Original Research Article

CHEMICAL AND ANTIMICROBIAL INVESTIGATIONS ON ESSENTIAL OIL OF *ROSMARINUS OFFICINALIS* LEAVES GROWN IN ETHIOPIA AND COMPARISON WITH OTHER COUNTRIES

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ABSTRACT:

This study has been concerned with determining the chemical composition and antimicrobial activity of essential oil extracted from *R. officinalis* leaves grown in Ethiopia. The essential oil was obtained by hydrodistillation and yielded clear and intense yellow brownish 1.1% (w/w) oil with pleasant smell. The oil was analyzed by GC/MS and resulted in the identification of 43 compounds, representing 100% of the total oil. The major components of *R. officinalis* oil were 1, 8-cineole (23.55%), verbenone (18.89%), camphor (15.06%), α-terpineol (6.43%), isoborneol (5.68%), tridecyl acrylate (5.57%), linalool (3.71%), bornyl acetate (3.57%), *trans*-caryophyllene (3.36%), terpine-4-ol (2.78%) and α-pinene (1.40%). *In vitro* antimicrobial activities of the essential oil of *R. officinalis* was determined by paper disk diffusion method and showed moderate antimicrobial activity in both 10 and 20 µL concentrations. *Escherichia coli* and *Staphylococcus aureus* were used as test bacterial strain where as *Apergillus niger* and *Fusarium oxysporum* were selected as test fungi. The presence of monoterpene and oxygenated monoterpene as the major constituents of the essential oil could be responsible for the moderate antimicrobial activity in this study. The results of this study showed some differences in the antimicrobial activities and chemical composition of the essential oil when compared with similar studies conducted in other countries.

Key Words: Antimicrobial activity, *Rosmarinus officinalis*, Essential oil, GC/MS, 1, 8-cineole

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INTRODUCTION

Rosemary (*Rosmarinus officinalis L.*), belonging to the Lamiaceae family, is a pleasant-smelling perennial shrub that grows in several regions of all over the world [1]. Rosemary is an aromatic, bushy, attractive evergreen shrub with pine needle-like leaves, native to the Mediterranean countries [2]. It grows up to 2 m high, with densely-leafy erect branches. Flowers are white to pale blue, in small clusters in the leaf axils at the tops of stems and last throughout the year mainly after the big rains from September to February. Honeybees collect pollen and nectar from the flowers. It is a well-known valuable medicinal herb that is widely used in pharmaceutical products [3]. In Ethiopia, it is known as "Siga metbesha or Yetibs Kitel" in Amharic and Kora in Oromifa. Rosemary also includes many other herbs. It is related to other well-known herbs such as the basils (*Ocimum*), thymes (*Thymus*) and mints (*Mentha*), but its closest relative is the genus *Salvia*, which includes at least 900 species. The main producers are Italy, Dalmatia, Spain, Greece, Iran, Turkey, France, Portugal, Ethiopia and North Africa (Morocco, Algeria, Tunisia, and Egypt) [4]. However, this aromatic plant can also be found in other countries such as Argentina, Brazil, Uruguay, and Cuba. Several countries cultivate rosemary as its essential oils, which can be used in food, perfume, cosmetic and pharmaceutical industries [5-6].

Traditionally, rosemary has been used by herbalists to improve memory, to relax muscles including the smooth muscles of the digestive tract and uterus, relieve muscle pain and spasm; stimulate hair growth, wedding ornaments, astringent, diuretic, diaphoretic, support the circulatory and nervous systems, for urinary ailments and recently in the prevention of cancer and its antibacterial properties [3]. Owing to its desirable flavor and antimicrobial and antioxidant activities, this plant has been widely employed as a spice and flavoring agent in the food processing and pharmaceutical industries [7-8].

