Chemical Profile and Antimicrobial Activity of Essential Oil of *Eucalyptus globulus* Leaves from Haramaya Campus and Entoto Park, Ethiopia

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Abstract:

Eucalyptus globulus trees are available abundantly in the highland part of Ethiopia and traditionally used for the treatment of various ailments including influenza, common cold, warts, febrile illness, and headache. In view of its traditional uses, an attempt was made to explore the chemical profile and antibacterial studies of the leaves of *Eucalyptus globulus* from two district area. The chemical composition of essential oil obtained by steam distillation extraction from the leaves of *Eucalyptus globulus* was analyzed by GC–MS. The extraction yields of essential oils of the leaves of *E. globulus* collected from Haramaya Campus, and Entoto Park were 0.23% and 0.24%, respectively. Twenty-one compounds, constituting about 99.20% of the total oil, were identified from the Haramaya University Campus, whereas eighteen compounds, constituting about 98.03% of the total oil, were identified from Entoto Park. The main constituents identified in both areas were 1, 8-cineole (68.25-72.10%), cis-sabinol (12.10-14.31%), Limonene (2.35-2.96%), α-pinene (2.15-2.50%) and α-terpineol (1.62-2.13. The antimicrobial activity of the oil was evaluated against four microorganisms using paper disk diffusion methods. Essential oils extract of *Eucalyptus globulus* leaves from Haramaya Campus showed comparatively less antibacterial activity than Entoto Park.

Keywords: Essential oil, *Eucalyptus globulus*, GC-MS, 1, 8-cineole, antibacterial activities

1. Introduction

In recent decades, the demand for plant derived products for therapeutic uses has been increased (Hermann & von Richter, 2012). In many countries worldwide aromatic herbs are used in primary health care, especially in rural areas (Kamatou et al., 2005), and 80% of the populations in developing countries use these traditional resources (Begossi, 1996). For this reason, the use of essential oils extracted from plants for clinical purposes have become an important topic in scientific research and industrial application thanks to the different biological activities of oils, which exercise antimicrobial (Lo, Shanmugaiah, & Iacobellis, 2009), antioxidant (Dutra, Leite, & Barbosa, 2008) and anti-inflammatory (Chao et al., 2005) activities It is not unusual for people living in the countryside to treat some common ailments using plants available around them. Dawit Abebe and Ahadu Ayehu in 1993 (Abebe & Ayehu, 1993) 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 (Abebe, 1986). Ethiopian people use different parts of plants for treatment of various diseases such as influenza (Alemayehu, Asfaw, & Kelbessa, 2015; Atnafu, Awas, Alemu, & Wube, 2018), common cold (Megersa, Asfaw, Kelbessa, Beyene, & Woldeab, 2013; Meragiaw, Asfaw, & Argaw, 2016), warts (Enyew, Asfaw, Kelbessa, & Nagappan, 2014), febrile illness (michi) (Araya, Abera, & Giday, 2015; Chekole, Asfaw, & Kelbessa, 2015; Kassa, Asfaw, & Demissew, 2016), itch (Kebebew & Mohamed, 2017) and headache (Meragiaw et al., 2016). 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 (Dagne, Bisrat, Alemayehu, & Worku, 2000).

E. globulus the "white eucalypt" locally known as "nech bahrzaf" one of them. It has been cultivated for medicinal oil production in many parts of the world. *Eucalyptus globulus* is an extremely adaptable species which grows well on a variety of soils and in a variety of climates. In Ethiopia it succeeds everywhere in the highlands, and does best at altitudes from 1800-2600m on loamy soils. *Eucalyptus* is known to be a rich source of secondary compounds with a variety of biological activities (Guo & Yang, 2006). The essential oil of *Eucalyptus globulus* contains 1,8-cineole as its major constituent that varies between 70-95%, however, some studies have shown as little as 4% in its oil (Iqbal, Bhatti, Ahmad, & Chatha, 2006; Whitman & Ghazizadeh, 1994). The use of *eucalyptus*

