



Original Article

Identification and Comparison of Some Essential Oils Components in Seven *Eucalyptus* Species Cultivated in Khoramabad

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Article History: Received: 15 September 2019/ Accepted in revised form: 28 April 2020

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Abstract

This study was laid out in Lorestan agricultural farm, Korramabad, Iran during 2016 and 2017. The experiment was designed based on a complete randomized design (CRD) with four replications. In this study, in the spring, fresh leaf samples were randomly collected seven species of *Eucalyptus*. The composition of essential oils determined using analytical gas chromatography coupled to mass spectrometry (GC-MS). The results showed that the effect of specie treatment was significant on essential oil yield, -Pinene, -Pinene, 1, 8-Cineole, -Terpinene, Pinocarvone, Epiglobulol and -Eudesmol. Based on our results, essential oil of *Eucalyptus camaldulensis* Dehnh. was obtained at rate of 3.43%. The measured oil yield was in range of 2.57% for *Eucalyptus nitens* (H. Deane & Maiden) Maiden, 1.34% for *Eucalyptus globulosusbicosata*, 5% for *E. globulus madidenii* 17746, 5.38% for *E. globulusmadidenii* 2130, 3.74% for *Eucalyptus viminalis* Labill. and 1.12% for *Eucalyptus suggrandis* L.A.S. Johnson & K.D. Hill. The main compounds of *Eucalyptus camaldulensis* Dehnh. oils were 1, 8-cineole (66.72%) followed by -pinned (17.88) in the samples. The major constituents of *Eucalyptus nitens* (H. Deane & Maiden) Maiden oils were 1, 8-cineole (46.67%), -pinene (14.56%) and -Pinene (4.51%). The main components of *E. globulus* oils were 1, 8-cineole (70.15%) and -pinene (3.65%). The main compounds of *E. globulusmadidenii* 17746 oils were 1,8-cineole (55.82%), -pinene (3.91%) and Veridiflorol (5.07%). The major constituents of *E. globulusmadidenii* 12130 were 1,8-cineole (60.29%), -pinene (15.5%), -Terpineol (6.11%) and Eudesmol (3.88%). The major constituents of *Eucalyptus viminalis* Labill. oils were 1, 8-cineole (56.43%), -pinene (3.91%). The major constituents of *Eucalyptus suggrandis* L.A.S. Johnson & K.D. Hill oils were 1, 8-cineole (30.94%), -pinene (18.52%), -Eudesmol (6.87%) and Pinocarvone (4.96%). The *E. globules maidenii* 12130 specie had highest rate of essential oil yield. Between all species *E. suggrandis* had maximum rate of -Pinene, -terpinene, Pinocarvone and -Eudesmol. Between essential oil compositions 1, 8-Cineole was in higher rate especially in *E. globules bicostata* specie.

Keywords: *Eucalyptus*, 1, 8-cineole, GC-MS, Essential oil, Khorramabad.

Introduction

The genus *Eucalyptus* belongs to *Myrtaceae* family is one of the most-extensively planted pulpwood is a native of Australia and Tasmania represented by up to 700 species distributed throughout the world and only about 1% are used for industrial purposes [1]. The *Eucalyptus* was successfully introduced in many other regions of the world [2]. *Eucalyptus* is an evergreen tree with fragrant foliage rich in some

components that is an excellent source of essential oil [1].

Eucalyptus is a rich resource of essential oils that used for medicinal and commercial purposes. Recently, the essential oils and their components have been of great interest as they have been the sources of natural products [3]. The chemical composition of a specific plant species essential oil varies from species to species and subspecies, plant chemo type and genotype and specific

