Akademik Ziraat Dergisi 10(1): 59-64 (2021)
ISSN: 2147-6403 e-ISSN: 2618-5881 DOI: http://dx.doi.org/10.29278/azd.748443

Research
(Araştırma)

Getting essential oil mixtures by different methods and determination of their compositions*

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*The study was produced from the Master's thesis.

Alınış tarihi: 5 Haziran 2020, Kabul tarihi: 4 Kasım 2020
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Abstract

Objective: The aim of the study is to determine the enhancement of the essential oil potential and find a more effective method with fewer substances.

Materials and Methods: For this purpose, Laurel and fennel essential oils obtained by the method of hydrodistillation in clevenger and their mixtures obtained by 2 different methods (Mix 1: separately obtained and mixed in appropriate proportions and Mix 2: obtained by placing together in the same glass flask) analyzed by Gc/Ms.

Results: When the main components of the Mix-1 mixture are examined, the main components of $R_{3/4}D_{1/4}$, $R_{1/2}D_{1/2}$ and $R_{1/4}D_{3/4}$ combinations are trans-anethhole (54.53%, 32.47% and 17.46%, respectively) and 1.8- cineol (18.16%, 33.11% and 39.37%, respectively). In the second method (Mix-2), the main components of essential oils were examined. The main components of $R_{3/4}D_{1/4}$, $R_{1/2}D_{1/2}$ and $R_{1/4}D_{3/4}$ were trans-anethhol (64.83%, 46.23% and 24.93%, respectively) and 1,8-cineol (11.47%, respectively, respectively). 23.97% and 37.67%).

Conclusion: As a result, it is clear that with essential oil blends, the components do not differ, while the proportions of the components do.

Key words: Laurel, Fennel, Mixture, Essential oil, Gc-Ms

Uçucu yağ karışımlarının farklı yöntemlerle elde edilmesi ve bileşimlerinin belirlenmesi

Öz

Amaç: Çalışmanın amacı, uçucu yağ potansiyelinin arttırılmasını belirlemek ve daha az madde ile daha etkili bir yöntem bulmaktır.

Materyal ve Yöntem: Bu amaçla, clevengerde su distilasyonu yöntemi ile elde edilen *Laurus nobilis* L. ve *Foeniculum vulgare Mill*. uçucu yağları ile bunların 2 farklı yöntemle elde edilen karışımları (Mix 1: Ayrı ayrı elde edilen ve uygun oranlarda karıştırılan ve Mix 2: Aynı cam balon içerisinde birlikte konularak elde edilen) Gc/Ms ile analiz edilmiştir.

Araştırma Bulguları: Mix-1 karışımının ana bileşenleri incelendiğinde, $R_{3/4}D_{1/4}$, $R_{1/2}D_{1/2}$ ve $R_{1/4}D_{3/4}$ kombinasyonlarının ana bileşenleri sırasıyla trans-anethol (sırası ile, %54.53, %32.47 ve %17.46) ve 1,8-sineol (sırası ile, %18.16, %33.11 ve %39.37) olarak belirlenmiştir. İkinci yöntemde (Mix-2), uçucu yağların ana bileşenleri incelenmiştir. $R_{3/4}D_{1/4}$, $R_{1/2}D_{1/2}$ and $R_{1/4}D_{3/4}$' ün ana bileşenleri sırasıyla trans-anethol (sırası ile %64.83, %46.23 ve %24.93) ve 1,8-sineol (sırası ile, %11.47, %23.97 ve %37.67) olarak belirlenmiştir.

Sonuç: Sonuç olarak, uçucu yağ karışımları ile bileşenlerin değişiklik göstermediği faka bileşenlerin oranlarının değişiklik gösterdiği açıkça görülmektedir.

Anahtar kelimeler: Defne, Rezene, Karışım, Uçucu yağ, Gc-Ms

Introduction

Essential oils can be obtained by different methods according to the amount, type and plant part of the essential oil in plants (Kaya and Günç-Ergönül, 2015). The methods used to obtain essential oil from plants are classified into two main categories as classical and advanced extraction methods. Classical methods are distillation (water distillation, steam distillation, hydrodiffusion, vacuum distillation, fractional distillation and water-vapor distillation), extraction (maceration, infusion, percolation, enfleurage, decoction) and mechanical methods (squeezing method, drawing method). Advanced extraction methods include pressure extraction, microwave assisted solvent extraction, supercritical fluid extraction and ultrasound-assisted extraction (Yaman and Kuleaşan, 2016)The most commonly used extraction procedure is the hydrodistillation method using a Clevenger type apparatus (Clevenger, 1928)

