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Chemical composition and antibacterial activity of the essential oil from the leaves of *Eucalyptus Globulus* collected from Haramaya University, Ethiopia

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ABSTRACT

Eucalyptus globulus, a plant from family myrtaceae, commonly known as blue gum, grows well in different parts of world and has been known since decades because of its rich ethanomedicinal and therapetic importance. Various phytochemicals isolated from the plant has been well known to possess various pharmacological effects. The aim of this study is to determine the chemical composition and antimicrobial properties of the essential oils of Eucalyptus globulus grown in Haramaya university main campus, Ethiopia. A total of twenty one compounds were identified from the essential oil, by Gas chromatography mass spectroscopy representing 99.85 % of the total oil.The dominant compounds being Eucalyptol (1,8-cineole) 62.38%, α- pinene 23.79% ,α-terpinyl acetate 5.41%, globulol 1.68%, β-pinene 1.1% . The results of the antimicrobial activity tests revealed that the essential oil of E. globulus has antimicrobial activity, especially against Staphylococcus aureus, and Staphylococcus epidermidis. Minimum inhibitory concentration (MIC) for the oil ranged from 0.72 to 2.75 µl/ml.

Key words: *Eucalyptus globules*,chemical composition, antibacterial activity.

INTRODUCTION

Plants have been used as a source of medicine in Ethiopia from time immemorial to treat different ailments. Due to its long history, traditional medicine has in fact become an integral part of the culture[1]. It is not unusual for people living in the countryside to treat some common ailments using plants available around them (e.g. *Hagenia abyssinica* (Bruce)J.F. Gmel. to expel tapeworm). Dawit Abebe and Ahadu Ayehu in 1993 [2] reported that 80% of the Ethiopian population depends on traditional medicine for their health care. More than 95% of traditional medical preparations are of plant origin[3]. Ethiopian people use different parts of plants for treatment of various diseases. Essential oil from leave of Eucalyptus plant is one of them. There are many species of Eucalyptus such as *E. camaldulensiq E. citriodora, E. daltympleana, E. dean E. delegatensis, E. globulus, E. orandis, E. nitens, E. saliana, E. teretiwrnis, E.urophylla and E. viminali* growing in Ethiopia[4].*E. globulus* the "white eucalypt" locally known as *netch buhrzaf* one of them. It has been cultivated for medicinal oil production in many parts of the world.Numbers of reports are available all around the world which shows the antibacterial[5-7] and antioxidant[8-11] of *Eucalyptus* Leaves.

The chemical profile of essential oils varies in the number of molecules, stereochemical properties of molecules, and also depends on the type of extraction. The extraction products may vary in quality, quantity and in composition according to climate, soil composition, plant organ, age and vegetative cycle stage[12-13]. Essential oils or some of their constituents are indeed effective against a large variety of organisms including bacteria and viruses[14] ,

fungi[15] and protozoa[16] .There are various studies available on chemical composition and antimicrobial activity of essential oil of Eucalyptus of Ethiopian origin[17-21] but to the best of my knowledge, no report is available on the chemical constituents and antibacterial activity of the *Eucalyptus globulus* leave essential oil grown in Haramaya university Ethiopia.

MATERIALS AND METHODS

Plant Material :
Leaves of *Eucalyptus globulus* were collected from main campus of Haramaya University, Ethiopia in September, 2013. The species were identified in Plant Science Department, Haramaya University, Ethiopia.

Description of the study area
Haramaya University is located at a latitude of 9°20' north of the equator and 42°03' longitude east of meridian. The university has a total area of about 46 km^2. It has a moderate average temperature of 16 °C, and the mean maximum and minimum annual temperature is 24.°2 and 9.73 °C, respectively [22].The mean annual rainfall is 780 mm. The 1980 m elevation of the area (Weinadega) ensures that it enjoys a relatively moderate and pleasant climate throughout the year. There were 12 beehives in the university. Among these 5 hives are traditional and 7 of them are modern beehives. *Eucalyptus globlus*, *Eucalyptus camnadulesis* (exotic), *Vernonia amygdalina* (indigenous), *Spathodea nilotica* (exotic), *Jacaranda mimosifolia* (exotic), *Pinus radiate* (exotic), *Olea africana*, *Cordial africana* and *Grevillea robusta* are dominant plants and vegetations in Haramaya university main campus[23] .