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environmental conditions [4]. Essential oils extracted from Eucalyptus are widely used in medicine, pharmaceuticals, cosmetics and food industries. Many species of the Eucalyptus have been used widely in folk medicine for a variety of medicinal applications [5]. However, Eucalyptus oil has been known for hundreds of years as antibacterial, anti-fungal and antiseptic in nature [1]. Eucalyptus essential oil had some components including lemon or lemon-scented Eucalyptus, Tasmanian blue gum, blue male and river red gum [6]. However, the value of Eucalyptus oil for medicinal purposes is based largely on the content of a particular oil constituent: 1, 8-cineole [7]. Eucalyptus species reduce atmospheric carbon dioxide levels directly and not only provide fuel biomass [8]. Zrira and Benjilali, [9] argued that two main components detected in *E. camaldulensis* were spathulenol and p-cymene, 1, 8-cineole, limonene and pinene. Doran and Brophy [10] told that some tropical Eucalyptus species leaf oil are rich in 1,8-cineole and they are potential commercial sources of medicinal-grade. Properties of three main components of Eucalyptus essential oil was shown in Table 1. Essential oils of Eucalyptus have many medicinal and commercial uses [11]. The Eucalyptus essential oil can be used as folk medicine and are antiperiodic, antiphlogistic, antiseptic, disinfectants, expectorant, febrifuge, fumigant, hemostat, sedative yet stimulant, supportive and vermifuge [12].

Differences between different species in essential oil components are related to natural origin, genetic and environmental properties. In this terms Cheng *et al.* [13] proved that *E. camaldulensis* oil from Taiwan were in majority composed by α -pinene (22.5%), β -phellandrene (20.1%), p-cymene (21.7%) and 1,8- cineole (9.4%) whereas *E.*

camaldulensis essential oil from Morocco had 1,8-cineole (42.3%), α -pinene (28.3%), β -terpinene (7.3%) and p-cymene (6.5%) as major compounds [14]. In the present work we have investigated the essential oil compositions obtained from some Eucalyptus species cultivated in Khorramabad environmental condition of Iran. We also have compared the main essential oil components in the Eucalyptus species with together.

Material and Methods




This study was laid out in Lorestan agricultural farm, Khorramabad, Iran during 2016 and 2017. The experimental area altitude and latitude was N48, 25.54 and E33, 26.40 respectively. The height from sea level was 1100m. Max and Min temperature were 47C and -13C with 519 mm annual rainfall. Soil texture was loam in semi-arid condition (Fig. 1). Plant Material Collection

Fresh and young leaves of seven Eucalyptus species (*E. camaldulensis e obtus*, *E. globules bicostata*, *E. niten*, *E. viminalis*, *E. suggrandis* sp, *E. globules maidenii* 17746, *E. globules maidenii* 12130) collected from mature tree in the Lorestan educational garden Khorramabad, Iran. Leaves dried for 7 days at room temperature.

Essential Oils Isolation

Crushed clean fresh leaves materials were separately subjected to 5 h hydro distillation using a Clevenger-type apparatus. The experiment was laid out in three repetitions. Oils floating on the water was collected in separate glass vials by discharging the water, dried by Sodium sulfate and saved in a freezer until used.

Table 1 Properties of three main essential oil components of Eucalyptus

Essential oil component name	Chemical form	Formula	Molar mass (g.mol ⁻¹)	Retention index
1,8-cineol		C10H18O	154.24	1022
α -pinene		C10H16	136.23	925
L-pinocarveol		C10H16O	152.23	1155

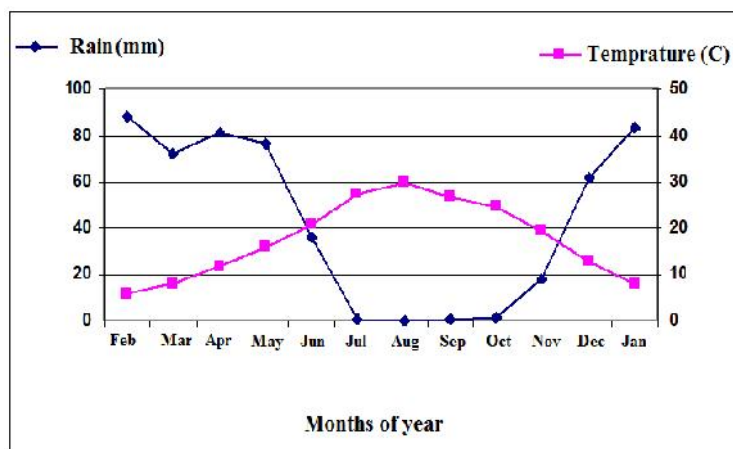


Fig. 1 The 25 year embryotic curves for Khorramabad

Analysis of Essential Oils and Identification of its Components

The analysis of essential oils was done using GC equipped with FID and GC-MS provided with a DB-5 column of 30 m X 0.25 mm i.e. and 0.25 μ m film thickness (A9, Shimadzu).

