foodborne pathogens agreed that, generally, EOs are slightly more active against gram-positive than gram-negative bacteria [42, 43]. Gram-negative organisms are less susceptible to the action of antibacterial is perhaps to be expected, since they possess an outer membrane surrounding the cell wall [44], which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering [45].

The EO is a very complex mixture containing a wide variety of components. Thus, it can be argued that the antimicrobial effect is observed as a result of the activity of the active compounds, as well as the possible synergistic effects between the minor components that accompany it [46]. From the antimicrobial results presented in Table 6 and Table 7, it was demonstrated that the EO of *Olea europaea* exhibited strong activity against the growth of the pathogens tested in this study. Thus, the EO displayed strong microbial activity against the selected gram-positive and gram-negative bacteria and the dermatophytes fungi indicating that the *Olea europaea* EO is a good antibacterial and antifungal source. This effect correlates with its folkloric uses and showed that it is an efficient antimicrobial plant that can be employed in alternative medicine for the treatment of microbial infection associated with skins. Earlier reports also showed that the major components of phenolics and organic acids in wood extracts can inhibit pathogenic fungi and bacteria [47, 48]. Therefore, the major phenolics, organic acids, terpene and other minor components contribute to the antibacterial and antifungal activities of the EO of *Olea erupaea*.

There are few reports in the literature on the Biological Activities of the EO of Oleaerupaea sub species Africana. It was found that the EO of Oleaerupaea inhibited the development of bacteria to a variable extent, which was more active against bacteria, *Citrobacter freundii* and *Pseudomonas aeruginosa* than *Staphylococcus aureus*, while *Bacillus subtilis* and *Escherichia coli* are resistant to the oil of this species [37], which are not in accordance with the present findings. It was postulated that individual components of EOs exhibits the different degrees of activity against gram-positives and gram-negatives and it is known that the chemical composition of EOs from a particular plant species can vary according to the geographical origin and harvesting period [49]. It is, therefore, possible that variation in composition between batches of EOs is sufficient to cause variability in the degree of susceptibility of gram-negative and gram-positive bacteria [50].

Antioxidant activity

Any sample possessing inhibition at 5 mg/mL (5000 ppm) is considered as active [41] and with IC50 values more than 240 µg/mL in DPPH assay is assumed to show negative results [51]. Experimental findings showed that the results of percentage scavenging activities of DPPH radical in the test solution at 5 mg/mL was strong when the percentage of scavenging are between 71-100, moderate when the percentage scavenging activity is between 41-70 and weak when the scavenging activities are ≤ 40 [41].

At the concentration of 0.15 mg/mL (149.090 µg/mL) (Table 7), the EO of *Olea erupaea* exhibited the highest percentage inhibition (95.03%) as compared to the range given in the literature [41]. The crude EO of *Olea erupaea* also gave strong antioxidant activities in DPPH radical scavenging test, with its IC50 values was 19.9 µg/mL (Table 8) and showed comparable antioxidant potential as compared to ascorbic acid. Ascorbic acid showed the percentages inhibition of 98.05% (IC50: 15.9 µg/mL) which serves as a standard [41].

The IC50 values of the EO and ascorbic acid (Figure 3), the standard reference, were determined by an online IC50 calculator/AAT Bioquest retrieved from the website https://www.aatbio.com/tools/ic50-calculator/, February, 2019.

Table 8. The percentage inhibition of DPPH (mean ± standard deviation, n=3) scavenging of EO of *Olea erupaea* at concentration of 0.15 mg/mL.

Samples	% Inhibition of DPPH scavenging activity	IC50 values (µg/mL)
Olea erupaea stem EO	95.03 ± 0.21	19.9
Ascorbic acid	98.05 ± 0.37	15.9
Samples	% Inhibition of DPPH scavenging activity	IC50 values (µg/mL)
Olea erupaea stem EO	95.03 ± 0.21	19.9
Ascorbic acid	98.05 ± 0.37	15.9
Samples	% Inhibition of DPPH scavenging activity	IC50 values (µg/mL)
Olea erupaea stem EO	95.03 ± 0.21	19.9
Ascorbic acid	98.05 ± 0.37	15.9