In Ethiopia, Rosemary is widely used to flavor meat dishes, stews, marinades, sauces, soups and salads. Related Lavandula species (lavender) has similar properties but is less often used in cooking [9]. Rosemary is a carminative and stomachic used to treat stomach cramps and flatulence, and to stimulate appetite and the secretion of gastric juices. The oil stimulates blood circulation and has antibacterial, antifungal, antiparasitic and mild analgesic effects. The leaves contain phenolic acids (rosmarinic acid, chlorogenic acid and caffeic acid); bitter substances (carnosol, rosmaridiphenol, rosmanol); volatile oil consisting camphor, camphene and borneol [10]. Its oil is used for hair care as it is believed to counter hair loss. It is also used for memory improvement, preventing Alzheimer's, treating baldness, body odor, fainting, and amenorrhea. *Rosmarinus officinalis* (*R. officinalis*) is also well-known for its substantial insecticidal activities against a number of insect pests in storage structures [11]. It was reported that rosemary essential oil have contact and fumigant toxicity. The essential oil of *R. officinalis*, commonly known as rosemary oil, has often been reported to inhibit osteoclast activity and to increase bone density in vitro [12].

At present, the demand for *R. officinalis* is increasing due to its use in traditional medicine, pharmaceutical industries, cosmetic fields and agribusiness, and for the quality of its essential oil. Due to their special flavor and biological activities, the essential oils have also been reported to be useful in aromatherapy [13], food preservation [14] and fragrance industries [15]. Significant variations in the chemical composition of the essential oils of Rosemary as well as its aroma and its medicinal characteristics (antimicrobial and antioxidant properties) have been reported with relation to the geographic origin [5].

Although the antimicrobial properties and chemical constituents of essential oils of *R. officinalis* have been reported worldwide, there has been a research gap till date on the antimicrobial activities and GC-MS analysis of essential oil composition of *R. officinalis* extracted by hydrodistillation in Eastern Ethiopia. Therefore, the aim of this study was to investigate the chemical composition and antimicrobial activities of *R. offcinalis* volatiles and comparing its components with other Countries.

MATERIALS AND METHODS

Collection of Plant Materials

The *R. officinalis* was purchased from local market of Haramaya town on 20th July, 2013. The plant materials (leaves) were identified by Mr. Abdurasek Abdulahi, and authenticated at the Herbariums of Plant Science Department, Haramaya University, Ethiopia. The shade dried plant material was chopped into small pieces and finally pulverized into fine powder using a sterile electric grinder.

Essential Oil Extraction by Hydro-distillation

A 150 g of the leaf powder of *R. officinalis* was mixed with 500 mL of distilled water in 1 L distillation flask and hydrodistilled using an apparatus of Clevenger type for 3 h [16-17]. The distillation flask was placed in heating mantle and allowed to boil the sample up on the addition of boiling chips until the distillation was completed. The distillate was collected in receiver apparatus (500 mL beaker). The extracted fractions of plant parts exhibited two distinct layers an upper oily layer and the lower aqueous layer. The oil was separated from aqueous portion by extracting it twice with chloroform using a separatory funnel. The chloroform (lower) layer was slowly drowned off until only the water layer

remains. After filtration, the solvent was eliminated in a rotary evaporator at 35 °C and reduced pressure distillation. The oil obtained as such was dried over anhydrous Na_2SO_4 in order to remove water traces, filtered, concentrated under vacuum, weighed its yield and stored in clean brown glass bottles at 4 °C in refrigerator until used for antimicrobial screening. The chemical constituents of the oil were determined by GC-MS at the research laboratory of Jawaharlal Nehru University, India.

GC-MS Analysis of Essential oil

The essential oil from leaves of *R. officinalis* was analyzed on GC-MS QP-2010 Plus (Shimadzu Company) using HP-5 MS column (30 m x 0.25 mm internal diameter x 0.25 μm film thickness) which was coated by 5% phenyl 95% methyl poly siloxane as the stationary phase. The syringe was washed with 8 μL of chloroform and 2 μL of the essential oil solution in chloroform was injected through autosampler and analyzed with the HP5 MS column.