oils, mostly obtained from the leaves, is grouped as medicinal, industrial, aromatic and flavoring, depending on their chemical composition (Li, Madden, & Potts, 1996; Ogunwande, Olawore, Adeleke, & Ekundayo, 2005; Sartorelli, Marquioreto, Amaral-Baroli, Lima, & Moreno, 2007). Numbers of reports are available all around the world which shows the antibacterial (Bachheti, Joshi, & Singh, 2011; Gilles, Zhao, An, & Agboola, 2010) and antioxidant (Bachheti, 2015; Barra, Coroneo, Dessi, Cabras, & Angioni, 2010; Marzoug et al., 2011), antiseptic agent (Song, Wang, & Liu, 2009) of *Eucalyptus* Leaves. Essential oils of *E. globulus*are constituted mainly by the monoterpenes 1, 8-cineole (eucalyptol, 60%), α-pinene (30%) and D-limonene (5%) and the sesquiterpene aromadendrene (Ammon, Barton, Clarke, & Tjandra, 1985). *Eucalyptol* has antifungal, antibacterial and antiviral activity in vitro (Morcia, Malnati, & Terzi, 2012). In addition, eucalyptol and aromadendrene have synergistic effects in regard to antioxidant and antimicrobial properties (Mulyaningsih, Sporer, Zimmermann, Reichling, & Wink, 2010). Moreover, it has been determined that *E. globulus* leaves contain several polyphenolic compounds such as ellagic acid, gallic acid, caffeic acid, chlorogenic acid, luteolin, rutinand quercetin in free or conjugated forms which have antioxidant and antimicrobial properties (Almeida et al., 2009).

Essential oils or some of their constituents are indeed effective against a large variety of organisms including bacteria and viruses (Duschatzky et al., 2005), fungi (Hammer, Carson, & Riley, 2002) and protozoa (Monzote et al., 2006). 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 (Masotti, Juteau, Bessière, & Viano, 2003). Therefore this study was designed to characterize the chemical profile of essential oils of *E. globulus* from two district area of Ethiopia and evaluate their antimicrobial activities in vitro.

2. Materials and Methods

2.1. Description of the Study Area

Medicinal plants (Fresh leaves of *Eucalyptus globulus*) used in this study were collected from Haramaya University (Eastern Ethiopia) is located 5km from Alemaya a town in the East Hararghe Zone, 17 km from the city of Harar and 40km from Dire Dawa at altitude range from 1400 to 2340 meters above sea level. It has mean annual rainfall and temperature of 1372 and 19ºC respectively and Enteto Natural Park (Central Ethiopia) is the highest peak north eastern rim city of Addis Ababa the capital of Ethiopia, slope of mountain. It reaches 3,200 meters above sea level. Its annual average rainfall and temperature are 1200mm and 14ºC respectively. It is situated at an altitude of between 2,600 and 3100 meters.

2.2. Chemicals and Reagents

The following chemicals, solvents, and drugs were used: Chloroform ($CHCl_3$), anhydrous Na_2SO4, dimethylsulfoxide (DMSO), Streptomycin 10, 20 µg, Sodium chloride (NaCl), Muller Hinton Agar (MHA), and Nutrient Agar was obtained from the Department of Pathology, Haramaya University.

2.3. Bacteria Pathogens

The bacterial test microorganisms used in this investigation were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Proteus mirabilis* which were donated by the Ethiopian Public Health Institute (EPHI).

2.4. Preparation of Essential Oil

One kilo gram of the leaves of *Eucalyptus globulus* from Haramaya Campus and Entoto area were collected. The collecting leaves were packed in plastic bags to reduce volatility from environmental factors (physical factors) until the experiment was conducted.

Figure 1: Leaves of *E.globulus* (locally Nech bahrzaf) from Entoto Park and Haramaya Campus

2.4.1. Steam-Distillation Essential Oil Extraction

A 500 g of the fresh leaf of *E. globulus* was mixed with 300 mL of distilled water in 1 L distillation flask and steam distilled using an apparatus of Clevenger type for 3 h. 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 essential oil was separated from aqueous portion by extracting 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 rotary evaporator at 40 °C under reduced pressure distillation. The collected oil was dried over anhydrous Na_2SO_4 in order to remove water traces and 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 Ambo University, Chemistry Department research laboratory.

2.5. GC-MS Analysis of Essential oil

The essential oil from leaves of *E. globulus* 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 µL 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 solutions in chloroform was injected through auto sampler and analyzed with HP5 MS column. Column temperature was programmed as follows: 50 to 120 °C at 204 °C/min, 120 to 150 °C at 4 °C/min, 150 to 250 °C at 20°C/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.