Extraction of essential oil: Freshly collected 100 g leaves were weighed and hydrodistilled for four hours for complete extraction of essential oil, using a commercial Clevenger-type apparatus. The oil sample obtained from hydrodistillation was freed from moisture by adding anhydrous sodium sulfate and an absolute oil sample was obtained.

Percentage Yield of Oil: **T**he amount of extracted oil was determined and yield% of the extracted oil from each sample on the basis of various eucalyptus leaves samples by using following formula:

$$\textit{\% age yield of oil} = \frac{\text{Weight of oil } x100}{\textit{Weight of Eucalyptus leaves}}$$

Microbial Strains: The antimicrobial activity of essential oil was determined against two Gram-postive and two Gram-negative bacteria. Two Gram-postive strains were *staphylococcus aureus*(MTCC 3160), *staphylococcus epidermidis*(MTCC 435) and two Gram-negative strain were *pseudomonas aeruginosa* (MTCC 7453) , *Klebsiella pneumonia*(MTCC 4030).The bacteria were procured from Institute of Microbiology Technology(IMTECH) Chandigarh, India.

Determination of Antimicrobial activity and Minimum Inhibitory Concentration (MIC).
Agar well diffusion method was carried out by allowing perforation of various oils dissolved in 10% DMSO. Petriplates containing 30 ml nutrient agar medium were kept for the solidification beforeinoculating the microorganism, desired numbers of wells of uniform diameter of 8mm were made after solidification, using sterile aluminum borer. 0.1 ml of each oil samples were poured into wells. After incubation for 24 hrs at 37 0C the plates were observed and the antibacterial activity was evaluated by measuring zone of inhibition (diameter mm). The tests were conducted in triplicate. Ciprofloxacin (10.0 µg/ml) was used as positive control. The negative control was 10% DMSO.

MIC of oils was determined by micro dilution technique as described by the National Committeefor Clinical Laboratories standards (NCCLS)[24]. The bacteria inoculums were prepared in 5 ml nutrient broth and incubated at 370C. The final inoculums were of approximately 106 CFU/ml (0.5 McFarland)[25]. Controls with 0.5 ml of culture medium without the samples and other without microorganisms were used in the tests. Tubes were incubated at 370C for 24 h.

