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Chemical Composition and Antibacterial activity of Essential oil of *Ocimum basilicum* of Northern Ethiopia

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Abstract

The constituents of essential oil isolated by hydro distillation of the aerial parts of Ocimum basilicum L, Lamiaceae family, from Ethiopia was examined by GC-MS. A total of 30 components were identified accounting for 76.7% of the oil of O.basilicum. The oil contained, as main components, copaene (25.5%), p-menth-2-en-1-ol (7.7%), eugenylacetae (4.8%), bornyl acetate (4.0%), γ – himachalene (3.6%), rosifoliol (3.0%) and α –cubebene(2.5%). The essential oil of O.basilicum showed significant anti bacterial activity against gram positive (Staphylococcus auerus) than gram negative bacteria (Escherichia coli).

Keywords: *O. basilicum* L., Lamiaceae, essential oil, antibacterial activity.

Introduction

The genus *Ocimum* belonging to family of Lamiaceae is widely distributed in tropical and warm temperate regions of the world. It is usually named as sweet basil and is an annual plant, with extraordinary medicinal properties and contains several antioxidant compounds. In traditional medicine, *Ocimum basilicum* has been used as an antiseptic, preservative, sedative, digestive regulator and diuretic[1-5]. It also has been recommended for the treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins[6-8]. Both *Ocimum* oil and its extracts were shown to exhibit antibacterial activities against gram positive and gram negative bacteria by various researchers[9-14].

Material and Methods

Plant Material: The aerial parts of *Ocimum basilicum* plant was collected during the month of January from Northern Ethiopia in 2012. The plant was identified by the authors and its herbarium sheet was deposited at the Chemistry department, Mekelle University, Mekelle, Ethiopia.

Chemical reagents: All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

Essential oil extraction: The shade dried aerial parts of *Ocimum basilicum* plants collected (1Kg) was subjected to hydro distillation in a Clevenger apparatus for 3h. The essential oil was separated from aqueous layer using a 100 mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was yellow-greenish liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until analysis. The yield of the oil was found to be 0.4% on fresh weight basis.

GC and GC-MS analysis: GC analyses were carried out in Agilent Technology 6890N gas Chromatograph data handling system equipped with a split-split less injector and fitted with a FID using N_2 as carrier gas. The column was HP-5capillary column (30m x 0.32mm, 0.25µm film thickness)and temperature program was used as follows: initial temperature of 60 ^{0}C(hold : 2 min) programmed at a rate of 3^{0}C/min to a final temperature of 220^{0}C (hold: 5 min). Both the temperature of injector and FID were maintained at 210^{0}C.

The GC-MS was performed by Perkin Elmer Clarus 500 gas chromatograph equipped with a split-split less injector (split rtatio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 min x 0.32 mm, 0.25µm film thickness). Helium was used as carrier gas at a flow rate of 1.0ml/min. The GC was interfaced with Perkin Elmer 500 mass detector operating in EI^+ mode. The mass spectra was recorded over 40-500 amu and revealed the total ion current chromatograms. The temperature program remained the same as in GC. The temperatures of injector and transfer line were kept at 210^{0}C and that of ion source at 200^{0}C.

Identification of the oil components was done by comparison of their mass spectra with the NIST/Wiley library as well as by comparing them with those reported in literature. The identification of each compound was also confirmed by comparison of its retention index with those of authentic compounds[15].

Antibacterial activity: The study was conducted by using standard disc diffusion method. In each experiment, microorganisms were cultured at 37°C for 24 hours and prepared to turbidity which is equivalent to 0.5 McFarland standards (National Committee of Clinical Laboratory Standards)[16-18].

Mueller-Hinton (MH) agar 38g was dissolved in 1000 ml of distilled water. Then it was boiled on heating mantle to dissolve the media completely and then sterilized by autoclaving at 15 lbs. and 121^{0}C for 15 min. After it was autoclaved at indicated conditions, it was poured to the sterilized petridishes and allowed to set at room temperature until the agar has solidified. It was then incubated at 37^{0}C for 24 hours to be ready for susceptibility test.

The stock solution of the crude *Ocimum* oil in Chloroform (20mg/ml) and test discs were prepared from Whatman filter paper[19].

