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Research Article

Investigation of Essential Oil Composition Variation with Age of *Eucalyptus Globulus* Growing in Ethiopia

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Abstract

Various types of aromatic and medicinal plants are available in Ethiopia from which the essential oil can be extracted for different purpose. *Eucalyptus* plant is most common among them. Despite its availability the variation of essential oils of *Eucalyptus globulus* with ages, the chemical constituents of *Eucalyptus globulus* essential oil of Ankober origin has not previously been examined. Thus, the aim of this study is to assess the variation of essential oil with ages in *Eucalyptus globulus* tree grown at Ankober district, Ethiopia. Most part of Ankober is covered by *Eucalyptus globulus*, locally known as 'Nech-Baharzaf'. Essential *Eucalyptus* leaves having different ages were collected and subjected to steam distillation to determine the quality and quantity of essential oil (main components of the oils). *Eucalyptus globulus* leaves aged 3, 5, 8, 12, 20, 35 and 100 were compared. All the leaves were collected from the same agro-ecological zone and in the same season to avoid oil content variation with climate change and agroecology. Chemical composition of the essential oils was analyzed by GC/MS and alpha-Pinene, d-limonene and cineol were the main constituents. The highest amount of eucalyptol (73.90%) presented in plant aged 100 years whereas alpha-Pinene (20.05%) and d-limonene (6.17%) detected in essential oil of plants aged three. The main constituent of the oil is cineole, ranging from 61.04%-73.90% which might suggest age variation has significant impact on essential oil content composition.

Keywords:

Eucalyptus globulus; Essential oil; Hydro-distillation; 1, 8-cineol; Age variation

INTRODUCTION

The genus *Eucalyptus* from Myrtaceae family, originally from Australia, has been distributed all over the world due to its easy adaptability, ease of cultivation, tolerance to a wide range of environmental conditions and fast growth. The *Eucalyptus* consists of approximately 900 species [1-3]. Reports indicate that more than 300 species of this genus are naturally rich in essential oil content in their leaves. Few of these species are known for their high content of 1, 8-cineole (more than 70%). Hydrocarbons, aromatic derivatives, terpenes and their oxygenated derivatives such as mono- and sesqui-terpenoids, alcohols and esters are the basic components found in essential oils [4,5]. The essential oil extracted from the leaves of *Eucalyptus* has been widely used in various sectors including in pharmaceutical, cosmetics, and food industries based on their compositions [6-8]. Cineole is the most important chemical constituent that determines the medicinal value of *Eucalyptus* oil [9]. According to British and European pharmacopeia the oil should contain a minimum of 70% 1,8-cineole to be considered as pharmaceutical grade *Eucalyptus* oil [10-12]. Currently there is growing tendency to use natural products as ingredients. Essential oil is one of the natural products that is gaining significant attention to be used as ingredients in drinks, foods, cosmetics and toiletries [13,14]. As a result, the scientific interest in this area is continuously increasing since demand of customers for essential oils are increasing for different applications. Many countries have approved extracts of *Eucalyptus* leaves as food additives [15]. For instance, United States Food and Drug Authority (FDA) have categorized the essential oil from *Eucalyptus* as safe and non-toxic. Europe has

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accepted the *Eucalyptus* essences as aromatizers in foods [16]. Likewise, Japan has listed the extract of *Eucalyptus globulus* as Food Additives due to its antioxidant nature [17]. It is common to observe vaporizing *Eucalyptus* oil in saunas with the assumption that it removes muscular pains through massaging with the oils, and also it has been widely used for many years by sportsmen for different purposes.