The GC settings were done as described by Cheng *et al.* [13] as follows: the initial temperature of the oven was set at 40°C for 5 min and increased to 140 °C at 3 °C/min, then increased from 140 to 250 °C at a rate of 10°C/min. Helium was the carrier gas at 1 mL/min flow rate.

The temperature of sample injection was maintained at 250°C. Diluted samples (1.0% in hexane) were injected (1 μ L) separately, with 1:10 split ratio. Spectra were obtained over the mass range 40-340 amu. The electron ionization energy was 70 eV and 230°C ion source temperature. The retention index calculation was performed using a homologous n-alkane series of C8-C20 (Sigma-Aldrich, USA). The identification of the oil constituents was performed using their mass spectra, relative retention indices and by using authentic reference compounds. The data were compared to the NIST MS library, Wiley/NBS

registry and reported mass spectra and retention indices [15,16].

Data Analysis

The experiment was designed based on a complete randomized design (CRD) with four replications. In the end of experiment data collected and analyzed using SPSS software and means compared using Duncan test.

Results

The results of analysis of variance shows that the effect of specie treatment was significant on essential oil yield, -Pinene, -Pinene, 1,8-Cineole, -terpinene, Pinocarvone, Epiglobulol and -Eudesmol (Table 2). The chromatogram curves for Eucalyptus species was shown in Fig. 2. The yield of essential oils ranged from 1.22% to 5.38% (w/w) for the seven different Eucalyptus species (Table 3). The maximum essential oil yield was obtained from *E. globules maidenii* 12130 (5.38%), followed by *E. globules maidenii* 17746 (5%) and their difference was not significant, while *E. suggrandis* gave the lowest essential oil yield at 1.12% range.

Table 2 Analysis of variance for essential oil yield and it components in Eucalyptus species

Eucalyptus species	DF	Essential oil yield	MS						
			-Pinene (%)	-Pinene (%)	1,8-Cineole (%)	-terpinene (%)	Pinocarvone (%)	Epiglobulol (%)	-Eudesmol (%)
Treatment (specie)	6	8.63**	14.74**	6.66**	522.63**	0.72**	8.53**	0.32**	17.48**
Error	14	2.85	0.76	0.69	0.78	0.68	0.79	0.78	0.67
Total	20								

** Significant at 1% level

Based on these results, there is no relationship between region and Eucalyptus essential oil yield because, species from the same region show different essential oil yield.

The results of mean comparison show that maximum rate of α -Pinene was recorded in *E. suggrandis* specie (18.52%) and its differences were significant in comparison with other species. However, *E. camaldulensis* specie had a higher rate of α -Pinene (17.88%) after *E. suggrandis* and their differences were significant. Minimum rate of α -Pinene was recorded in three Eucalyptus species namely *E. viminalis*, *E. globules maidenii* 17746 and *E. globules bicostata* (3.91, 3.9 and 3.65% respectively) and their differences were not significant (Table 3).

Also the results show that maximum rate of β -Pinene was recorded in *E. nitens* specie (4.51%) and its differences were significant in comparison with all other species. The β -Pinene rate in *E. viminalis* specie was 1.33% and minimum rate of β -Pinene was recorded in *E. camaldulensis* specie (0.32%) that was significantly lower than *E. viminalis* and *E. viminalis* species.

The amount of 1, 8-Cineole in all Eucalyptus species was ranked in rate of 30.94-70.15% in *E. suggrandis* and *E. globules bicostata* species respectively. Differences between *E. globules maidenii* 17746 (55.82%) and *E. viminalis* (56.43%) was not significant.