Column temperature was programmed as follows: 50 to 120 °C at 20 °C/min, 120 to 150 °C at 4 °C/min, 150 to 250 °C at 20 °C/min (with 10 min hold time) and 3.5 min solvent delay. The temperature of the injector was fixed to 260 °C and that of the detector (FID) to 270 °C. Carrier gas was helium (1 mL/min) with 69.8 kPa and a split ratio of 100:1. The interface temperature was 280 °C. The mass spectra were recorded in electron ionization mode at 70 eV with scanning from 40 to 600 m/z at 0.5 s and ion source temperature was set at 230 °C [18]. The percentage of each constituent in the oil was determined based on GC peak areas. The constituents of the essential oil were identified by their retention index, MS Library search (NIST 08 and WILEY 8 libraries) and by comparison with the spectra and retention index data available in the literature.

Antimicrobial Activity of Essential Oil of the leaves of *R. officinalis*

The oil extracted by hydrodistillation was evaluated *in vitro* for antimicrobial activity by using the paper disc diffusion method against gram positive bacterium (*Staphylococcus aureus* (*S. aureus*)) and gram negative bacterium (*Escherichia coli* (*E. coli*)) and two fungi, *Aspergillus niger* (*A. niger*) and *Fusarium oxysporum* (*F. oxysporum*) [19]. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA) (and incubated at 37 °C). Fungal cultures were inoculated into Potato Dextrose Agar (PDA) and incubated at 27 °C. The bacteria were obtained from plant pathology laboratory of the school of plant sciences, Haramaya University. *A.niger* and *F.oxysporum* were obtained from infected fruits (onion) and grain (sorghum), respectively. Chloroamphenicol (CAL) was used as standard drug against bacteria where as bavistin was used against fungi. Dimethyl Sulfoxide (DMSO) was also used as a negative control.

Preparation of inoculums

The test bacterial strains were transferred from the stock cultures and streaked on MHA plates and incubated for 24 h at 30 °C oven. Well separated bacterial colonies were then used as inoculums. Then spores of the test fungi were harvested by washing the surface of the colony using 10 mL sterile distilled water. The mycelial plugs of fungi from stock cultures were transferred to PDA plates and incubated for 7 days at 27 °C oven. The MHA and PDA medias were autoclaved at 121 °C and 1.03 bars for 15 minute inorder to be sterilized and cooled to about 45 °C in a water bath. The microorganisms were then transferred to their media using sterile loop and mixed by gentle swirling the flasks and then poured to sterile petri plates, allowed to solidify and used for the bioassay test.

Testing for antimicrobial activity

Filter paper discs of 6 mm diameter placed in a beaker were sterilized in an oven at 180 °C for 1 h. Then 10 and 20 μL of the solutions of the oil extract were pipetted to the discs in three replications. The paper discs impregnated with the sample were then transferred with sterile forceps to medias seeded with spore suspension of test fungi and bacterial strains as described above. The inhibitory activity of

the oil was evaluated by measuring the zone of inhibition against the test organisms after an incubation period of 24 h and compared to that of the commercial drugs [20].

RESULTS AND DISSCUSION

Essential oil composition of *R. officinalis*

The essential oils yield of *R. officinalis* purchased from Haramaya town (Ethiopia) was obtained by hydrodistillation of the fresh leaves and the yield was 1.1%. It was almost colorless to pale yellow liquid with a characteristic, refreshing and pleasant odor. It is relatively higher than other aromatic plants industrially exploited as a source of essential oils: Artemisia herba-alba (0.59%) and Artemisia absinthium (0.57%) [21], Thymus (1%) [22], menthe (0.5-1%), neroli (0.5-1%), *R. officinalis* (0.5-1.0%) [23] and Laurel (0.1-0.35%) [24]. However, the rosemary oil of the current study was relatively less than Turkish *R. officinalis* (1.9%) [1] and Italian *R. officinalis* (1.75%) [25]