A 0.5 McFarland standard was prepared as described in National Committee of Clinical Laboratory Standards (NCCLS)[20,21]. One percent V/V solution of sulfuric acid and 1.175% W/V solution of barium chloride were prepared and made it turbidity standard. A small volume of this turbid solution was transferred to a screw capped tube and vigorously shaken on a mechanical vortex mixer to have a uniform turbid appearance and stored in the dark at room temperature.

Purely cultured Mueller-Hinton agar petridishes were labeled with different names of bacteria. Then 5 ml of sterile Normal Saline Solution (NSS) was pipetted out into a three different sterile screw-cap tubes. These tubes were labeled according to the type and number of bacteria used to test (*E. coli* and *S. aurous)*. To prepare inoculums, 3 well isolated colonies of the same morphological types were selected from an agar plate culture. The top of each colony is touched with a loop, and growth was transferred into a tube containing 5 ml of NSS that corresponds to each bacterium names. These inoculums containing tubes were mixed by using mechanical vortex mixer and their turbidity was compared accurately.
The sterile discs which were prepared by office perforator were inserted in to different concentrations of *Ocimum* oil with stock solution of 20mg/ml. It was impregnated in to negative and positive controls petroleum ether and chloroform, and amoxicillin respectively. After that, discs with different concentrations were placed on the inoculated plates using a pair of sterile forceps. Seven discs were placed on a 90 cm diameter petridish plate and the space between each disc was given as 24 mm gap from center of the disk to the center of petridish. The pressed discs were completely stacked the agar surface, plates were inverted and placed in an incubator at 37 C for 24 hour. After overnight incubation, the diameter of each zone (including the diameter of the disc) were measured and recorded.

Results and Discussion

The composition of essential oil of *Ocimum basilicum* is shown in the table 1. A total of 76.7% was identified. The major components identified were copaene (25.5%), p-menth-2-en-1-ol (7.7%), eugenylacetae (4.8%), bornyl acetate (4.0%), γ – himachalene (3.6%), rosifoliol (3.0%), α –cubebene (2.5%). The class of compounds identified from the oil of *Ocimum basilicum.L.* by the authors contain monoterpenes (6 .2%), sesquiterpenes (34.8%), ketones (1.9%), esters (10.9%) and alcohols (17.8%). These identified constituents in the present study are same as reported in early studies[22-25].

A few of the identified constituents in *Ocimum* oil in our study are un reported. They include rosifoliol (3.0%), isophytol (0.4%our), eugenyl acetate(4.8%), thunbergol (0.6%), farnesyl acetone (0.2%), 1, 2, 3, 4, 5-pentamethyl-1, 3-cyclopentadiene (2.8%), tricyclo [6.3.0.0(3, 7)] undec-1(8)-en-3-ol(0.2%) and uncineol (1.1%). These variations in composition at different regions in chemotypes of sweet basil were reported [26-27].

The crude oil of *Ocimum basilicum* showed considerable antibacterial activity (table 2) against gram positive bacteria (*S.aureus*) than gram negative (*E.coli*) bacteria.

Conclusion

The major compounds of the oil from *Ocimum basilicum L*, from Ethiopia are *copaene (25.5%), p-menth-2-en-1-ol (7.7%), eugenylacetae (4.8%), bornyl acetate (4.0%), γ – himachalene (3.6%), rosifoliol (3.0%) and α –cubebene(2.5%)* showed significant anti bacterial activity against gram positive (Staphylococcus auerus).

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Table-1
Chemical compositions of the essential oil of *Ocimum basilicum.L*