The results also shows that between all Eucalyptus species maximum amount of γ -terpinene was recorded in *E. suggrandis* (0.64%) and had a significant differences in comparison with other Eucalyptus species. After *E. suggrandis* the maximum amount of γ -terpinene was founded in *E.*

viminalis (0.54%) and their difference was significant. Based on mean comparison results the minimum amount of γ -terpinene was recorded in *E. globules bicostata* (0.24%) and *E. globules maidenii* 17746 (0.24%) species respectively.

Between differences Eucalyptus species extract, percentage of Pinocarvone was higher in *E. suggrandis* specie (4.89%) and its different was significant in comparison with other species. However, that percentage of Pinocarvone in *E. suggrandis* specie was 22.9 times higher than its percentage in *E. globules maidenii* 12130 specie (0.21%) and it's very notable. The percentage of Pinocarvone in the *E. globules maidenii* 12130 specie was significantly lowers that other species.

Based on the results, amount of Epiglobulol component was vary between all seven Eucalyptus species, but between *E. globules maidenii* 17746 and *E. viminalis* there were no significant differences and produced higher amount of Epiglobulol compared to other species (1.06 and 1.08% respectively), but their differences were not significant. Morever, minimum amount of Epiglobulol was recorded in *E. camaldulensis* and *E. globules maidenii* 12130 species and these two species had no significant difference with together too.

The mean comparison results show that all seven Eucalyptus species produced different rate of δ -Eudesmol. The *E. suggrandis* specie produced higher amount of δ -Eudesmol (6.87%) followed by *E. globules maidenii* 12130 (3.88%) and *E. viminalis* (3.76%) species. Minimum amount of δ -Eudesmol was recorded in *E. camaldulensis* (0.38%) that was 18 times lower than *E. suggrandis* specie and was very notable.

Table 3 Mean comparison for essential oil yield and it components in Eucalyptus species

Eucalyptus species	Essential oil yield (%)	α -Pinene (%)	β -Pinene (%)	1,8-Cineole(%)	γ -terpinene (%)	Pinocarvone (%)	Epiglobulol (%)	δ -Eudesmol (%)
<i>E. camaldulensis</i>	3.43 b	17.88 b	0.32 e	66.72 b	0.51 b	0.46 c	0.33 e	0.38 f
<i>E. nitens</i>	2.57 c	14.52 c	4.51 a	46.67 e	0.34 c	0.35 d	0.82 b	1.79 c
<i>E. globules bicostata</i>	1.43 d	3.65 d	0.38 d	70.15 a	0.24 d	0.71 b	0.68 c	0.66 e
<i>E. globules maidenii</i> 17746	5 a	3.9 d	0.47 d	55.82 d	0.24 d	0.39 d	1.06 a	0.64 e
<i>E. globules maidenii</i> 12130	5.38 a	15.05 c	0.78 c	60.29 c	0.38 c	0.21 e	0.31 e	3.88 b
<i>E. viminalis</i>	3.74 b	3.91 d	1.33 b	56.43 d	0.54 b	0.56 c	1.08 a	3.76 d
<i>E. suggrandis</i>	1.12 d	18.52 a	0.68 c	30.94 f	0.64 a	4.89 a	0.41 d	6.87 a

Means by at least 1 common letters had no significant difference based on LSD test.