In different countries including Ethiopia, the extracts of *Eucalyptus* have been used traditionally to treat a wide range of ailments. For instance, hot water extracts of dried and fresh leaves of *Eucalyptus globulus* are used as analgesic, anti-inflammatory and antipyretic remedies for common cold, flu and sinus infection since it is a unique natural products having antiseptic properties and the ability to clear the nasal passages and bronchial tubes making it easier to breathe. They also boil the *Eucalyptus* leaves in water and inhale the vapor coming out of it to get relief especially from common cold. Numerous investigations have shown that the essential oils of *Eucalyptus* species possess important biological activities including diaphoretic, disinfectant, antimalarial, antiseptic, analgesic, anti-inflammatory, antibacterial and antioxidant properties [18-21]. And hence traditionally it has been widely employed as effective alternatives or complements to synthetic drugs. With the rising significance in the use of EO in both food and pharmaceutical industries, a systematic assessment of the phytochemical extracts has become increasingly essential [22-24]. Today the essential oil from *Eucalyptus* is marketed in the world in different forms (with other ingredients). For instance, the product named "Vicks Vaporub" which contains *Eucalyptus* oil as one of the components is manufactured and sold in Europe and North America for different uses.

Eucalyptus has been planted for decades in large parts of Ethiopia for different purposes including firewood, construction and as means of afforestation. Ankober district is a part of the Amhara Region of Ethiopia, located at the eastern edge of the Ethiopian highlands in the North Shewa Zone. This district is one of the areas in which more plantations of *Eucalyptus* trees has been done for long, and trees with different ages are available. And we have already started to make use *Eucalyptus* oil from these trees as one of the ingredients to produce laundry detergents and toiletries with good deodorizing and antiseptic properties. Even though several studies have been made on the essential oil content variation with agro-ecological and other factors, no reports indicated the essential oil content variation with the age of trees in the study area except botanical studies. In this work, we focus on the investigation of essential oil chemical content variation with the age of *Eucalyptus* trees.

MATERIALS AND METHODS

Study area

The study was conducted in the area of Ankober woreda. Ankober is one of the woredas in the Amhara Region of Ethiopia, Located at the eastern edge of the Ethiopian highlands in the Semien Shewa Zone at an elevation of about 2,465 meters. It is 42 kilometers to the east of Debre Birhan and about 90 miles (140 km) northeast of Addis Ababa.

Collection of plant materials

Leaves of *Eucalyptus globulus* were collected from locally grown *Eucalyptus globulus* trees aged 3, 5, 8, 12, 20, 35 and 100 years growing in the Ankober woreda of Ethiopia. The leaves were air-dried in the shade at the ambient temperature, protected from the direct light, until further analysis. The identity of the plant specimen was confirmed at the Department of biology by Botanist at Debreberhan University.

Extraction of essential oil

Extraction of oil from *Eucalyptus* leaves was carried out by a hydro-distillation. Freshly collected 500 g leaves were weighed and hydro-distilled for three hours for complete extraction of essential oil, using full glass Clevenger-type apparatus. The oil sample obtained from hydro distillation was freed from water by adding anhydrous sodium sulfate. The essential oil of from *Eucalyptus* leaves was yellow in color.

Identification of chemical composition

Determination of the chemical composition of the extracted EO from *Eucalyptus globulus* was carried out by Gas Chromatography-Mass Spectroscopy (GC/MS). GC/MS analysis was performed with HP5890 series II coupled with mass spectrometry HP5972 series detector and an electron ionization system, equipped with a capillary column HP5 (30 m, 0.53 mm, 0.25 μm film). The carrier gas was helium, with a gas flow of (0.5 mL/min). Oven temperature was kept at 50°C for 4 min and programmed to 280°C at a rate of 30°C/minutes. Injector temperature was 250°C and the detector temperature was 280°C. Mass spectra were taken at 70 eV. The samples were diluted in chloroform before analysis. The detected compounds were identified by processing the raw GC-MS data and comparing with mass spectral database and from retention times and mass spectra of standard compounds. Relative amounts of detected compounds were calculated based on GC peak areas.

Component identification

Identification of the chemical components was based on the comparison of their GC/retention time while passing through GC columns, which determines the retention time of chemical components present in the essential oil, and then compared with authentic compounds through computer matching with commercial mass spectral libraries.