The constituents of *R. officinalis* from Haramaya are listed in order of their elution on the HP-5 MS column (Figure 1). All of the 43 volatile compounds in the leaves oil, representing 100% of the total composition, were identified by means of their retention times, retention indices, by comparison with the spectra data in the literature and mass spectral fragmentation patterns as well as by comparing their mass spectra with the NIST 08 and WILEY 8 libraries of mass spectra (Table 1). This total oil composition (100%) is higher than other *R. officinalis* studies example, in Iran (99.74%) [7], in Brazil (90.6%) [26] and in Algeria (98.2%) [27]. Monoterpene and Oxygenated Monoterpene hydrocarbons were found to be the major group of compounds in this study. The most abundant component found in the leaf oil was 1,8-cineole (eucalyptol) (23.55%), other predominant components were verbenone (18.89%), camphor (15.06%), α-terpineol (6.43%), isoborneol (5.68%), tridecyl acrylate (5.57%), linalool (3.71%), bornyl acetate (3.57%), *trans*-caryophyllene (3.36%), terpine-4-ol (2.78%) and α-pinene (1.40%). Considerable differences were observed in previous studies on the chemistry of Ethiopian rosemary with regard to the essential oil composition of the leaves. More specifically, the oils of Ethiopian rosemary grown in Sebeta garden was found to be rich in 1,8-cineole (24.6%), α-pinene (12.9%), camphor (12.6%), citronellal (6.6%), linalool (4.5%), cinnamaldehyde (4.5%), estragole (3.6%), camphene (3.5%), β-pinene (2.3%), citronelly formate (2.3%) and unknown (3.7%) [28].

A comparison of the results of this work with those of previously reported for essential oil composition of *R. officinalis* grown in different geographical origins reveal significant differences (Table 2). The α-pinene (18.25%) was the highest component in the leaves of Morocoian *R. officinalis* (Atlas median origin) followed by camphor (6.02%), 1.8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%) [17] where as the *R. officinalis* essential oil of Rabat origin contained 1, 8-cineole (50.49%), α-pinene (15.82%), camphor (11.61%), camphene (6.80%), β-pinene (4.75%), broneol (2.58%), p-cymene (2.16%), broneol acetate (2.08%) and myrcene (1.70%) [29]. Similarly, the hydrodistillation of Brazilian *R. officinalis* essential oil was found to be rich in α-pinene (40.55 to 45.10%), 1,8-cineole (17.40 to 19.35%), camphene (4.73 to 6.06%) and verbenone (2.32 to 3.86%) [30]. The Iranian *R. officinalis* essential oil (Lalehzar origin) was found to be rich in α-pinene (43.9%), 1,8-cineole (11.1%), camphene (8.6%), β-myrcene (3.9%), broneol (3.4%), camphor (2.4%) and verbenol (2.3%) where as the *R. officinalis* essential oil of Kerman origin contains α-pinene (46.1%), 1,8-cineole (11.1%), camphene (9.6%), camphor (5.3%), sabinene (4.6%), β-myrcene (3.9%), broneol (3.4%), bornyl acetates (2.8%), verbenone (2.3%) and linalool (2.1%) [31].

However, Turkish *R. officinalis* oil was characterized with a high content of p-cymene (44.02%), linalool (20.5%), γ-terpinene (16.62%), thymol (1.81%), β-pinene (3.61%), α-pinene (2.83%) and eucalyptol (2.64%) [1]. Moreover, α-pinene (7.9–10.9%), myrcene (17.9–20.4%), 1,8-cineole (14.5–15.3%), camphor (9.0–9.3%) and β-caryophyllene (14.5–15.3%) were the main constituents of Argentinean

rosemary essential oil [32]. Furthermore, the essential oil extracted from the fresh leaves of Egyptian *R. officinalis* was rich in Verbenone (12.3%), camphor (11.3%), α-pinene (9.3%), 1,8-cineole (9.0%), bornyl acetate (7.6%) and limonene (7.1%) [33] (Table 2). The relative amount of each compound was different in type and in composition between the oils of this study and those in the literatures. This stresses the importance of analysis of this oil and could explain differences in biological properties. These differences in oil composition are correlated with different regions or countries where the plant is cultivated [34]. In general, the difference in percentage and composition of essential oils of *R. officinalis* from Ethiopia and the other countries could be markedly affected by geographical environment, plant population density, physical and chemical characteristics of soil, time of harvest, distillation equipment, methods of extraction, condition of the twigs and leaves, the plant age, growing media and management [35].