2.6. Antibacterial Activity of Essential Oil

The essential oil extracted by steam distillation was evaluated *in vitro* for antimicrobial activity by using the paper disc diffusion method against the selected three intestinal bacterial species included *Staphylococcus aureus*, *Pseudomonas aeruginosa Proteus mirabilis* and *Streptococcus pyogenes*. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA) and incubated at 37°C. The antimicrobial activity test was conducted plant pathology laboratory school of plant sciences, Haramaya University. Streptomycin was used as standard drug against the selected bacteria. Streptomycin and DMSO were used as a positive and negative control respectively. The percentage yield of the essential oil was determined gravimetrically using the volume of the extract (x) and the weight of wet leaves sample material (y) as follows

$$percentage\ yield = \frac{x}{y}\ X\ 100$$

2.6.1. Media Preparation

Thirty nine grams of Mueller-Hinton agar was suspended in 1000 ml of sterile distilled water and then sterilized by autoclaving at 15 Lbs pressure (120°C) for 15 minutes. Immediately after autoclaving, the medium was allowed to cool in a 48 to 50°C water bath and poured aseptically into a sterile Petri dish in the safety biological hood at room temperature until it solidifies.

2.6.2. Preparation of Inoculums for Antimicrobial Activity Test

Four well isolated bacterial strains were transferred from the stock cultures and streaked on MHA plates and incubated for 24 h at 37°C oven. Well separated bacterial colonies of *S. aureus*, *P. aeruginosa*, *Proteus mirabilis* and *S. pyogenes*, were then used as inoculums. The MHA media was autoclaved at 121°C and 1.03 bars for 15 minute in order to 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 dishes plates, allowed to solidify and used for the bioassay test.

2.6.3. Determination of Antibacterial Activities

The antibacterial activities of the essential oil plant extracts were tested using disc diffusion method. The dried surface of a Mueller-Hinton agar plate was aseptically inoculated with bacterial suspension by swabbing over the entire agar surface in the safety hood. This procedure was repeated by swabbing two more times, rotating the plate approximately 90° and 60° each time to ensure an even distribution of inoculums. As final step the edge of the agar was swabbed. After inoculation, the lids of the plates were left partly open for 3-5 minutes to allow surface moisture to be absorbed before applying the respective plant extracts impregnated discs, sterile distilled water and standard drug.

2.6.3.1. Application of antimicrobial agent, sterile distilled water and test drug impregnated discs onto inoculated agar plates

Filter paper discs of 0.6 mm diameter placed in a beaker were sterilized in an oven at 180 °C for 1 h. Then10 and 20 µL of the solutions of the essential oil extract (antimicrobial agent), standard drug (streptomycin) and distilled water were added to the discs surface in three replications by using a micropipette. All discs used for predetermined sets of antimicrobial agents and distilled water as well as antibiotics impregnated discs were applied onto the surface of the inoculated Mueller-Hinton agar plates using sterile forceps. Each disc was pressed down individually to ensure complete contact with the agar surface. 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 commercial drugs.

2.6.3.2. Reading plates and interpreting results

After 24 hours of incubation, each plate was examined for zone of inhibition. The diameters of the zone of complete inhibition (judged by the unaided eye) including the diameters of the disc were measured to the nearest whole millimeter using a caliper. The zone margin was taken as the area showing no obvious visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with magnifying lens of colony counter at the edge of the zone of inhibition, was ignored. The experiment was

repeated three times and the results were expressed as mean values of zone of bacterial growth inhibition by each essential oil plant extract.

2.7. Data Analysis

Data were analyzed using computer software SPSS. Results of the study were expressed as mean ± standard error (M ± SEM). Statistical significant differences values were determined using one way analysis of variance (ANOVA).

3. Results and Discussion

3.1. Yield of Extract

The extraction yields of essential oils of the leaves of *E. globulus* collected from Haramaya Campus and Entoto Park were 0.23% and 0.24%, respectively as shown in Table 1. There are many literature reported for *E. globulus* where the essential oil yield was 0.8 – 2.0 % in Northern Ethiopia (Subramanian, Gebrekidan, & Nigussie, 2012), 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 yield variability in the total essential oil of *E. globulus* species may be attributed to the climatic conditions, ecological, soil conditions, age of plant and the season of harvest.

