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Identification and comparison of the yield and composition of essential oil constituents of four Eucalyptus species adapted to the climatic conditions of Khorramabad

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ABSTRACT

Introduction: Eucalyptus has more than 400 species, while only a few species of this genus have been imported in Iran. In this study we aimed to investigate chemical compounds of the essential oils of Eucalyptus species adapted in Lorestan climate.

Methods: In this study, the fresh young leaves of four different Eucalyptus species including *E. suggrandis*, *E. globulus* subsp. *bicostata*, *E. nitens* and *E. globulus* subsp. *maidenii* were collected in spring (the middle of May) in khorramabad, Iran. The powder of air-dried leaves of different species of Eucalyptus was subjected to hydro-distillation using a Clevenger-type apparatus. The chemical compositions of different essential oils were detected using gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) and determination of their retention time (RT), retention index (RI) and Mass Spectra. The obtained data were analyzed using SPSS software.

Results: The results of this study indicated that there was significant difference ($P<0.01$) among the yield and chemical compounds of the essential oils of studied species. The results showed that the yield of essential oil extracted from *E. suggrandis*, *E. globulus bicostata*, *E. nitens* and *E. globulus maidenii* were 1.12%, 1.34%, 2.57% and 5.38%, respectively.

Conclusion: The essential oil constituents of four Eucalyptus species plant were different in among of some compounds. *E. globulus maidenii* had most content of essential oil and 1.8-Cineole compound so it is necessary to identify quality and quantity characteristics of compounds available in this plant.

Implication for health policy/practice/research/medical education:
E. globulus maidenii has most content of essential oil and 1.8-Cineole compound so it is necessary to identify quality and quantity characteristics of compounds available in this plant.

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Introduction

Developed countries tend to use medicinal plants and natural compounds in pharmaceutical, cosmetics and food industries and the public welcome to use these plants need applied research. Damages resulting from the use of chemical drugs despite their significant therapeutic effects in many diseases have been reported. Because of synthetic compounds that there are in chemical drugs, adverse side effects may occur in patients and consumers treated with these drugs. So developed countries gradually eliminated all chemicals in food, pharmaceutical, cosmetic and health products and such industries have been transferred to developing countries. Some developed countries such as America, Germany, France, Belgium and the UK have shown significant advancements in the field of medicinal plants so the herbal medicines can be

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sold several times higher than chemical ones by these countries. Climate variability and unique ecological conditions of Iran, especially in Zagros region areas such as Lorestan has led to more diversity and richness of plant species, especially in the case of medicinal plants. In the research performed to identify the chemical composition of the extract of *Nardostachys jatamansi* in Iran, a total of 29 components were identified using GC/MS analysis (1). According to the researches carried out in Lorestan, there are approximately 1670 plant species of which 250 species are known to have medicinal properties (2). One of the species with medicinal properties is Eucalyptus that is imported in Iran. Even though there are more than 400 species of Eucalyptus in the world, only a few species were imported in Iran and have been planted in areas with mild winters, such as the north and south of Iran (3). According to the performed researches during ten years in Khorramabad, 7 species of Eucalyptus were compared in terms of survival and adaptation (4). Beside of drug usage, Eucalyptus is used to drain the everglades, prevent soil erosion and produce timber and green space. The main chemical components of Eucalyptus, which have many applications in medicine and other industries are Eucalyptol and 1, 8-cineol. In this study we aimed to investigate chemical compounds of the essential oils of Eucalyptus species adapted in Lorestan climate.

Materials and Methods

Plant collection and extraction of essential oils

Fresh young leaves of four different Eucalyptus species including *E. suggrandis*, *E. globulus* subsp. *bicostata*, *E. nitens* and *E. globulus* subsp. *maidenii* were collected in spring (the middle of May) in Khorramabad, Iran. The leaves were spread on the cloth and dried during 7 days in the laboratory. Air-dried leaves of different species of Eucalyptus (100 g) were powdered and subjected to hydro-distillation using a Clevenger-type apparatus. The oils were separated from water and dried over anhydrous sodium sulfate and kept in sealed vials at low temperature before analysis.

Identification of essential oil constituents

The essential oils extracted from leaves of different Eucalyptus were subjected to gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). After injection of oils, using Retention Rime (RT), Retention Index (RI) and Mass spectra of components and their comparison with those of standard ones or by computer matching against the library spectra, the compositions of the oils were studied qualitatively and quantitatively (5).