RESULTS AND DISCUSSION

Yield of *Eucalyptus* essential oil

The yields of *Eucalyptus* oils extracted from the collected leaves of *Eucalyptus globulus* varied from 0.95 to 1.32% on fresh weight basis as presented in Table 1. The maximum percentage value of EO was obtained for the age of 3 years old plant (1.32%) while the lowest was 100 years old plants (0.95%). The yield of the oil was gradually decreased as the age of the plant increased suggesting that the extraction of the oil depends on age of the trees and maturity of the leaves. That means, young leaves tend to have higher oil yield than adult ones. Several reports also

shows similar trends in *Eucalyptus globulus* essential oil yields. For example 0.8-1.21% of essential oil (w/w, based on the fresh weight of the mature leaves) in Ethiopia [25], 1.87% of essential oil (w/w, based on the fresh weight of the leaves) in Algeria [26], 0.08-3.5% of essential oil (w/w, based on the fresh weight of the mature leaves) in Bangladesh [27], 1.1% of essential oil (w/w, based on the fresh weight of the mature leaves) in India [28]. It is noted that there are several factors that affect the yields of *Eucalyptus* oils such as tree age, leaf age, altitude, season, harvest time and fertilizer [29]. In terms of age, young leaves contain more oil than old leaves, while leaves from older trees gave slightly higher yield [29] that is consistent with our result.

Table 1: Essential oil (EO) yields of *Eucalyptus* at different age.

Age of *E. globulus* (year)	Weight of leaves extracted (g)	Weight of oil collected (g)	Yield (%)	Location collected
3	300	396	1.32	Ankober
5	300	336	1.12	Ankober
8	300	330	1.10	Ankober
12	300	321	1.07	Ankober
20	300	312	1.04	Ankober
35	300	291	0.97	Ankober
100	300	285	0.95	Ankober

Chemical composition of essential oil

A several chemical components, with different retention times, were eluted from the GC column
as indicated by the chromatogram in supporting information (Figures S1-S7) and were further analyzed based on their retention time and mass spectra library search. The relative amount of individual components was calculated based on GC peak areas. The GC chromatogram obtained revealed a high concentration of 1, 8 cineol indicated by presence of large peaks which eluted at about 10 minutes i.e., the main principal component in each sample (in all ages of *Eucalyptus globulus*) is eucalyptol. Furthermore, the color of the volatile oil extracted from all ages of *Eucalyptus globulus* trees hydro-distillation were light yellow.

The GC/MS total ion chromatogram of the EO was obtained under the conditions described in the procedure section, and the major identified chemical components are summarized in Table 2. GC/MS analysis revealed the presence of 18, 19, 18, 29, 15, 29 and 15 compounds in Y-3, Y-5, Y-8, Y-12, Y-20, Y-35 and Y-100 representing 99.69%, 99.69%, 99.98%, 99.91%, 99.54%, 99.49% and 99.04% of the total essential oils respectively. However, those chemical components with very small composition compared to other components were ignored and not included in the Table 2. All the *Eucalyptus globulus* leaves with the different ages studied contain alpha-Pinene, d-limonene and eucalyptol as the main constituent. The highest amount of eucalyptol (73.90%) detected in Y-100 whereas alpha-Pinene (20.05%) and d-limonene (6.17%) are highest in percentage in essential oil of Y-3. Figures S1-S7 shows the chromatograms of *Eucalyptus globulus* having different ages with major peaks. In general, it is evident that adult leaves have higher contents of eucalyptol than younger ones. Other components which are small in concentration are also detected in each sample as indicated in Table 2 and Figures S1-S7.

Table 2: Chemical composition of *Eucalyptus globulus* essential oils with tree of different ages.