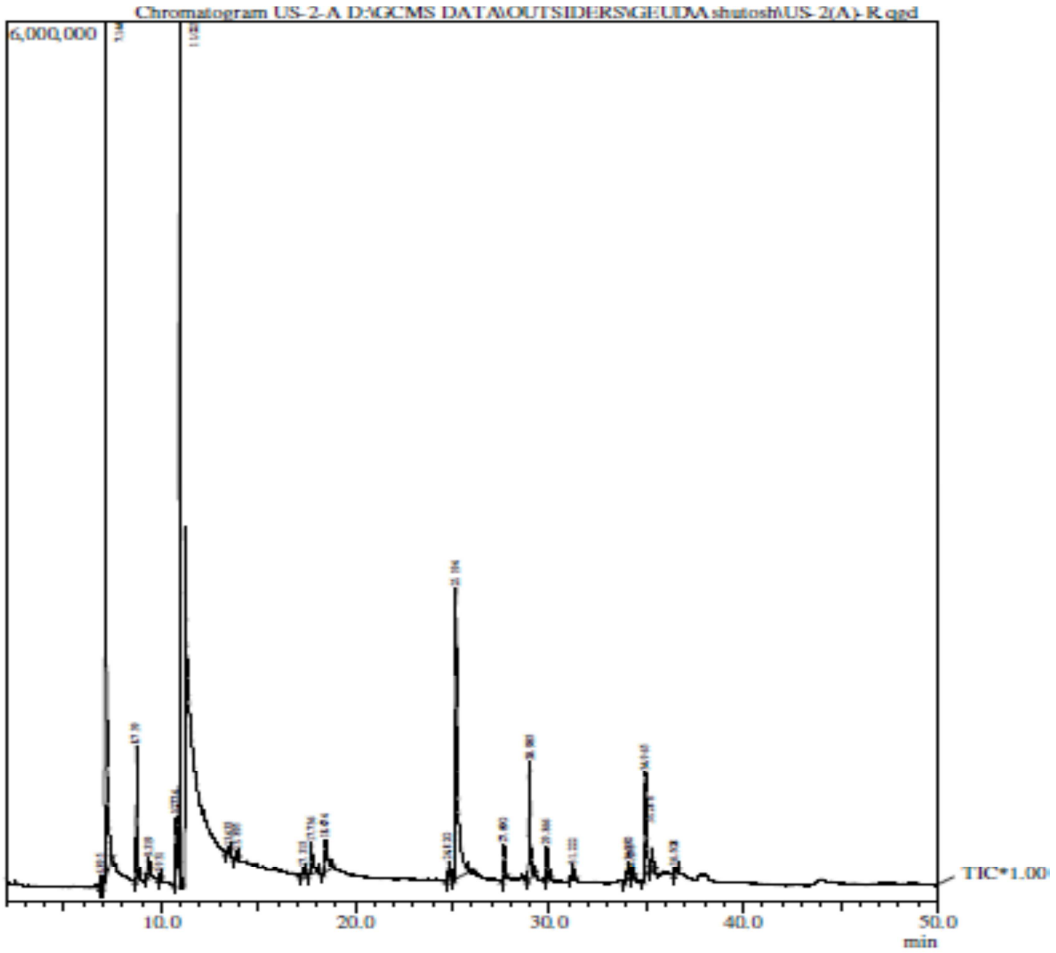

Figure 1. GC-MS chromatogram of *Eucalyptus Globulus* essential oil

Table 1.Chemical composition of *Eucalyptus Globulus* essential oil

Peak#	Retention time	%	Name of compound
1	6.895	0.07	alpha Thujene
2	7.144	23.79	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl
3	8.739	1.01	Beta.-pinene
4	9.319	0.17	1,6-octadiene, 7-methyl-3-methylene
5	9.931	0.05	alpha Phellandrene
6	10.774	0.57	Cymene <para->
7	11.023	62.38	Eucalyptol (1,8-cineole)
8	13.895	0.07	3-Oxatricyclo[4.1.1.0(2,4)]octane, 2,7,7-trimethyl-
9	17.315	0.09	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo-
10	17.736	0.59	1-isopropyl-4-methyl-3-cyclohexen-1-ol
11	18.474	0.70	Terpineol <alpha->
12	24.820	0.23	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate
13	25.194	5.41	alpha Terpinyl acetate
14	27.690	0.35	alpha Gurjunene
15	28.985	1.53	1h-cycloprop[e]azulene, decahydro-1,1,7-trimethyl
16	29.866	0.41	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene
17	31.222	0.12	Viridiflorene
18	34.050	0.21	1,1,4,7-tetramethyldecahydro-1h-cycloprop
19	34.965	1.68	Globulol
20	35.286	0.34	Veridiflorol
21	36.508	0.10	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl

GC-MS Analysis of Essential oil

The essential oil from leaves of *Eucalyptus globulus* was analyzed on GC-MS QP-2010 Plus (ShimadzuCompany) 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 stationary. The syringe was washed with 8 µL of chloroform and 2 µL essential oil solution in chloroform was injected through autosampler and analyzed with HP5 MS column.Column temperature was programmed as follows: 50 to 120 °C at 20, 120 to 150 °C at 4 °C/min, 150 to 250 °C at 20 oC/min (10 min hold time) and 3.5 min solvent delay. The temperature of the injector was fixed to 260 °C and 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 . The percentage of each constituent in the oil was determined based on GC peak areas. The constituents of 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 in the literature.

RESULTS AND DISCUSSION

Hydrodistillation of the *E. globulus* leaves yielded 1.21% of essential oil (w/w, based on the fresh weight of the mature leaves). The results obtained are similar to those reported in the literature for Ethiopian *E. globulus* where the yield was 0.8 – 2.0 % [21] There are many literature reported for *E. globulus* where the essential oil yield was 1.9–2.7% (w/w, based on the fresh weight of the young leaves) in Morocco[26], 2.68% (w/w, based on the fresh weight of the adult leaves) in Argentina[27] and1.05% in India[5].