Statistical analysis

To ensure normal distribution of obtained data, the Kolmogorov-Smirnov test was used. The significance of differences among the yield and components of samples were determined by analysis of variance (ANOVA), using the Duncan test of SPSS. Differences were considered significant at the $P<0.01$ level.

Results

Compositions of essential oils obtained from different Eucalyptus species

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. globulus maidenii* and 38 compounds in the essential oil of *E. suggrandis* were identified.

Compositions of essential oil of E. nitens

Of the 36 components identified in the essential oil of *E. nitens*, 1, 8-cineol (46.67%) was the main constituent. α-pinene (14.56) was in the second place and the other constituents were Aromadendrene (9.02), Viridiflorol (5.65) and β-pinene (4.51). All compounds that were identified in the essential oil of *E. nitens* are recorded in Table 1.

Table 1. The composition of essential oil extracted from *E. nitens*

	Chemical composition	Compound (%)	Retention time	Katz index
1	α- Pinene	14.56	11.64	935
2	β-Pinene	4.51	13.61	975
3	Myrcene	0.35	14.32	988
4	1.8-Cineole	46.67	16.77	1038
5	Gamma-terpinene	0.34	17.90	1061
6	Terpinolene	0.32	19.25	1087
7	Methybuthyl2-methylbutanoate(3)	0.22	20.11	1104
8	Trans-Pinocarveol	1.15	21.88	1140
9	Pinocarvone	0.35	22.85	1160
10	Terpinene-4-ol	0.54	23.69	1170
11	α-Terpineol	0.94	24.45	1192
12	Myrtenol	0.20	24.65	1196
13	Trans-p-Mentha-1(17).8-dien-2- ol	0.32	26.13	1228
14	Geranial	0.27	26.64	1239
15	Delta-Elemene	0.40	31.05	1336
16	Isoledene	0.23	32.62	1371
17	Geranyl acetate	0.20	33.08	1382
18	α-Ggurjunene	0.56	34.27	1409
19	Trans-Caryophllene	0.39	34.68	1419
20	β-Copaene	0.39	35.93	1432
21	Aromadendrene	9.02	35.93	1449
22	Alloaromadendrene	1.68	36.60	1465
23	α-Gurjunene	0.33	36.92	1473
24	Naphthalene,1,2,3,5,6,7,...	0.59	37.59	1489
25	Viridiflorene	0.60	38.03	1500
26	Gamma-Cadinene	0.15	38.60	1514
27	Delta-Cadinene	0.38	38.96	1523
28	Epiglobulol	0.82	40.54	1564
29	Globulol	0.63	40.78	1570
30	Veridiflorol	5.65	41/81	1596
31	1H-Cycloprop(e)azulenn-4-ol	1.34	42.05	1602
32	Leptospermone(iso)	0.65	42.76	1622
33	Eremoligenol	0.62	43.04	1629
34	(gamma)Eudesmol	0.62	43.04	1629
35	2-Naphthalenemathanol, 1, 2, 3, 4,...	0.32	43.60	1645
36	β-Eudesmol	1.79	44.16	1660
	-	99.01	-	-

Compositions of essential oil of E. globulus bicostata

Twenty-seven compounds were identified in the essential oil of *E. globulus bicostata* of these, 1, 8-cineol as the major component with the 70.15% was significantly higher than the other compounds. Aromadendrene with 6.92%, Viridiflorol with 4.49% and α-pinene with 3.65% were the other main constituents. All compounds that were identified in the essential oil of *E. globulus bicostata* are presented in Table 2.

Compositions of essential oil of E. globulus maidenii

As presented in Table 3, 31 compounds were identified in the oil of *E. globulus maidenii,* of which 1, 8-cineol detected as the main constituent with 60.29%. The other major components were α-pinene, α-terpineol and β-eudesmol with 15.05%, 6.11% and 3.88%, respectively.

Compositions of essential oil of E. suggrandis

38 compounds were identified in the oil obtained from *E. suggrandis*. 1, 8 –cineol, α-pinene, Trans-pinocarveol, β-Eudesmol and Pinocarvone were the major detected constituents with 30.94%, 18.52%, 14.89%, 6.87% and 4.96%, respectively. All compounds that were identified in the essential oil of *E. suggrandis* are presented in Table 4.