The complex structures of essential oils are represented by three major categories. These are i) terpenes and terpenoids usually having a low molecular weight, ii) aromatic and iii) aliphatic compounds (Dhifi et al, 2016; Danila and et.al, 2018a)

The antimicrobial effects of essential oils and their chemical components have been accepted by several researchers in the past. Furthermore, studies have shown the synergistic effect of any two or more components of essential oils against pathogens (Pinto et al, 2009, Swamy et al, 2016, Nazzaro et al, 2013). Mixing essential oils is more effective than using them alone. This provides the evidence that combinations of essential oils can be evaluated for synergistic activity to reduce their minimum effective doses (Nestor Bassolé et al, 2012). For these reasons, essential oils are used alone or in various combinations for the treatment of different infectious diseases. In addition to the side effects produced by most antibiotics, essential oils and mixtures there of are used to reduce or prevent resistance (Danila et al, 2018b). Essential oils obtained from plants such as fennel, bay laurel, oregano, mint, chamomile, lemon, lavender, clove etc. have been shown to have antifungal and antibacterial effects (Pinto et al, 2009, Soylu et al, 2007). In this study, it is aimed to produce an antimictobial product by obtaining an appropriate mixture of essential oils which have antifungal and antibacterial effect. Fennel essential oil with a strong antifungal effect (Soylu et al, 2005) and laurel essential oils having a strong antibacterial effect (Chmit et al, 2014) and suitable mixtures of these oils were used in the present study. In this study, fennel with a strong antifungal effect and laurel plants with a low antifungal effect were preferred. The antifungal activities of essential oils arise from the active ingredients. The aim of this study is to create a more effective essential oil and achieve a synergistic effect by pull the active ingredients to the desired level with the different combination of laurel essential oils with less antifungal activity and more effective fennel essential oils. The laurel essential oil used in the study is easier to find and less costly due to the fact that it is more in the flora than fennel essential oil. The other aim of this study is to determine to increase their potential and to find a more effective method with less substance.

Materials and Methods

Plant material

In the study, fennel essential oil obtained from fennel seeds has a strong antifungal effect (Soylu et al, 2005) and laurel essential oils obtained from laurel leaves with a strong antibacterial effect (Chmit et al, 2014) and suitable mixtures of these oils are used The plant materials used in the study were collected by hand from flora of Hatay. Fennel seeds and bay laurel leaves were collected at the end of September.

Isolation of the essential oils

Plant materials dried under room temperatures (25±1°C) were obtained in neo-clevenger by classical hydrodistillation method. Firstly, fennel seeds and bay laurel leaves were ground. Fennel seeds (25 g) and laurel leaf (25 g) were weighed and placed in a round bottom flask with 1 liter volume of distilled water; mixture was refluxed about 3-4 h, during which the oil was collected in the side arm of the system (having a density less than water, oil separates out of the water). The installation was allowed to stand for about half an hour to prevent the oil to reach room temperature, the oil was dried over anhydrous sodium sulphate and then stored in dark colour (amber) glass bottles and keep to refrigerator (about 4°C) until the use for GC/MS analysis. The obtained volatile oil is a clearly liquid, slightly yellowish and has characteristic smell.

Getting mix EO

Two different methods have been applied for obtaining essential oil mixtures. Mixtures of essential oils obtained by two different methods are shown in Figure 1 and 2.

Firstly, the *F. vulgare* (R) and *L. nobilis* (D) essential oils obtained separately with hydrodistillation previously have been mixed in particular ratios as fennel and bay laurel (75-25%), (50-50%), (25-75%). These different ratios of essential oils are coded as $R_{3/4}D_{1/4}$, $R_{1/2}D_{1/2}$, and $R_{1/4}D_{3/4}$, respectively.

In the second method, mixture of *F. vulgare* and *L. nobilis* plants in suitable ratios have been obtained with hydrodistillation method by putting them together into in a round bottom flask as fennel and bay laurel (75-25%), (50-50%), (25-75%). These different ratios of essential oils are coded as $R_{3/4}D_{1/4}$, $R_{1/2}D_{1/2}$, and $R_{1/4}D_{3/4}$, respectively.

