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Influence of Drying and Extraction Methods on Yield and Chemical Composition of the Essential Oil of *Eucalyptus sargentii*

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ABSTRACT

Many parameters affect essential oil yield and chemical composition of aromatic plants. For obtaining the highest quantity and quality of essential oil, it is necessary to know the proper methods of drying and distillation. The aim of this research was to investigate the influence of drying and extraction methods on the yield and chemical composition of the essential oil of *Eucalyptus sargentii*. The fresh leaves of *Eucalyptus sargentii*, cultivated in Kashan (central region of Iran), were collected in the middle of spring and dried by five different drying methods: sun-drying, shade-drying, and oven-drying at 30, 40 and 50°C. The essential oils of every treatment were obtained by hydro-distillation in three replication. In addition, the essential oil of shade-dried sample was obtained by two other distillation methods, namely, water- and steam-distillation and direct steam-distillation. The oils were analyzed by capillary GC and GC-MS. Statistical analysis showed significant difference between oil yield (w/w) of the shade-dried sample (3.39%) compared to oven-dried at 40°C (2.92%), sun-dried (2.66%), oven-dried at 30°C (2.59%) and oven-dried at 50°C (2.30%). The oil content of the shade-dried sample obtained by hydro-distillation (3.39%) was higher than those of the water and steam distillation (2.89%) and steam distillation (1.35%). Twenty-three components were identified in the oil of *E. sargentii* in the different drying methods, including 1,8-cineole (57.9-65.8%) and α-pinene (11.3-28.3%) as main components. Twenty-four compounds were characterized in the oils of different distillation methods, including 1,8-cineole (61.2-66.6%) and α-pinene (19.7-28.3%) as the major compounds. Among the different drying methods, shade-dried samples produced the highest oil yield and 1,8-cineole content, while in different distillation methods, hydro-distillation produced the highest oil yield, but the highest percentage of 1,8-cineole was obtained by water and steam distillation.

Keywords: Drying, Essential oil, *Eucalyptus sargentii*, Extraction, 1,8-cineole.

INTRODUCTION

The genus *Eucalyptus*, which is native to Australia and some islands to the north of it, comprises over 600 species of trees and belongs to the family of Myrtaceae. It has spread worldwide, particularly in Africa, because of its easy adaptability and fast growth. More than 300 species of this genus have been shown to contain volatile oil in their leaves and less than 20 of these have ever been exploited commercially for oil production [1- 2].

The leaves and oils of many *Eucalyptus* species are especially used for respiratory aliment such as bronchitis and croup [3-6].

The main uses of Eucalyptus oils are for the pharmaceutical industry (those that are rich in 1,8-cineole), perfumery (those that are rich in citronellal) and for industrial use (those that have piperitone and α-phellandrene as their main constituents [7].

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The Eucalyptus oils and their main component (1,8-cineole) are largely employed in the preparation of liniments, cough syrups, ointments, toothpaste and pharmaceutical flavourings. Also, they are used in veterinary practice and dentistry, as fragrance component in soaps, detergents and toiletries, and have limited use in perfumes. The oils of Eucalyptus species also have antioxidant properties [8] and anti-inflammatory effects [9].

The aim of this study was to test the effect of drying method (sunshine, shade and oven drying at 30, 40 and 50°C) and also distillation method (hydro-distillation, water and steam distillation and steam distillation) on the essential oil content and composition of *Eucalyptus sargentii*.

A literature search showed that drying method had a significant effect on oil content and composition of aromatic plants [10-13]. For example, the oil content of shade-dried leaves of *Mellisa officinalis* was higher than oven-dried [14]. In another research, the oil content of shade-dried flowers of *Tanucetum parthenium* cv. Zardband was found to be higher (0.48% w/w) than those of oven-dried at 40°C (0.42%) and sun-dried (0.27%). Drying methods had no effect on the proportion of the various components [15]. Similar results were obtained for *Roman chamomil* [16].

The effect of different distillation methods on oil content and composition of aromatic plants have also been previously reported. For example, one study showed that the main component in the essential oil of *Eucalyptus dealbata* in three different distillation method (hydro-distillation, water and steam distillation and direct steam distillation) was 1,8-cineole and hydrodistillation gave the highest percentage of 1,8-cineole [17]. Also, hydro-distillation of *Eucalyptus camaldulensis* gave the higher oil yield and 1,8-cineole percentage than steam-distillation [18].