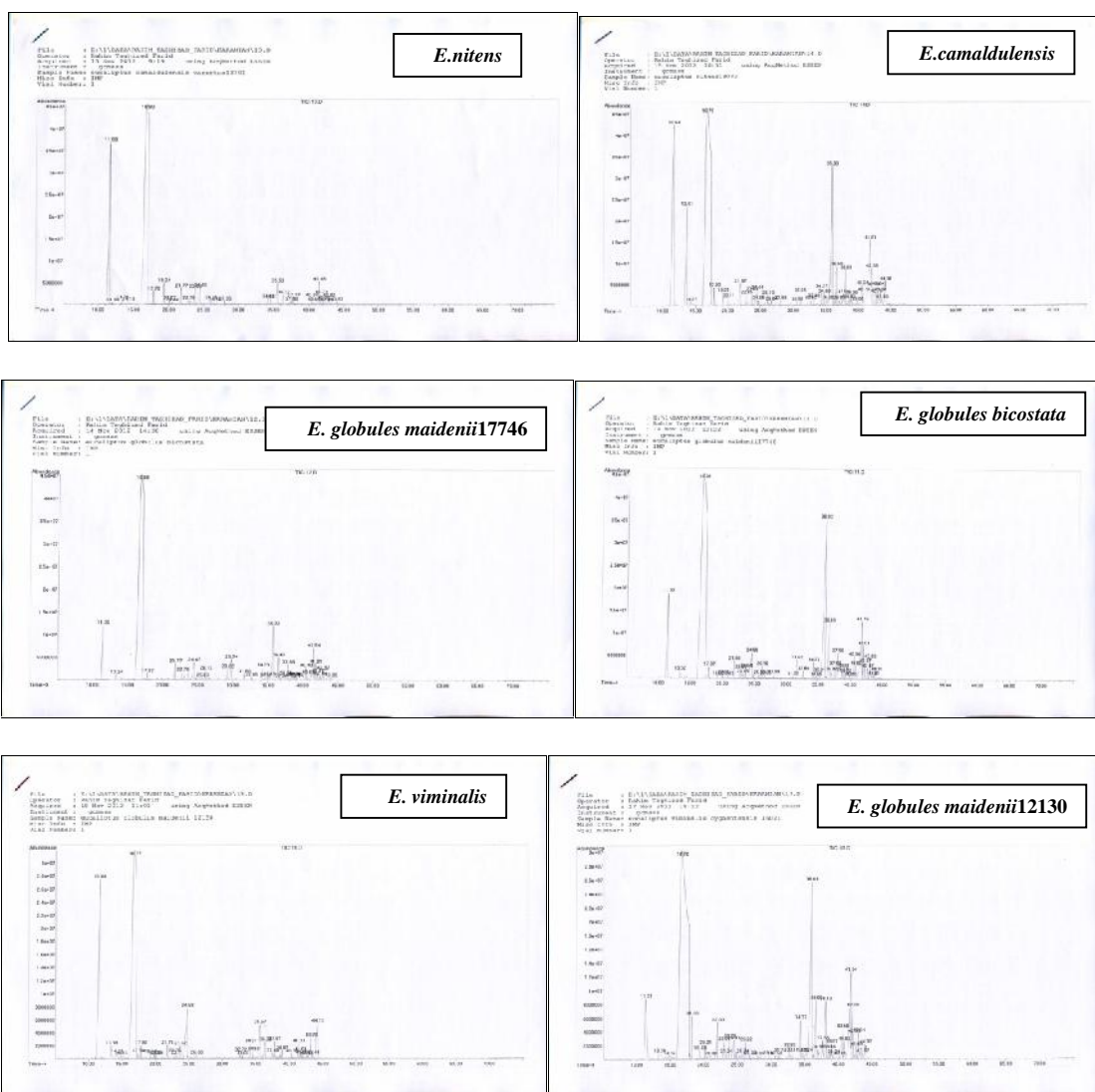
Discussion

The present results revealed that the seven species of Eucalyptus from Iran had different essential oil yield and its composition. There is a difference between all seven Eucalyptuses for essential oil yield, so that maximum yield was recorded in *E. globules maidenii* 12130 specie (5.38%).

The yield and chemical composition of Eucalyptus oil are influenced by several factors such as age of leaves, the harvest date, geographical origin and distillation method [17].

Also, the external factors such as altitude, soil nutrient, light irradiation, and climate are very

strong factors. In the present study essential oil yields varied according to Eucalyptus species [18]. In the same study revealed that yield of essential oil extracted from *E. suggrandis*, *E. globulus bicostata*, *E. nitens* and *E. globules maidenii* were 1.12%, 1.34%, 2.57% and 5.38%, respectively in Khorramabad region of Iran [19]. They also told that in total, 37 compounds in the essential oil of *E. nitens*, 27 compounds in the essential oil of *E. globulus bicostata*, 31 compounds in the essential oil of *E. globules maidenii* and 38 compounds in the essential oil of *E. suggrandis* were identified.