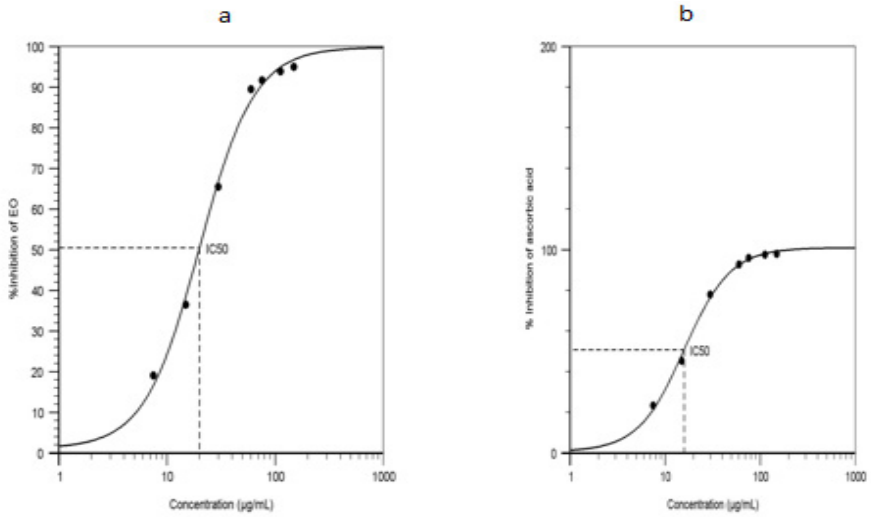

Figure 3. The DPPH radical scavenging activity of EO of *Olea erupaea* and ascorbic acid; a): IC50 value of the EO (19.9µg/mL); b): IC50 value of ascorbic acid (15.9µg/mL).

The AAI was calculated considering the mass of DPPH and the mass of the tested compound in the reaction, resulting in a constant for each compound, independent of the concentration of DPPH and sample used. Rodrigo Scherer and Helena Teixeira Godoy considered the EO to show poor antioxidant activity when AAI<0.5, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when AAI>2.0 [27]. The final concentration of DPPH solution was determined by conversion factors from its initial concentration, 4.2 mM, molar mass 394 g/mol, initial and final volume 30 mL and 2 mL in sequence gives a concentration of 110.32 µg/mL DPPH solutions. Hence, using the formula developed by Rodrigo Scherer and Helena Teixeira Godoy, the Antioxidant Activity Index (AAI) is calculated as follows:

$$AAI=(110.32\ \mu g/mL)/(19.9\mu g/mL)=5.54 \quad (3)$$

Thus, this result showed that the EO of *Olea erupaea* exhibited very strong radical scavenging activities as its AAI >2.0.

In the present study, the strongest antioxidant activity of *Olea erupaea* EO could be due to the presence of syringol type components, guaiacol type components, Catechol and D-Limonene which have potential antioxidant activities as if all they are phenolic compounds except limonene. This is in line with the observation of Gallo [52] who found Phenolic compounds in EO have strong antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In general, the EO obtained from *Olea erupaea* showed a highest scavenging capacity for the free radical (DPPH) which revealed its potential to donate electron or hydrogen to free radical thereby making them stable.

Little information is available in the literature on the antioxidant activity of *Olea europaea* L. EO. In the recent reports, the antioxidant level of *Olea erupaea* leaves EO was found to be 74.44 ± 0.79% at 5 mg/mL concentration which was lower than the results obtained in the present study. This is due to its major components which are not phenolics rather α-pinene (52.70%), 2.6-dimethyloctane (16.57%), 2-methoxy-3-isopropylpyrazine (6.01%), tetracosane (4.38%) and docosane (3.58%) [38].

Conclusion

The dry distillation method was found to be one of the promising techniques for the extraction of EO from the stem of plants. The results of this study suggested that the EO of *Olea erupaea* has the potential to be exploited as a good source of antioxidants and antimicrobial agents of plant origin. The study also showed that the EOs exhibited a broad spectrum of antimicrobial activities against the gram-positive bacteria, Staphylococcus aureus, gram-negative bacteria *Salmonellae enterica, E. coli*, and dermatophytes fungi, *Candida Albicans*. This activity is suspected to be associated with the predominant compounds such as phenolics, terpenes, and other miscellaneous molecules known for their antimicrobial activity. This adds value to the ongoing exploration for the new and safer plant-based compounds that are readily available and also as models for new drug discovery, towards effective and yet affordable antioxidant and antimicrobial agents. The results, thus, obtained supported the proposed hypothesis and the rationale for the use of *Olea erupaea* in folk medicine.

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Conflict of Interest

The author's report has no declarations of interest.

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