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Eucalyptus sargentii* were collected from Research Station of Dry and Desert Regions of Kashan in Isfahan province (central region of Iran) in the middle of spring.

To study the effect of drying method, five methods of drying i.e. sun-drying, shade-drying and oven-drying at 30, 40 and 50°C, were investigated. For oven drying, the fresh leaves were spread in calibrated oven with controlled temperature and dried in phytochemistry laboratory in the Research Institute of Forests and Rangelands. The required drying times for all samples were determined when their humidity reached values less than 5%. In the case of sun- and shade-drying, 0.5 kg fresh leaves was spread over 1 m^2 area.

Isolation Procedure

Dried leaves of every treatment (40-80 g, three replications) were subjected to hydro-distillation of 2 hours using an all glass Clevenger-type apparatus, according to the method recommended by the European Pharmacopoeia (1983) [19] to produce oils in yields presented in Table 1.

To study the effect of distillation method on oil content and compositin of *E. sargentii*, two other methods of distillation,

Table 1. Oil yields of *Eucalyptus sargentii* by different methods of drying obtained by hydro-distillation.

Drying Method	Means of oil yield (%)
Shade drying	3.39a
Oven drying at 40°C	2.92b
Sun drying	2.66c
Oven drying at 30°C	2.59c
Oven drying at 50°C	2.30d

Dfferent letters in oil yield shows significant difference.

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water and steam distilation and direct steam distillation, were used for shade-dried sample. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (2°C) before analysis.

Gas Chromatography

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica coloumn (30 m×0.25 mm id, film thickness 0.25 μm). Oven temperature was started at 60°C and then programmed to 210°C at a rate of 3 °C min^{-1} and finally the temperature was increased from 210 to 240°C at a rate of 20 °C min^{-1} and was held at this temperature for 8.5 minutes. Detector (FID) temperature was 280°C and Injector temperature was 300°C; helium was used as carrier gas.

Gas Chromatography-Mass Spectroscopy

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica coloumn (30 m×0.25 mm id, film thickness 0.25 μm); oven temperature was similar to that in GC. Injector temperature was adjusted 10°C more than final-temperature (250°C). Carrier gas was helium with a liner velocity of 31.5 cm s-1, ionization energy was 70 eV and mass range was 40-300 amu.

Identification of Componenets

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [20-22]. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

Statistic Analysis

The data were analyzed by SAS software and using Duncan's test.

RESULTS AND DISCUSSION

The oils isolated by different methods of distillation from the leaves of *E. sargentii* and dried under different conditions were yellow liquids in yields shown in Tables 1 and 2. Lengths of dryness for sun-dried, shade-dried and oven-dried at 30, 40 and 50°C samples were 2, 4, 6, 3 and 1 days, respectively. The analysis of variance for different distillation and drying methods are also shown in Tables 3 and 4.

The analysis of variance (Table 3) showed that the distillation method had a significant effect on the oil content of *Eucalyptus sargentii* (α= 5%). The highest oil yield was obtained by hydro-distillation and the lowest by steam-distillation. This may be due to the fact that, in the steam-distillation method, the characteristics of plant material, such as type of plant material, mode of comminution, mode of charging, and grade of insulatin are much more important than in the other distillation method. These results

Table 2. Oil yields of *E. sargentii* by different distillation methods (shade-dried samples).

Distillation method	Means of oil yield (%)
Hydro-distillation	3.39a
Water and steam distillation	2.89b
Steam-distillation	1.35c

Dfferent letters in oil yield shows significant difference.

Table 3. Analysis of variance for distillation methods.

Source of variation	DF	Mean Square
Distillation method	2	3.3913*
Error	4	0.0123
Total	6	-

Coeff Var= 4.3592. * Significant at 5%.

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Table 4. Analysis of variance for drying methods.

Source of Variation	DF	Mean Square
Drying Method	4	0.4622*
Error	7	0.0152
Total	11	-

Coeff Var = 4.3878. * Significant at 5%.

are in agreement with the previous studies on the effect of distillation methods on oil content and composition of other essential oil-bearing plants [23- 24].