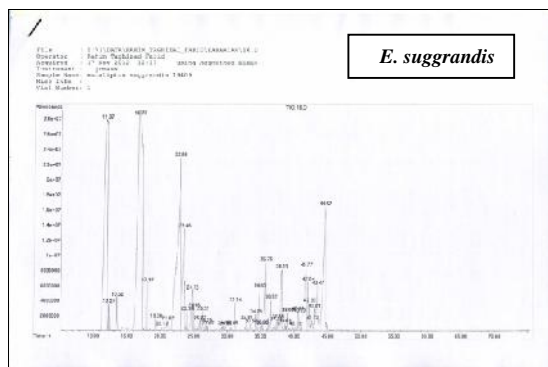


Fig. 2 The chromatogram pattern for *Eucalyptus* species

Between all species the *E. suggrandis* provided much higher essential oil components including -Pinene, -terpinene, Pinocarvone and -Eudesmol significantly in comparison with other species. However, based on the results, it was cleared that higher amount of -Pinene, 1,8-Cineole and Epiglobulol was recorded in *E. nitens*, *E. globules bicostata* and *E. globules maidenii* 17746 respectively. Benjemaa *et al.*, [18] reported that that chemical composition varied with Tunisian *Eucalyptus* species. The seven *Eucalyptus* essential oils contained 1,8-cineole and -pinene as major common compounds. The rate of 1,8-cineole in seven *Eucalyptus* species was 30.94% to 70.19%. Between all *Eucalyptus* essential oil components 1,8-cineole had higher percentage in comparison to other components. Each species has a slightly different biosynthesis matrix pathway that is governed by gene expressions. In the same study in Iran, essential oil composition of two *Eucalyptus microtheca* F.Muell. and *Eucalyptus viminalis* was obtained using hydro-distillation method using a Clevenger-type apparatus and analyzed by GC/MS. The results showed that there are about 101 compounds representing 100%, were identified, which among them, - phellandrene (16.487%), aromadendrene (12.773%), -pinene (6.752%), globulol (5.997%), ledene (5.665%), P-cymen (5.251%), and -pinene (5.006%) were the major constituents for *E. microtheca* leaves. They also told that there was 88 compounds representing 100%, were identified in which -pinene (16.246%), O-cymen (13.522%), -pinene (11.082%), aromadendrene (7.444%), - phellandrene (7.006%), globulol (5.419%), and 9-octadecenamamide (5.414%) were the major components in *E. microtheca* flowers [20]. *Eucalyptus* species contain volatile oils that are most plentiful in the plant leaves [21]. In another

study on *Eucalyptus* species reported chemical composition of the essential oils of six *Eucalyptus* species were 1, 8-cineole (64.80%), terpinen-1-ol (7.20%), and -pinene (5.70%); in *E. largiflorens* were 1, 8-cineole (47.0%), P- cymene (10.60%), and -terpineol (8.50%); in *E. kingsmillii* were 1, 8-cineole (77.0%), -pinene (8.70%), and camphene (3.80%); in *E. dealbata* were 1, 8-cineole (70.60%), -pinene (13.0%), and terpinen-1-ol (3.70%), from South West of Iran [22]. Gene expressions have a template that was inherited through generations. This may be due to genetic effects [17]. Internal factors that affected the chemical composition are the nature of oil biosynthesis in the plant. According to Pino *et al.* [23] *Eucalyptus* oils rich in 1,8-cineole are widely used in the pharmaceutical industry that is in line with the present results. 1,8-cineole is the major compound in *E. globules bicostata* essential oils with 70.15% that was 2.3 times higher than *E. suggrandis* specie (30.94%). These rates of 1,8-cineole were very close to those found by Panahi *et al.* [24] and Lima *et al.* [25] in *E. amaldulensi* soils (54.4% and 46.7%, respectively). Also Sebei *et al* [17] argued that all essential oils contain -pinene, 1,8-cineol and pinocarveol-trans for all *Eucalyptus* species studied. They also told that 1,8-cineol was the major compound in all species that is in line with the present findings. However the results of the present study show that the amount of 1,8-cineole had not significant differences between *E. globules maidenii* 17746 and *E. viminalis* species in rates of 55.82% and 56.43% respectively. The minimum rate of *Eucalyptus* essential oils was -terpinene that was in range of 0.24% in *E. globules bicostata* and *E. globules maidenii* 17746 species and 0.64% in *E. suggrandis* specie. However, we are concluded that between seven *Eucalyptus* species there are some differences and similarities