Table 2. The composition of essential oil extracted from *E. globulus bicostata*

	Chemical composition	Compound (%)	Retention time	Katz index
1	α- Pinene	3.65	11.35	929
2	β-Pinene	0.38	13.34	970
3	1.8-Cineole	70.15	16.80	1039
4	Gamma-terpinene	0.24	17.77	1058
5	Trans-Pinocarveol	1.3.	21.77	1138
6	Pinocarvone	0.71	22.79	1159
7	α-Terpineol	2.54	24.43	1192
8	Trans-carveol	0.18	25.63	1217
9	Trans-p-Mentha-1(17).8-dien-2-ol	0.55	26.10	1227
10	Thymol	2.48	29.22	1294
11	α-Terpinylacetate	0.27	31.60	1348
12	Isoledene	0.20	32.60	1371
13	α-Gurjunene	0.62	34.23	1408
14	Trans-Caryophyllene	0.13	34.61	1417
15	β-Copaene	0.36	35.16	1431
16	Aromadendrene	6.92	35.72	1444
17	Alloaromadendrene	0.22	36.84	1471
18	Propanoic acid,2,2-dimethyl-2-phenylethyl	0.40	37.51	1487
19	Ledene	0.93	37.85	1495
20	Gamma-Cadinene	0.13	38.55	1513
21	Cycloisolongifol-5-ol(trans)	0.16	38.76	1518
22	Delta-Cadinene	0.16	38.90	1522
23	Epiglobulol	0.68	40.43	1561
24	Viridiflorol	4.49	41.54	1589
25	1H-Indene	0.44	42.12	1604
26	β-Eudesmol	0.66	42.87	1625
27	α-Cadinol	0.24	43.99	1656
	-	99.24		

Table 3. The composition of essential oil extracted from *E. globulus maidenii*

	Chemical composition	Compound (%)	Retention time	Katz index
1	α- Pinene	15.05	11.60	934
2	β-Pinene	0.78	13.39	971
3	Myrcene	0.58	14.21	988
4	α-phellandrene	0.47	14.83	1000
5	1.8-Cineole	60.29	16.77	1038
6	(z-β) Ocimene	0.17	17.34	1050
7	Gamma-terpinene	0.38	17.80	1059
8	Terpinolene	0.16	19.17	1086
9	Linalool	0.16	19.81	1098
10	Isopenntyl 2-methylbutanoate (3)	0.09	20.06	1103
11	Trans-Pinocarveol	0.68	21.71	1137
12	Pinocarvone	0.21	22.75	1157
13	Delta-Terpineol	0.31	23.11	1165
14	Terpinene-4- ol	0.62	23.62	1176
15	α- Terpineol	6.11	24.70	1197
16	1,7-Octadien-3-one,2-methyl-6-methylene	0.29	26.08	1227
17	α-Copaene	0.30	32.72	1373
18	Geranial acetate	0.11	30.00	1380
19	α- Gurjunene	0.60	34.20	1407
20	Trans-Caryophyllene	0.24	34.60	1417
21	Aromadendrene	3.32	34.75	1440
22	Aristolene	0.39	37.48	1486
23	Viridiflorene	0.87	37.81	1494
24	Delta- Cadinene	0.5	38.87	1521
25	Epiglobulol	0.31	40.32	1558
26	(-)-Globulol	0.73	41.33	1584
27	Viridiflorol	0.26	41.61	1591
28	Guaiol	0.25	41.80	1596
29	Gamma- Eudesmol	1.53	43.20	1634
30	Hinesol	0.19	43.43	1640
31	β-Eudesmol	3.88	43.13	1659
	-	99.82	-	-

Discussion

The results of this study indicated that the yield of obtained essential oil was varied in case of some species of Eucalyptus in the studied area. Based on the comparison of the averages of the yield of oil by Duncan test, *E. globulus maidenii* had the highest oil yields (5.38%) and *E. suggrandis* showed the lowest oil yields with approximately 1.12% (Figure 1). 7 similar compounds identified in the extracted oils from different species of Eucalyptus that were studied in this study (Table 5). As presented in Table 5, three compounds including 1, 8-cineol, α-pinene and β-pinene had the highest amount in terms of composition between different species.