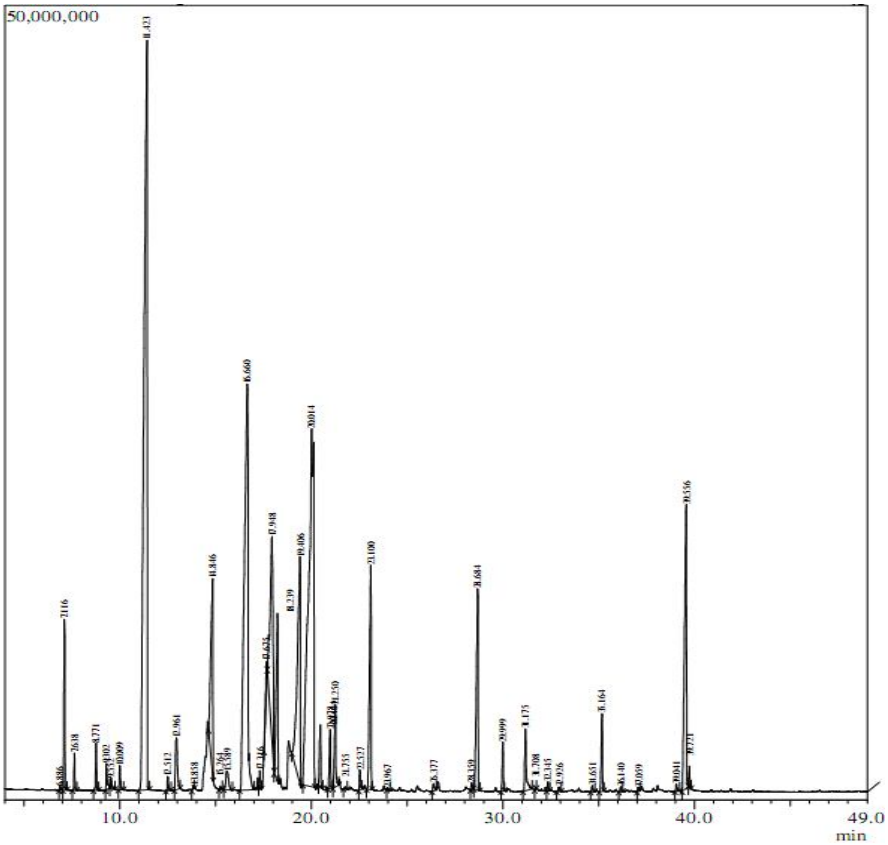

Figure 1. GC of the constituents of essential oils of *R. officinalis* leaves

Antimicrobial of *R. officinalis* Essential Oil

The antimicrobial activities of the essential oil of *R. officinalis* leaves were tested by the disc diffusion method and are shown in Table 3. The inhibition of each microorganism by the hydrodistilled oil was measured as the average of two cross diameters after 24 h of inoculation of the microorganism. The oil of *R. officinalis* showed moderate antifungal activities against *A. niger* and *F. oxysporum* and antibacterial activities against *S. aureus* and *E. coli* at 10 and 20 µL concentrations (Table 3).

The antibacterial and antifungal inhibition effect of the hydrodistilled oil was increasing with increasing concentrations. It was noted that the oil has low antifungal activity *A. niger*. Table 3 also reveals that the inhibitory effects of Bavistin and CAL were greater when compared with the oil. DMSO was used as control and showed no antimicrobial activities. Oil composition analyses indicated that the antimicrobial activity of *R. officinalis* essential oil was strictly related to its chemical composition [8, 39].