S. No	Retention Time (R_T)	Identified compounds	Percentage composition (%)	Method of identification
1.	7.09	3-Carene	1.6	GC, GC-MS
2	10.992	Ocimene	0.7	GC,G-MS
3.	11.775	1,2,3,4,5 pentamethyl-1,3-Cyclopentadiene	2.8	GC,GC-MS
4.	12.065	P-menth-2-en-1-ol	7.7	GC,GC-MS
5.	14.657	6-methyl-5-(1-methylethylidene)-6,8-Nonadien-2-one,	0.9	GC,GC-MS
6.	16.188	3,6-dimethyl-4,5,6,7-tetrahydrobenzofuran	1.7	GC,GC-MS
7.	19.944	1-ethenyl-1-methyl-2-(1-methylethenyl)- 4- (1-methylethylidene) cyclohexane	0.6	GC,GC-MS
8.	21.426	1, 7, 7-trimethlbicyclo [2.2.1] heptan-2-ol	0.7	GC,GC-MS
9.	21.432	Bornyl acetate	4.0	GC,GC-MS
10.	22.284	4-methyl-1-(1-methylethyl) Cyclohexene	0.4	GC,GC-MS
11.	22.830	γ – Elemene	0.6	GC,GC-MS
12.	22.926	1-ethenyl-1-methyl-2-(1-methyl) Cyclohexane	0.7	GC,GC-MS
13.	24.021	α -Cubebene	2.5	GC,GC-MS
14.	24.805	Eugenyl acetate	4.8	GC,GC-MS
15.	24.785	Un identified	2.2	GC,GC-MS
16.	25.305	Copaene	25.5	GC,GC-MS
17.	25.911	1, 4-dimethyl-7-(prop-1-en-2-yl)-1, 2, 3, 4, 5, 6,7,8-octahydroazulene	2.1	GC,GC-MS
18.	26.594	2, 4, 5, 6, 7, 8- Hexahydro- 1, 4, 9, 9 -tetramethyl -3H-3a, 7-Methanoazulene	1.9	GC,GC-MS
19.	28.955	2,2,4,8 Tetramethyltricyclo [5.3.1.0(4,11)]undecan-7-ol	3.7	GC,GC-MS
20.	34.260	γ – Himachalene	3.6	GC,GC-MS
21.	34.266	Un identified	3.3	GC,GC-MS
22.	34.925	9.β-Acetoxy-3.β.-hydroxy-3,5.α,8 trimethyltricyclo [6.3.1.0(1, 5)]dodecane	2.8	GC,GC-MS
23.	37.899	Unidentified	0.3	GC,GC-MS
24.	38.071	Unidentified	1.1	GC,GC-MS
25.	39.089	Un identified	0.3	GC,GC-MS
26.	40.036	Isoeugenyl acetate	2.1	GC,GC-MS
27.	40.543	Uncineol	1.1	GC,GC-MS
28.	40.874	β-Eudesmol	0.5	GC,GC-MS
29.	42.290	Rosifoliol	3.0	GC,GC-MS
30.	42.847	6, 10, 14-trimethyl- 2-Pentadecanone.	0.7	GC,GC-MS
31.	44.150	Tricyclo[6.3.0.0(3,7)]undec-1(8)-en-3-ol	0.2	
32.	44.553	Unidentified	0.2	GC,GC-MS
33.	45.120	Farnesylacetone	0.2	GC,GC-MS
34.	46.212	Isophytol	0.4	GC,GC-MS
35.	46.573	Un identified	0.5	GC,GC-MS
36.	48.672	Thunbergol	0.6	GC,GC-MS
37.	49.280	1,5,6,7-Tetramethylbicyclo [3.2.0] hept-6-en-3-one	0.8	GC,GC-MS
38.	50.981	Unidentified	0.6	GC,GC-MS
39.	51.179	Unidentified	0.6	GC,GC-MS
Total Identified Compounds		30 compounds	76.7	GC,GC-MS
Total unidentified or unknown compounds			9.1	GC,GC-MS

Table-2
In vitro* antibacterial activity of crude essential oil of *Ocimum basilicum.L

S.No	Test Organisms		Zone of inhibitions(mm)						
			Concentrations of *Ocimum* oil (µg/ml)					Negative control	Positive control
			10	20	40	80	St.	Chloroform	AM (30µg/disk)
1.	*Gram Positive*	*S.aureus*	1.04 ± 0.099	1.467 ± 0.085	1.00 ± 0.082	1.003 ± 0.148	1.133 ± 0.169	-	13.084 ± 0.282
2.	*Gram negative*	*E.coli*	0.833 ± 0.062	0.817 ± 0.070	0.803 ± 0.078	1.183 ± 0.178	0.950 ± 0.071	-	7.981 ± 0.428

All the values are given as mean ±STD which were analyzed in triplicate, St: - Stock solution, -:- Has no activity, AM: - Amoxicillin, *S.aureus:- Staphylococcus aureus, E.coli:- Escherichia coli*.

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