The chemical composition of the hydrodistilled *E. globulus* essential oil is shown in Table 1. GC-MS analyses revealed the presence of twenty one compounds representing 99.85 % of the total oil. The dominant compounds being Eucalyptol (1,8-cineole) 62.38%, α pinene 23.79% ,α terpinyl acetate 5.41%, globulol 1.68%, β pinene 1.1% . Eucalyptol (1-8 cineole) is a cyclic ether with empirical formula $C_{10}H_{18}O$ and systematic name 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane. It is sometimes traded commercially as "eucalyptol".Cineole determines the commercial value of the oil and its importance as a raw material for different industries. Different percentages of 1,8-cineole in *E. globules* leaf oil have been reported: 64.5% in Uruguay, 77% in Cuba, 86.7% in California, 58% to 82% in Morocco, 48.7% in Africa, and 50% to 65% in Argentina [27].

Other compounds identified in the oil obtained (as shown in table1)were, 1,6-octadiene, 7-methyl-3-methylene 0.17%,Thujene alpha 0.07%, Phellandrene alpha 0.05%,Cymene <para0.57%, 3-Oxatricyclo[4.1.1.0(2,4)]octane 2,7,7-trimethyl 0.07%, Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo 0.09%, 1-isopropyl-4-methyl-3-cyclohexen-1-ol 0.59%,2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate 0.23%,Terpineol alpha 0.07%, Terpinyl acetate alpha 0.70%,Gurjunene alpha 0.35%, 1h-cycloprop[e]azulene, decahydro-1,1,7-trimethyl 0.41%, 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene 0.21%, Viridiflorene 0.12% 1,1,4,7-tetramethyldecahydro-1h-cycloprop Veridiflorol 034% 2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl 0.01%

Antibacterial activity: The result of antibacterial activity of essential oil are shown in the table 2. Maximum antibacterial activity is shown against *Staphylococcus aureus* (28 ±0.4) while minimum antibacterial activity is shown against *Pseudomonas aeruginosa* (23±0.03).Various reports are there which show the almost similar result on the antimicrobial activity of essential oil of *E. globules* [5, and 28-30].Minimum inhibitory concentration(MIC) for the oil ranged from 0.72 to 2.75 µl/ml.as shown in Table 3.

Table 2 Antibacterial activity of *E. globules* essential oil

S.No	Microorganism	Inhibition zone(mm)	ciprofloxacin	(-)control
1	*Staphylococcus aureus*	28±0.4	27±0.1	---
2	*Staphylococcus epidermidis*	27.5±0.1	21±0.5	---
3	*pseudomonas aeruginosa*	23±0.3	32±0.1	---
4	*Klebsiella pneumonia*	24.5±0.1	23±0.2	---

Table 3 Minimum Inhibitory Concentration (µl/ml)of *E. globules* essential oil

S.No	Microorganism	Concentration (µl/ml)	ciprofloxacin
1	*Staphylococcus aureus(3160)*	0.74	1.15
2	*Staphylococcus epidermidis(435)*	0.72	0.30
3	*pseudomonas aeruginosa(7453)*	1.50	0.60
4	*Klebsiella pneumonia(4030)*	2.75	1.20

The antimicrobial activity of *E. globulus* essential oil is due to the presence of a mixture of monoterpenes and oxygenated monoterpenes.[31].In this study percentage of oxygenated monoterpenes Eucalyptol (1,8-cineole) 62.38 is high which is responsible for antibacterial activity.Essential oils are potential sources of novel antimicrobial compounds [32] especially against bacterial pathogens. An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable [33- 34]. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death [35].

CONCLUSION

This study has shown that essential oil of *Eucalyptus globulus* grown in Haramaya university Ethiopia possesses rather a significant activity against different microorganisms, These results confirm the potential use of *E. globulus* essential oil in different industries such as food industries and pharmaceutical industries., Also the essential oil of *Eucalyptus globulus* may be useful as an alternative antimicrobial agent in natural medicine for the treatment of numerous infectious diseases.

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