Table 1 Chemical composition of essential oils of *R. officinalis* leaves

Peak	Constituents	MF	RT(min)	RI (iu)	RI Lit	% Comp.
1	α-thujene[M]	$C_{10}H_{16}$	6.886	927	973	0.03
2	α-pinene[M]	$C_{10}H_{16}$	7.116	961	969	1.40
3	Camphene[M]	$C_{10}H_{16}$	7.638	943	998	0.30
4	β-pinene[M]	$C_{10}H_{16}$	8.771	978	1040	0.42
5	Amyl vinyl carbinol[OA]	$C_8H_{16}O$	9.302	969	-----	0.32
6	Myrcene[M]	$C_{10}H_{16}$	9.552	991	991	0.11
7	α-phellandrene[M]	$C1_0H_{16}$	10.009	1007	1006	0.26
8	1,8-Cineole[OM]	$C_{10}H_{18}O$	11.423	1032	1059	23.55
9	γ-terpinene[M]	$C_{10}H_{16}$	12.512	1058	1061	0.15
10	4-Thujanol[OM]	$C_{10}H_{18}O$	12.961	1124	------	1.00
11	α-terpinolene[M]	$C_{10}H_{16}$	13.858	1086	1080	0.05
12	Linalool[OM]	$C_{10}H_{18}O$	14.846	1101	1082	3.71
13	Fenchol[OM]	$C_{10}H_{18}O$	15.264	1138	1110	0.03
14	Chrysanthenone[OM]	$C_{10}H_{14}O$	15.589	1122	1120	0.51
15	Camphor[OM]	$C_{10}H_{16}O$	16.660	1121	1122	15.06
16	Pinocarvone[OM]	$C_{10}H_{14}O$	17.316	1164	------	0.15
17	Borneol[OM]	$C_{10}H_{18}O$	17.675	1088	1138	0.46
18	Isoborneol[OM]	$C_{10}H_{18}O$	17.948	1138	------	5.68
19	Terpinene-4-ol[OM]	$C_{10}H_{18}O$	18.239	1137	1137	2.78
20	α-terpineol[OM]	$C_{10}H_{18}O$	19.406	1121	1174	6.43
21	Verbenone [OM]	$C_{10}H_{14}O$	20.014	1119	1195	18.89
22	2,3-Pinanediol[OM]	$C_{10}H_{18}O_2$	20.464	1276	------	0.77
23	*Cis*-Myrtanol[OM]	$C_{10}H_{18}O$	20.978	1180	1244	0.73
24	*Trans*-Myrtanol[OM]	$C_{10}H_{18}O$	21.250	1270	1251	1.16
25	Piperitone[OM]	$C_{10}H_{16}O$	21.755	1267	------	0.02
26	Isopiperitenone[OM]	$C_{10}H_{14}O$	22.527	1190	------	0.22
27	Bornyl acetate[OM]	$C_{12}H_{20}O_2$	23.100	1277	1277	3.57
28	Isoascaridole[OM]	$C_{10}H_{16}O_2$	23.967	1306	------	0.03
29	Thymoquinone[OM]	$C_{10}H_{12}O_2$	26.377	1344	------	0.12
30	Methyl eugenol[OM]	$C_{11}H_{14}O_2$	28.359	1361	1401	0.08
31	*Trans*-caryophyllene[S]	$C_{15}H_{24}$	28.684	1424	1422	3.36
32	α-humulene[S]	$C_{15}H_{24}$	29.999	1452	1453	0.54
33	1-Dodecanol[OA]	$C_{12}H_{26}O$	31.175	1457	------	0.94
34	Valencene[S]	$C_{15}H_{24}$	31.708	1492	------	0.02
35	β-Bisabolene[S]	$C_{15}H_{24}$	32.345	1508	------	0.09
36	β-Sesquiphellandrene[S]	$C_{15}H_{24}$	32.926	1523	------	0.07
37	Viridiflorol[S]	$C_{15}H_{24}$	34.651	1594	------	0.08
38	Caryophyllene oxide[OS]	$C_{15}H_{24}O$	35.164	1587	1506	0.94
39	Humulene epoxide II[OS]	$C_{15}H_{24}O$	36.140	1613	------	0.06
40	Cubenol[OS]	$C_{15}H_{26}O$	37.059	1580	------	0.05
41	α-Bisabolol[OS]	$C_{15}H_{26}O$	39.041	1688	------	0.10
42	Tridecyl acrylate[O]	$C_{16}H_{30}O_2$	39.556	1769	------	5.57
43	Decyl Propanoate[O]	$C_{13}H_{26}O_2$	39.721	1481	------	0.21
	Total Identified Compounds					**100%**
	Essential oil yield					**1.1%**