in aspect of oil compositions. In another study identified 22 components in the oil of *E. microtheca* from Isfahan province of Iran, and revealed that the major components were 1, 8-cineole (34.0%), P-cymene (12.40%), α -pinene (10.70%), β -pinene (10.50%), and viridiflorene (5.20%) [26]. In some of studied indicate that the major constituent of *E. microtheca* leaf oils from Semnan province of Iran was 1, 8-cineole (48.51%), followed by aromadendrene (18.31%), β -pinene (9.47%), and alloaromadendrene (4.67%) as the other dominant constituent [27]. Similar to the present study, many other researches on composition of *Eucalyptus* showed that the main constituents of the oil of *E. sargentii* from Isfahan province were 1, 8-cineole (55.48 %), β -pinene (20.95 %), aromadendrene (6.45 %), and trans-pinocarveol (5.92%) [28].

Between all *Eucalyptus* species β -pinene amounts were higher in *E. suggrandis* essential oils in comparison with other species. In this case Mondello *et al* [29] argued that the rate of β -pinene in *E. Alba* specie from Bangladesh was higher than other species. The *E. globules maidenii* 17746 and *E. viminalis* oils had Epiglobulol as major compounds in their essential oils. Also they had lower rates of β -Pinene than those contained in the *E. suggrandis* soil. This result is in accordance with the results of Cimanga *et al.* [30]. However the essential oils of the seven species, *E. camaldulensis var obtus*, *E. globules bicostata*, *E. niten*, *E. viminalis*, *E. suggrandis* ssp, *E. globules maidenii* 17746, *E. globules maidenii* 12130 studied showed quantitative and qualitative differences of composition. Thus, the amount of β -Pinene, α -terpinene, Pinocarvone and β -Eudesmol were present in *E. suggrandis* significantly in comparison to others. This result indicated that *E. suggrandis* specie had the higher amount of four important compositions in essential oil of *Eucalyptus* species. The same results proposed by Sefidkon *et al* [5] that found a qualitative difference in the composition of oils from four *Eucalyptus* species (*E. microtheca*, *E. spathulata*, *E. torquata* and *E. largiflorens*) of Iran. The differences between *Eucalyptus* species is related to differences of their origin, variety, harvest season (i.e. stage of the plants), storage of biomass can influence the chemical composition of essential oils [31]. Based on the present results and other literatures, there are many variations due to differences between environments, geographic,

growing conditions, physiological and biochemical states of plants, genetic factors, and different extraction and analytical method between researchers [32] but the main purpose of these studies are reach to exact identification of essential oil compositions in different environmental and geographical conditions for extraction in order to medicinal goals.

Conclusion

Many different environmental and inheritance factors effect on plant essential oil and its compositions. In this study, the essential oil yield and composition in *Eucalyptus* species are influenced by some internal and external factors. In the present study, there are differences between *Eucalyptus* species for essential oil yield and its compositions. The present results show that *E. globules maidenii* 12130 specie had highest rate of essential oil yield but don't have any compositions in higher rate. Between all species *E. suggrandis* had maximum rate of β -Pinene, α -terpinene, Pinocarvone and β -Eudesmol. Between essential oil compositions 1, 8-Cineole was in higher rate especially in *E. globules bicostata* specie. Based on the differences between essential oil yield and compositions can selected a specie for special purpose.

Acknowledgment

This study was supported by the grants Razi Herbal Medicines Research Center of Lorestan University of Medical Sciences, Khorramabad, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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