	Entoto Park (sample material)	Haramaya University (sample material)
Weight of leaves(g)	500	500
Volume of extract oil(ml)	1.19	1.15
Percentage of yield(% ml/g)	0.24	0.23

Table 1. The yield of the essential oils from *Eucalyptus globulus* Entoto Park and Haramaya Campus

3.2. Essential Oil Analysis

The chemical composition of the leaves of *E. globulus* essential oil was shown in (**Table 2, Figure 2 & 3).** GC-MS analyses revealed the presence of twenty one (21) compounds representing 99.20 % collected from Haramaya Campus and eighteen (18) compounds representing 98.03% of the total oil collected from Entoto Park. The essential oil composition collected from Haramaya Campus was revealed 92.37 % and 6. 83% monoterpenes and sesquiterpenes, respectively, whereas the essential oil composition collected from Entoto Park were revealed 93.37 % and 4. 66% monoterpens and sesquiterpenes, respectively. The main constituents of the essential oil in the *E. globulus* leaves collected from Haramaya Campus were 1, 8- cineole (68.25%), cis-Sabinol (14.31%), Limonene (2.35%), α-Terpinolene (1.24%), and α-pinene (2.15%), wereas *E. globulus* leaves collected from Entoto Park were 1, 8- cineole (71.30%), cis-Sabinol (12.10%), Limonene (2.96%), α-Terpinolene (0.90%), and α-pinene (2.50%).

The major identified compounds in these oils were 1,8-cineole in agreement with other observations: 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 (Viturro, Molina, & Heit, 2003), 63.8 to 75.8% I Portugal (Silvestre, Cavaleiro, Delmond, Filliatre, & Bourgeois, 1997), 72.71% in China (Song et al., 2009), 66.28 to 75.36% in Ethiopia (Subramanian et al., 2012). Oxygenated and hydrocarbon monoterpenes had shown the higher composition due to the presence of 1, 8-cineole (71.30%) and cis-sabinol (12.10%). *E. globules* leaves essential oil were contained more oxygenated terpenes derivatives mainly monoterpenes (84.56 -86.20%) compared to monoterpenes hydrocarbons (6.20-8.32%). Moreover among sesquiterpenes, the oxygenated species were also detected in a higher percentage than hydrocarbons in agreement with literature (Akolade et al., 2012).

We thus note significant differences in the chemical composition of *E. globules* leaves essential oil collected from Haramaya Campus and Entoto Park. These qualitative and quantitative differences in the chemical composition of essential oils could be attributed to several factors such as geographical location (Usman et al., 2010) , climatic

effects of the plants, harvest season, nature of the soil, age of the plant parts, the part of the plant used (Emara & Shalaby, 2011) , and time of collection (Marzoug et al., 2011) with consequent influence on biological activities (Saliu, Usman, Sani, Muhammad, & Akolade, 2011).

Table 2. Major Chemical constituents of the leaves essential oil of *Eucalyptus globulus Labill*

S.No	Chemical Components	Plant Sample Collected from			
		Haramaya Campus		Entoto park	
		Retention Time (min)	Composition (%)	Retention Time (min)	Composition (%)
1	Limonene	5.05	2.35	5.01	2.96
2	α-Terpinolene	5.93	1.24	6.53	0.90
3	β-Phellandrene	6.15	1.20	6.91	1.51
4	Camphene	6.20	0.76	6.95	0.87
5	cis-Sabinol	6.56	14.31	7.18	12.10
6	α-terpinol acetate	6.91	0.56	7.23	0.36
7	α-Pinene	7.07	2.15	7.73	2.50
8	1,8-Cineole	7. 29	68.25	9.27	71.30
9	cis-Carveol	7.55	0.78	9.35	0.48
10	Globulol	7.75	1.33	10.58	1.60
11	Aromadendrene	8.05	0.35	-	-
12	Pulegone	8.70	0.89	10.84	1.00
13	γ-Terpinene	9.27	0.79	11.15	0.37
14	β-pinen	10.31	1.12	12.04	0.42
15	4-terpineol	10.56	1.62	12.06	2.13
16	β-myrcene	10.83	0.34	12.34	0.30
17	α-Fenchene	11.15	0.42	-	-
18	p-Cymen	12.03	0.25	12.44	0.23
19	Spahulenol	12.35	0.24	12.50	0.37
20	Caryophyllene oxide	12.44	0.82	-	-
21	tr-p-Mentha-1,7- 8dien-2-ol	13.24	0.63	12.77	0.14
		Total Content (%)	**99.20**		**98.03**