The different drying methods had also significant effect on oil yield of *Eucalyptus sargentii* (Table 4). Plant materials dried in shade had higher essential oil content (3.39% w/w) in comparison with oven-dried at 40°C (2.92%), sun-dried (2.66%), oven-dried at 30°C (2.59%) and oven-dried at 50°C (2.30%) samples. The results showed by increasing the temperature of drying, less essential oil were obtained. Such decrease in the oil yield could be due to evaporation of more volatile components of the oil at higher temperature.

Twenty-three components that constituted 93.5-97.1% of the oils were identified in the essential oil of *E. sargentii* by different drying methods. The chemical composition of the oils can be seen in Table 5. The components are listed in order of their elution on the DB-5 column.

By hydro-distillation, the main components of the oil of oven-dried leaves at 30°C were 1,8-cineole (57.9%), α-pinene (15.6%), β-eudesmol (7.8%) and trans-pinocarveol (4.9%). The major components of the oil of oven-dried leaves at 40°C were 1,8-cineole (58.2%), α-pinene (19.9%), β-eudesmol (6.3%), and trans-pinocarveol (3.8%) and for the oven-dried leaves at 50°C, they were 1,8-cineole (65.8%), α-pinene (11.3%), trans-pinocarveol (6.1%) and γ-eudesmol (4.8%). In the case of sun-dried leaves, the most important compounds of the oil were 1,8-cineole (64.6%), α-pinene (18.0%), trans-pinocarveol (4.9%) and β-eudesmol (3.2%). The major compounds of the oil of Shade-dried were 1,8-cineole (61.3%), α-pinene (28.3%), trans-pinocarveol (2.1%) and γ-eudesmol (1.7%).

The drying method caused some variation in the relative proportions of the components. The major compounds, 1,8-cineole and α-pinene, had no sharp difference in five drying methods, but higher amount of 1,8-cineole was obtaind by drying in oven at 50°C. Relative increase of 1,8-cineole in this situation resulted from evaporation of more volatile components like α-pinene. In other words, evaporation of volatile hydrocarbon monoterpens caused higher relative percentage of 1,8-cineole (as oxygenated monoterpene) in the oil that is desirable for *Eucalyptus* oil. This result was in agreement with those obtained for *Satureja hortensis* [25].

Using different distillation methods, twenty-four components were identified that constituted 91.1%-97.1% of the oils in the essential oil of *E. sargentii* (shade-dried) . The chemical composition of the oils can be seen in Table 6. The components are listed in order of their elution on the DB-5 column.

The main components of the oil extracted by water and steam distillation were 1,8-cineole (66.6%), α-pinene (21.5%) and trans-pinocarveol (3.1%), while in the case of steam distillation, they were 1,8-cineole (61.2%), α-pinene (19.7%) and trans-pinocarveol (3.1%).

Comparison of the results showed that different drying methods had a significant effect on the percentage of the main components.

Research on *E. sargentii* in Iran (Khuzistan province) showed sixteen compounds among which 1,8-cineole (56.7%), β-eudesmol (6.0%) and α-pinene (4.9%) were the major ones (26). In our study, nineteen componenets were identified with the main constituent being 1,8-cineole

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Table 5. Comparison of essential oil components of *Eucalyptus sargentii* using different drying methods (by hydro-distillation).