RT = Retention Time, RI = Retention Index , RI Lit = Retention Index Literature,
Comp.=composition, MF=Molecular Formula, M=Monoterpenes, OM = Oxygenated Monoterpenes,
OA = Oxygenated Aliphatic, S = Sesquiterpenes, OS = Oxygenated Sesquiterpenes, O = Others

Table 2. Comparison of the chemical composition (%) of the major constituents of Ethiopian Rosemary oil with Other Countries

Compound	Haramaya	Sebeta	Brazil	Iran	Mororco	Spain	France	Algeria	Cuba	Argentina	Egypt	Italy	Turkey
1, 8-Cineole	23.55	24.6	19.35	11.1	47.44	18.9	5.30	29.5	11.00	14.5	9.0	20.64	2.64
Verbenone	18.89	-	3.86	2.3	0.46	-	4.80	-	0.25	0.3	12.3	4.76	-
Camphor	15.06	12.6	2.42	5.3	7.9	18.9	3.00	11.5	34.80	9.0	11.3	10.26	-
α-terpineol	6.43	-	0.92	0.4	-	-	-	9.2	-	-	-	-	-
isoborneol	5.68	-	-	-	-	-	-	-	-	-	-		-
Camphene	0.3	3.5	6.06	9.6	3.62	11.2	8.30	5	5.18	5.1	-	5.52	-
β-Myrcene	0.11	-	1.71	3.9	1.57	4.9	2.10	-	-	17.9	-	0.55	-
Borneol	0.46	-	3.10	3.4	2.97	4.5	4.44	9.4	11.60	1.1	-	13.70	-
Bornyl acetate	3.57	-	1.39	2.8	0.23	1.0	14.30	-	-	0.9	7.6	-	-
tridecyl acrylate	5.57	-	-	-	-	-	-	-	-	-	-	-	-
Linalool	3.71	4.5	-	2.1	0.7	1.0	1.61	-	3.05	0.9	-	1.82	20.5
trans-caryophyllene	3.36	-	-	0.1	-	-	-	-	-	-	-	-	-
terpine-4-ol	2.78	-	0.75	0.1	-	-	-	-	-	-	-	-	-
α-pinene	1.40	12.9	45.10	46.1	12.51	24.7	35.80	7.5	8.17	10.9	9.3	25.16	2.83
4-Thujanol	1.0	-	-	-	-	-	-	-	-	-			-
Trans-Myrtanol	1.16	-	-	0.4	-	-	-	-	-	-			-
Caryophyllene oxide	0.94	-	-	0.1	-	-	-	-	-	-			-
β-pinene	0.42	2.3	2.70	1.9	7.2	3.4	4.30	3.2	2.49	4.8		1.05	3.61
β-Caryophyllene	-	-	-	1.1	3.31	2.2	1.40	-	0.88	8.3		0.97	-
Limonene	-	-	3.81	1.2	1.9	3.1	3.40	-	2.80	2.9	7.1	1.33	-
References	This study	[28]	[30]	[31]	[36]	[36]	[36]	[27]	[37]	[32]	[33]	[38]	[1]

Table 3. Antimicrobial Activities of *R. officinalis* Essential Oil

Compound	**Average inhibition (I) (mm) of Microorganism**							
	Gram(-)Bacteria		Gram (+)Bacteria		Fungi			
	E.coli		*S.aureus*		*A. niger*		*F. oxysporum*	
	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL
Oil	10	11	11.7	13.7	-	8	11.7	13.4
Bavistin	-	-	-	-	20.3	21.8	20.9	21.8
CAL	26.0	29.3	22.5	27.7	-	-	-	-
DMSO	-	-	-	-	-	-	-	-