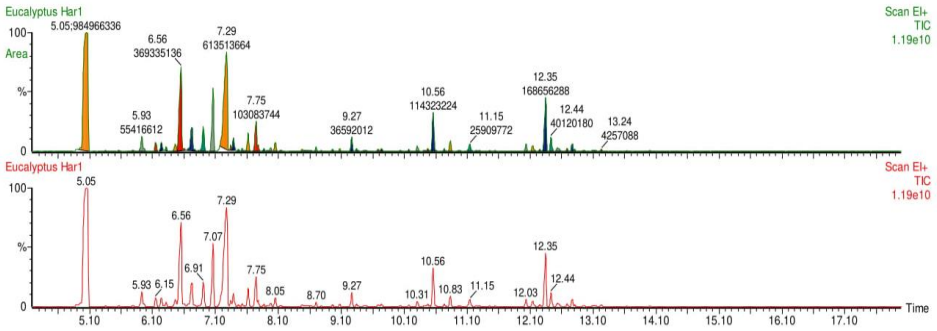

Figure 2: GC-MS Chromatogram of *E. globulus* essential oils collected from Harmaya Campus

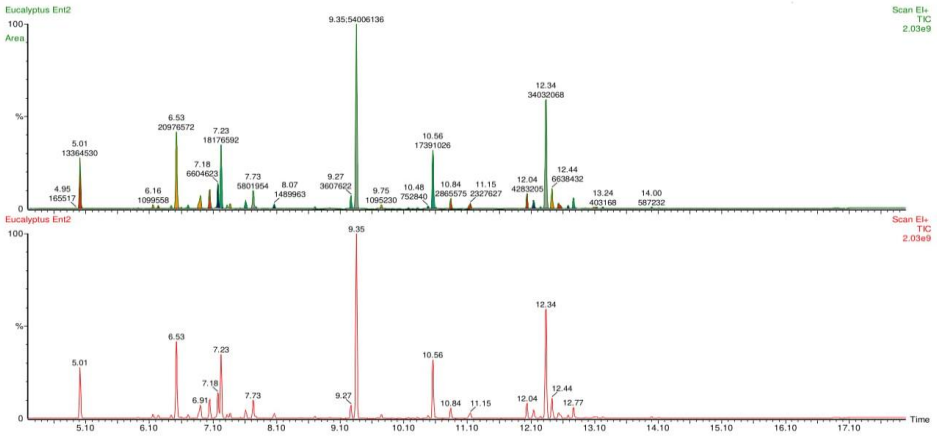

Figure **3:** GC-MS Chromatogram of *E. globulus* essential oils collected from Entoto Park

The medicinal properties of the oil are specified by minimum quantity of constituents, which are defined in the British pharmacopoeia (Pharmacopoeia, 2004) require e*ucalyptus* oil to contain not less than 70% 1,8-cineole and be practically free of phellandrene. Therefore, the oils extracted from all were free of phellandrene, and the 1,8-cineole content of Entoto Park were greater than 70%, therefore, the trees under study except Haramaya Campus could be used for medicinal purpose while the case of Haramaya Compus needs further purification so as to increase the 1,8-cineole content.

3.3. Antibacterial Activity of *Eucalyptus globulus* leaves essential oil extract

The study showed that the essential oil extracts investigated were active against Gram positive and Gram negative bacteria .The antibacterial activities of essential oils extract of *Eucalyptus globulus* leaves were tested by paper disk diffusion method. The essential oils extract of *E. globulus* leaves has exhibited effective antimicrobial activity *P.aeruginosa*, *P.mirabilis*, *S.aureus* and S.*pyogenes*at concentrations of 10 and 20µm/ml. Essential oils of *E.globulus* leaves extract from Entoto Park was produced maximum zone of inhibition (14.43±0.15mm) against *S.pyogenes* while minimum zone of inhibition (6.57±0.26mm) against *P.mirabilis* wereas essential oil extracts of *E.globulus* leaves from Haramaya Campus showed maximum zone of inhibition (14.23±0.11mm) against *S.aureus* while the minimum zone of inhibition (6.47±0.21mm) against *P.aeruginosa*. The inhibition zones obtained were intermediate between those obtained for Gram negative bacteria and Gram positive bacteria. Essential oils extract of *Eucalyptus globulus* leaves from Haramaya Campus showed comparatively less antibacterial activity. Similarly among the gram negative and gram positive group bacteria (*P.aeruginosa*, *P. mirabilis, S. aeruginosa and S. Pyogenes*) was observed less zone of inhibition while essential oils *Eucalyptus globulus* leaves extract from Entoto Park showed high antibacterial activity with Gram negative

and gram positive *(P.aeruginosa*, *P. mirabilis, S. aeruginosa and S. Pyogenes*) was observed with good zones of inhibition (Table 3).