Conclusion

Based on the results of this study, it can be concluded that the development of culturing of *E. globulus* in Khorramabad weather conditions and exploitation of its leaves to obtain essential oil in large extent is recommendable economically (more than 5% essential oil and 1, 8-cineol up to 66.72%). Therefore, with regard to the specific application and required compound, developing

Table 4. The composition of essential oil extracted from *E. Suggrandis*

	Chemical composition	Compound (%)	Retention time	Katz index
1	α- Pinene	18.52	11.87	940
2	Camphenne	0.41	12.24	947
3	β-Pinene	0.68	13.52	974
4	1.8-Cineole	30.94	16.77	1038
5	Gamma-terpinene	0.64	17.97	1062
6	Metha-Cymenene	0.27	19.30	1088
7	Methybuthy12-methylbutanoate(3)	0.10	20.12	1105
8	-Campholenalα	0.51	21.07	1124
9	Trans-Pinocarveol	14.89	22.87	1160
10	Pinocarvone	4.96	23.46	1172
11	Terpinene-4-ol	0.33	23.94	1182
12	-Terpineolα	1.57	24.73	1198
13	Myrtenol	0.46	24.97	1203
14	Trans-Carveol	0.26	25.87	1222
15	Trans-p-Mentha-1(17).8-dien-2-ol	0.38	26.31	1232
16	2,3-Butanedioldiacetate	0.05	27.07	1248
17	Carvacrol	0.22	29.39	1298
18	Delta-Elemene	0.62	30.65	1326
19	-Copaeneα	0.21	32.81	1375
20	-Elemeneβ	0.15	33.52	1392
21	α- Gurjunene	0.30	34.29	1410
22	Trans-Caryophyllene	0.85	34.80	1422
23	1H-Cycloprop(a)naphthalene	0.08	35.06	1428
24	β -Gurjunene	0.11	35.23	1432
25	Aromadendrene	2.21	35.75	1445
26	Alloaromadendrene	0.83	36.52	1463
27	Germacrene-D	0.30	37.28	1482
28	-Selinneneβ	0.45	37.55	1488
29	Bicyclogermacrene	1.96	38.11	1502
30	Gamma-Cadinene	0.15	38.63	1515
31	Delta- Cadinene	0.38	39.00	1524
32	Epiglobulol	0.41	0.52	1563
33	Globulol	0.42	40.78	1570
34	Viridiflorol	3.67	41.77	1595
35	1H-Cycloprop(e)azulenn-4-ol	1.25	42.04	1602
36	1H-Cycloprop(e)azulenn-1A-2-3,...	0.43	42.67	1619
37	-Naphthalenemethanol, 1,2,3,4,...	2.10	43.48	1642
38	β-Eudesmol	6.87	44.57	1671
	-	98.94		

and planting of mentioned species can be suggested.

Authors' contributions

All contributed to the study. RK, AM, EH, LA, NA, MK, KB acquired data and prepared the drafting. MK revised it critically for important intellectual content and submitted it. KB prepared the main drafting.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

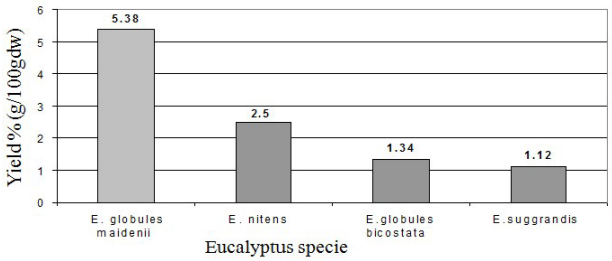

Figure 1. The yield of extracted essential oils from different four species of Eucalyptus

Table 5. The same compounds in the extracted oil of different four Eucalyptus species

	Nitens	*Suggrandis*	*Globulus bicostata*	*Globulus* maidenii 12130
α-Pinene	14.56	18.52	3.65	15.05
–Pineneβ	4.51	0.68	0.38	0.78
1, 8- Cineole	46.67	30.94	70.15	60.29
Gammaterpinen	0.34	0.64	0.24	0.38
Pinocarvone	0.35	4.96	0.71	0.21
Epigloubluol	0.82	0.41	0.68	0.31
-Eudesmolβ	1.79	6.87	0.66	3.88

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