RT	identified compound	Percentage composition of the extracted oil						
		Y-3	Y-5	Y-8	Y-12	Y-20	Y-35	Y-100
6.13	Alpha-Pinene	19.39	19.05	17.24	16.29	15.55	13.8	13.73
7.35	Beta-Pinene	0.83	0.77	0.54	0.5	0.53	0.36	0.48
7.82	Beta-myrcene	0.87	0.74	0.39	0.29	-	-	0.22
8.28	Alpha-Phellandrene	0.31	0.51	0.15	0.09	-	0.1	0.17
9	P-Cymene	0.31	0.5	0.43	0.45	1.37	1.06	0.66
9.15	D-Limonene	5.87	6.17	4.86	3.51	3.51	2.93	4.03
9.38	Eucalyptol	61.04	62.06	65.1	68.45	72.24	72.19	73.9
10.32	Gamma-terpinene	0.69	0.56	0.29	0.15	0.6	0.35	-
10.81	1-Octanol	-	-	1.06	-	-	-	-
11.53	CMM	0.59	0.69	0.32	0.25	0.36	-	0.37
11.98	Undecane	-		1.26	-	-	-	-
13.54	Pinocarveol, trans-		0.31	0.97	-	-	-	-
13.62	Isopinocarveol	-	-	-	1.27	1.91	1.46	1.6
14.57	Pinocarvone	-	-	0.29	0.48	0.95	0.89	0.6
15.22	Terpinene-4-ol	0.33	0.28	-	0.19	-	0.24	-
15.67	Trans-p-mentha-1(7), 8-dien-2-ol	-	-	-	0.28	0.34	0.39	0.26

15.86	Alpha-terpineol		1.04	1.03	0.97	-	1.4	-
22.75	3CMA4A	2.31	3.01	4.23	3.48	-	-	1.34
25.21	Alpha-Gurjunene	0.87	0.39	-	0.14	-	-	-
26.4	Aromandendrene	2.43	1.59	0.91	0.99	0.94	1.01	1.05
27.27	Alloaromadendrene	0.75	0.44	0.29	0.27	0.24	0.37	0.26
28.7	Viridiflorene	0.86	0.48	-	0.11	-	-	-
31.22	Epiglobulol	-	0.28	-	0.18		0.26	-
32.17	(-)-Globulol	0.77	0.82	0.62	0.72	0.55	1.05	0.37
32.46	Ledol	-	-	-	0.11	-	0.16	-
		99.36	99.69	99.98	99.17	99.09	99.49	99.04

Y: year; CMM: Cyclohexane, 1-methyl-4-(1-methylethylidene); 3CMA4A: 3-Cyclohexe-1-methanol alpha-alpha 4-trimethyl-acetate.

A comparison was also made regarding the content of monoterpenes in oil samples of *Eucalyptus* that were collected from Ankober woreda at the age of 3, 5, 8, 12, 20, 35 and 100. The results showed that the volatile oil of the plant possessed a particular quantitative and qualitative chemical composition. 1, 8-cineole determines the business value of the oil and its significance as a raw material for diverse industries. Components such as 1, 8-cineole was found to be the dominant and its percentage composition is agreement with the same tree species reported in other region of the world [25]. Different percentages of 1, 8-cineole in *E. globulus* leaf oil have been mentioned in the literature: 51.08% in Algeria, 62.38%-5.37 in Ethiopia, 67% in Bangladesh, 54.79% in India [25-28], 72.71% in China [30] in which variation might be due to different agro-ecological zones and other factors. Our finding suggests that as the age of the plant increases, percentage composition of 1, 8-cineole increases whereas percentage composition of Alpha-Pinene (the second most dominant compound) decreases as shown in Table 2.

CONCLUSION

The extraction of the essential oil from the *Eucalyptus globulus* aged 3, 5, 8, 12, 20, 35 and 100 was carried out by hydro-distillation method. The essential oil content analyzed by GC/MS was found to be in the range of 0.95-1.32% depending upon the ages of the plant. The GC/MS analysis revealed the presence of several compounds in all samples. All the essential oils contain alpha-Pinene, d-limonene and eucalyptol as the main constituent. The 1,8-cineole constituent of the oil is ranging from 61.04%-73.90%. The highest Cineole (73.90%) was obtained for the oldest *Eucalyptus globulus* tree (100 years old plant) in this work suggesting age difference causing oil content variation. The oils obtained from aged *Eucalyptus globulus* plant could be used for medicinal purpose but in the case of lowest age needs purification to make its 1,8-cineole content greater than 70%. In general, these findings will help to access the quality of the *Eucalyptus* oil which is important in the production of high value essential oils.

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CONFLICTS OF INTEREST

The author declares that no conflicts of interest.

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