No	Compound	RI	Oven 30°C (%)	Oven 40°C (%)	Oven 50°C (%)	Sun (%)	Shade (%)	Methods of idntificatin
1	*α*-Pinene	933	15.6	19.9	11.3	18.0	28.3	RI[a], MS[b], CoI[c]
2	Camphene	950	0.2	0.1	0.3	0.1	0.1	RI, MS
3	*β*-pinene	971	0.4	0.5	0.3	0.4	0.6	RI, MS
4	Myrcene	996	0.3	0.3	0.4	0.3	0.2	RI, MS
5	*p*-Cymene	1016	0.6	0.1	0.2	0.2	0.4	RI, MS
6	*1,8*-Cineole	1023	57.9	58.2	65.8	64.6	61.3	RI, MS, CoI
7	*α*-Campholenal	1116	0.1	0.1	0.1	-	-	RI, MS
8	*Trans*-Pinocarveol	1133	4.9	3.8	6.1	4.9	2.1	RI, MS, CoI
9	Pinocarvone	1154	1.5	1.2	2.2	1.5	0.1	RI, MS, CoI
10	Borneol	1160	0.2	0.2	0.2	0.1	0.1	RI, MS
11	Terpinene-4-ol	1170	0.1	0.2	0.1	0.1	-	RI, MS
12	*p*-Cymene-8-ol	1184	0.6	0.2	0.4	0.3	0.4	RI, MS
13	*α*-Terpineol	1189	0.2	0.5	0.5	0.5	-	RI, MS
14	*trans*-Carveol	1216	0.5	0.3	0.4	0.3	0.2	RI, MS
15	*E*-Caryophyllene	1406	0.2	0.2	0.4	0.2	0.1	RI, MS
16	*α*-Guaiene	1423	0.2	0.2	0.4	0.2	0.1	RI, MS
17	Aromadendrene	1441	0.1	-	0.1	0.1	-	RI, MS
18	*Allo*-Aromadendrene	1464	0.2	0.2	0.2	0.1	0.3	RI, MS
19	Spathulenol	1568	0.5	0.6	0.5	0.3	0.1	RI, MS
20	Caryophyllene oxide	1574	0.8	0.2	0.4	0.3	0.2	RI, MS
21	Viridiflorol	1582	0.4	-	0.2	0.1	0.1	RI, MS
22	*γ*-Eudesmol	1625	0.2	0.2	4.8	0.1	1.7	RI, MS, CoI
23	*β*-Eudesmol	1640	7.8	6.3	-	3.2	0.7	RI, MS, CoI
	Total	-	93.5	93.5	95.3	95.9	97.1	-

[a] Retention indices in elution order form DB-5 column; [b] Mass Spectroscopy, [c] Co-Injection.

Table 6. Comparison of essential oil components of *Eucalyptus sargentii* using different distillation methods (shade drying).

No	Compound	RI[a]	Hydro-distillation (%)	Water and steam distillation (%)	Steam distillation (%)
1	*α*-Pinene	935	28.3	21.5	19.7
2	Camphene	951	0.1	0.1	0.2
3	*β*-Pinene	972	0.6	0.5	0.6
4	Myrcene	996	0.2	0.2	0.3
5	*α*-Phellandrene	1010	-	-	0.1
6	*p*-Cymene	1017	0.4	0.6	-
7	*1,8*-Cineole	1026	61.3	66.6	61.2
8	*α*-Campholenal	1119	-	-	0.1
9	*Trans*-Pinocarveol	1135	2.1	3.1	3.1
10	Pinocarvone	1157	0.1	0.1	1.2
11	Borneol	1163	0.1	0.1	0.1
12	Terpinene-4-ol	1173	-	0.2	0.1
13	*p*-Cymene-8-ol	1182	0.4	0.5	0.2
14	*α*-Terpineol	1186	-	-	0.5
15	*trans*-Carveol	1218	0.2	0.2	0.3
16	*E*-Caryophyllene	1408	0.1	0.1	0.2
17	*α*-Guaiene	1425	0.1	0.1	0.4
18	Aromadendrene	1442	-	-	0.2
19	*Allo*-Aromadendrene	1463	0.3	0.4	0.2
20	Spathulenol	1568	0.1	0.2	0.4
21	Caryophyllene oxide	1575	0.2	0.1	0.5
22	Viridiflorol	1583	0.1	-	0.1
23	*γ*-Eudesmol	1626	1.7	1.7	0.1
24	*β*-Eudesmol	1641	0.7	-	1.3
	Total	-	97.1	96.3	91.1

[a] Retention indices in elution order form DB-5 column.

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(61.3%). This difference is probably caused by differences in ecological conditions.

In another study in Iran (Kashan from Isfahan province) on *E. sargentii* subsp. *Sargentii* Maiden, there were seventeen compounds in the essential oil with 1,8-cineole (75.5%), α-pinene (8.3%) and β-eudesmol (4.1%) as the main ingredients [27]. Different harvesting times may cause different percentage of 1,8-cineole in the oils.

A study on *E. microtheca*, *E. spathulata*, *E. largiflorens* and *E.torquata* cultivated in Iran (Kashan, in Isfahan province) showed 22, 21, 26 and 16 compounds in the essential oils of these species, respectively. The major compound was 1,8-Cineole (34.0, 72.5, 37.5, and 66.9%, respectively) [28].

In essential oil of *E. tereticornis* from France, 23 constituents were identified with para-cymene (31.4%), β-phellandrene (9.8%), spathulenol (8.3%), γ-terpinene (7.0%) and α-phellandrene (6.8%) as the major constituents that have anti-microbial activity [29], whereas the main compound in *E. sargentii* was 1,8-cineole.