Table 3: In vitro antimicrobial activity of *E.globulus* leaves essential oil extracts and standard antibiotics against test- bacteria.

Compounds	**Zone of inhibition(I)(mm) of Test Microorganisms**							
	Gram(-) Bacteria				**Gram(+) Bacteria**			
	P. aeruginosa		*P. mirabilis*		*S. aurous*		*S. pyogenes*	
	10µg/ml	20µg/ml	10µg/ml	20µg/ml	10µg/ml	20µg/ml	10µg/ml	20µg/ml
EPEO	**7.77±** 029	12.2± 0.84	7.5± 0.28	12.23± 0.16	10.01± 0.10	14.43± 0.15	10.17± 0.40	12.43± 0.69
HCEO	6.47**±** 0.21	11.83± 0.31	6.57± 0.26	11.87± 0.33	10.0± 0.12	14.23± 0.11	9.87± 0.14	12.57± 0.28
SM	10.07± 0.29	15.9± 0.11	9.73± 0.17	15.83± 0.21	13.8± 0.07	17.20± 0.09	13.97± 0.04	16.73± 0.06
DMSO	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Key: P=*Pseudomonas aeruginosa,* S=*Staphylococcus aurous,* P=*proteus mirabilis*,

S: *Streptococcus pyogenes,*Gram (-) = Gram negative, Gram (+) = Gram positive, EPEO=Entoto park essential oil, HCEO= Haramaya campus essential oil, **-** = No inhibition was observed, DMSO= dimethyl sulfoxide as a negative control, SM= streptomycin as a positive control.

The antimicrobial activity of *E. globulus* essential oil is due to the presence of a mixture of monoterpenes and oxygenated monoterpenes (Damjanović-Vratnica, Đakov, Šuković, & Damjanović, 2011). In this study percentage of oxygenated monoterpenes Eucalyptol (1,8-cineole) 65.25 and 71.30 from two different place (Haramaya University campus and Entoto Park) is high which is responsible for antibacterial activity. Essential oils are potential sources of novel antimicrobial compounds (Daroui-Mokaddem et al., 2010) 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 (Knobloch, Weigand, Weis, Schwarm, & Vigenschow, 1986; Mitscher, Drake, Gollapudi, & Okwute, 1987). Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (Sikkema, de Bont, & Poolman, 1994).

4. Conclusions

The result of the present study have revealed that the chemical Profile and antibacterial activity of essential oils of *E. globulus*, varied greatly depending upon different type of geographical location. The main constituent of Haramaya University Campus and Entoto Park *E. globulus* oil are 1, 8-cineole *(Eucalyptol)*, ranging from 68.25%-71.10% and cis-sabinol, ranging from 12.10%-14.31%. The slightly difference in composition might be attributed due to different agro-climatic conditions and soil composition in the region. Twenty one compounds were detected from Haramaya University Campus and eighteen chemical components were identified from Entoto Park *E. globulus* essential oils. The essential oils obtained from Entoto Park could be used for medicinal application and/or pharmaceutical agents but in the case of Haramaya University Campus it needs further purification and enrichment so as to make 1, 8-cineole composition greater than 70%.

The results of the antibacterial activity of essential oils from different area of Ethiopia may be directly associated with their major constituents or the presence of synergy between the major and minor constituents within the essential oil. The relationship evaluated between the chemical composition of the oil and the antibacterial activity was suggested that the presence of oxygenated monoterpene 1, 8- cineole as the principal component

of the essential oil which is very responsible for the potent antibacterial activities. In general, the strong antibacterial activity was not related only to a high composition of major component such as 1, 8-cineole, but also the presence of other moderate and minor chemical constituents of the oil. The Gram (-) bacteria (*P.aeruginosa*and *P.mirabilis*) was the most resistant to the oils in comparison with the Gram (+) bacteria *(S. pyogenes* and *S. aureus)*. Thus, *E. globulus* leaves essential oils chemical components can be used against for selected pathogenic and some microorganisms, and may provide better alternatives or supplements to the conventional antibacterial.

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