Susceptibility (inhibition zone ≥ 7 mm) and (-) absence of susceptibility

The GC and GC/MS results of this study showed that the antimicrobial activities of the oil of *R. officinalis* could be because of presence of 1,8-cineole (23.55%), verbenone (18.89%), camphor (15.06%), α-terpineol (6.43%), isoborneol (5.68%), tridecyl acrylate (5.57%), linalool (3.71%), bornyl acetate (3.57%), *trans*-caryophyllene (3.36%), terpine-4-ol (2.78%) and α-pinene (1.40%).

Comparison of the current findings with literature showed that Ethiopian rosemary oil has less antimicrobial inhibitory effect when compared with other countries. *In vitro* disk diffusion method depicted that the essential oil of the leaves of *R. officinalis* obtained from Morocco has potent antibacterial activity against *E.coli, Pseudomonas aeruginosa, S.aureus, Kellebsiella pneuomonae, Salmonella typhi, Staphylococcus intermedius, Bacillus subtilis, Streptococcus mutans, Micrococcus luteus*, and *Proteus* at 10.4 and 23 µg/mL concentrations. The presence of monoterpenes as the major constituents of the essential oil extracted from leaves of *R. officinalis* such as α-pinene (18.25%), camphor (6.02%), 1,8-cineole (5.25%), camphene (5.02%), β-pinene (4.58%), bornylacetate (4.35%), limonene (3.56%), borneol (3.10%), α-terpineol (2.89%) and cymene (2.02%) could be responsible for the potential antibacterial activity [19]. The antimicrobial properties of essential oil from the leaves of *R. officinallis* is presumably related to its high contents in 1, 8-cineole, verbenone, camphor, α-terpineol, isoborneol, borneol and α-pinene. These compounds were previously reported to display marked antimicrobial effects [39-41]. The major component of this oil, 1, 8- cineole, was also previously described to display antimicrobial activity against various bacterial and fungal strains (*E. coli, S. typhi*, *S. aureus*, *S. intermedius, Pseudomonas aeruginosa* and *Bacillus subtilis*) [42]. However, the role of other minor compounds should not be neglected. Gill *et al.* [43] and Wang *et al.* [44] have concluded that whole essential oils have a greater antibacterial activity than a mixture of major components of the same essential oils which suggests that the minor components may have a synergistic effect or potentiating influence. The findings presented in the current work indicate that the rosemary oil exhibited moderate antimicrobial activities. The antimicrobial activities of the oil of *R. officinalis* may be attributed to 1,8-cineole (23.55%), verbenone (18.89%), camphor (15.06%), α-terpineol (6.43%), isoborneol (5.68%), tridecyl acrylate (5.57%), linalool (3.71%), bornyl acetate (3.57%), *trans*-caryophyllene (3.36%), terpine-4-ol (2.78%) and α-pinene (1.40%). Ethiopian rosemary oil has less antimicrobial inhibitory effect when compared with other countries. This may be attributed due to the chemical composition differences. This study suggest that this oil has a number of promising properties for certain applications, particularly in the food, cosmetic and pharmaceutical industries, as a safe and cost effective natural additive to substitute toxic synthetic food additives.

CONCLUSION

This study has confirmed that most of the identified components and the composition of essential oils of *R. officinalis* from Ethiopia are different from other countries are different. It also showed some differences in the antimicrobial activities. This could be markedly affected by geographical environment, physical and chemical characteristics of soil, time of harvest, method of extraction, relative humidity, distillation equipment, condition of the twigs and leaves, plant age, plant cultivation techniques, plant population density, climate and management [35]. Therefore, for different use of essential oils of rosemary different geographic origins may be considered for growing rosemary. From 43 compounds identified, 16 of them are found to be detected for the first time in essential oil of *R .officinalis*.

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