In Australia the highest amount of 1,8-cineole was reported in the essential oil of *E. mannensis* Boomsma subsp. Mannensis (86.1%) [30], but the highest amount of 1,8-cineole was in essential oil of *E. globulus* ssp. Bicostata from Argentina (90.7%) [31].

In this study, the highest amount of 1,8-cineole (46.0%) was obtained by water- and steam-distillation. The percentage of 1,8-cineole in the other two distillation methods was the same. The higher amount of α-pinene was obtained by hydro-distillation. In addition, the higher amount of oil yield in different distillation methods was obtained by hydro-distillation.

Finally, it could be concluded that drying of *Eucalyptus sargentii* leaves in the shade is more suitable for obtaining higher amount of oil yield and percentage of 1,8-cineole. Although the highest amount of 1,8-cineole was obtained by oven-drying at 50°C, the amount of oil yield obtained was the lowest by this method while the difference between percentage of 1,8-cineole in the shade-dried and oven-dried at 50°C was small. Therefore, we can choose shade-dried as the suitable method for drying of *E. sargentii* leaves. Moreover, oil extraction of these leaves by hydro-distillation could be recommended for obtaining higher oil yield. Also, water and steam distillation produced more 1,8-cineole percentage in the oil compared to other distillation methods.

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تأثیر روشهای خشک کردن و اسانس گیری بر مقدار و ترکیب شیمیایی اسانس *Eucalyptus sargentii*

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چکیده

عوامل زیادی بر روی بازده و کیفیت اسانس گیاهان معطر اثر می‌گذارند. برای دستیابی به بالاترین میزان و کیفیت از اسانس، دانستن مناسب ترین روش خشک کردن و اسانس گیری ضروری است. هدف از این تحقیق مطالعه تأثیر روش خشک کردن و استخراج بر کمیت و کیفیت اسانس *Eucalyptus sargentii* بوده است. برگهای تازه این گیاه، کشت شده در کاشان (مناطق مرکزی ایران)ددر اواسط فصل بهار جمع آوری شده و تحت پنج شرایط مختلف (آفتاب، سایه آون ۳۰°C، ۴۰°C و ۵۰°C) خشک شدند. اسانس هر نمونه در سه تکرار با روش تقطیر با آب استخراج شد. بعلاوه، اسانس نمونه خشک شده در سایه با دو روش دیگر استخراج (تقطیر با آب و بخار آب و تقطیر با بخار آب مستقیم) نیز تهیه شدند. اسانسها با استفاده از دستگاههای GC و GC-MS مورد تجزیه و شناسایی قرار گرفتند. آنالیز آماری نشان داد بازده اسانس نمونه خشک شده در سایه (۳/۳۹٪) تفاوت معنی داری با بازده اسانس نمونه خشک شده در آون ۴۰°C (۲/۹۲٪)، نمونه خشک شده در آفتاب (۲/۶۶٪)، نمونه خشک شده در آون°C ۳۰ (۲/۵۹٪) و نمونه خشک شده در آون ۵۰°C (۲/۳۰٪) دارد. بازده اسانس نمونه خشک شده در سایه به روش تقطیر با آب (۳/۳۹٪) بیش از روش تقطیر با آب و بخار آب (۲/۸۹٪) و تقطیر با بخار آب (۱/۳۵٪) بود. بیست و سه ترکیب در اسانس *E. sargentii* در روشهای مختلف خشک کردن شناسایی شد که ۸،۱-سینئول (۶۵/۸٪-۵۷/۹٪) و آلفا-پینن (۲۸/۳٪-۱۱/۳٪) اجزای اصلی بودند. بیست و چهار ترکیب نیز در اسانس این گونه در روشهای مختلف تقطیر شناسایی شد که ۸،۱-سینئول (۶۶/۶٪-۶۱/۲٪) و آلفا-پینن (۲۸/۳٪-۱۹/۷٪) اجزای اصلی بودند. نتایج نشان داد که در روشهای مختلف خشک کردن، نمونه های خشک شده در سایه بیشترین مقدار اسانس و ۸،۱-سینئول را تولید می کنند و در روشهای مختلف تقطیر، بیشترین مقدار اسانس در روش تقطیر با آب و بیشترین مقدار ۸،۱-سینئول در روش تقطیر با آب و بخار آب حاصل می شود.

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