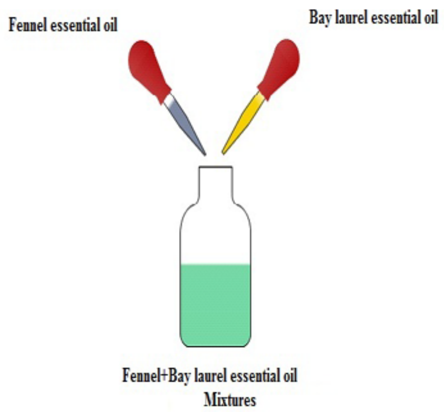

Figure 1. First mix method

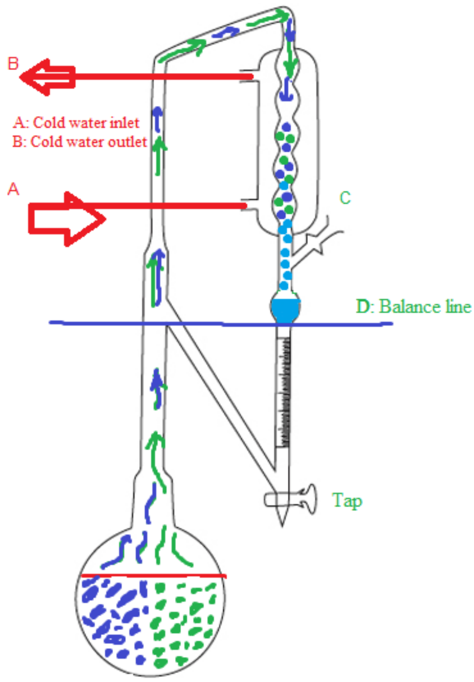

Figure 2. Second mix method

Characterization by GC-MS of essential oils

Analysis of the essential oils obtained by classical hydrodistillation method was performed using a Thermo Scientific Focus Gas Chromatography equipped with a Mass Spectrometer, autosampler and TR-5MS capillary column (5% phenyl-polisilfenilenesiloxan, 30 m x 0.25 mm inner diameter, film thickness 0.25). The carrier gas was helium (99.9%) with a flow rate of 1 mL/ min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scanning mode. Transfer line temperature of the mass spectrometer was 220°C; the temperature of orifice injection was 220°C. The samples were injected with a split ratio of 250. The injection volume was 1 mL. The temperature of oven was programmed in the range of 50 to 220°C at 3°C/min. The structure of each compound was identified by comparison of their mass spectra (Wiley 9 library). The data were processed using Xcalibur software.

Results and Discussions

Composition of essential oils

The main components of laurel essential oil (D) obtained by classical hydrodistillation method were determined as 1,8-cineole (52.88 %), α-terpineol acetate (11.77 %), sabinene (8.05 %) and α-pinene (5.32%). When the main components of fennel essential oil (R) were examined, it was determined as *trans*-anethole (81.55%), limonene (5.88%) and estragole (4.25%), respectively. The same major components were determined for laurel and fennel essential oils in previous researches (Taban et al., 2018, Todorovska et al., 2018, Özcan and Chalchat, 2005, Taoudiat et al., 2005).

When the main components of suitable mixtures obtained from essential oils were investigated; the main components of essential oils obtained by putting them different amounts in the same balloon were given in Table 1 (Mix-1). When the main components of fennel and laurel mixtures were analyzed; main components of $R_{3/4}D_{1/4}$ essential oil mixture were determined as *trans*-anethole (54.53%) and 1,8-cineole (18.10%); main components of the $R_{1/2}D_{1/2}$ mixture were 1,8-cineole (33.11%) and *trans*-anethole (32.47%). When the main components of the $R_{1/4}D_{3/4}$ mixture were examined, 1,8-cineole and *trans*-anethole were detected as a ratio of 39.37% and 17.16%, respectively. In Table 1, while *trans*-anethole was highest (81.55%) in fennel (R), 1,8-cineole was highest (52.88%) in laurel essential oil (D). As expected, in terms of 1,8-cineole, the highest 18-cineole area was observed in the $R_{¼}D_{¾}$ mix containing 75% D. On the other hand, the highest *trans*-anethole area was detected in the $R_{¾}D_{¼}$ mix containing 75% R.

Table 1. Essential oil composition of fennel-laurel mixtures ($R_{¾}D_{¼}$, $R_{½}D_{½}$, $R_{¼}D_{¾}$) boiled ın same round bottom flask (Mix 1)

				Mix-1		
RT	Compound Name	R	D	$R_{¾}D_{¼}$	$R_{½}D_{½}$	$R_{¼}D_{¾}$
3.87	α-Pinene	0.53	5.32	2.22	3.30	4.10
4.64	Camphene	-	0.22	0.10	0.14	0.18
5.49	β-Pinene	0.16	3.65	1.40	2.28	3.05
5.80	Sabinene	0.20	8.05	3.63	5.54	7.98
6.91	β-Myrcene	-	0.67	0.30	0.49	-
7.33	α-Terpinene	-	0.35	0.14	0.32	0.29
7.59	Dehydro-1,8-cineole	-	0.29	0.06	0.10	0.12
7.88	Limonene	5.88	1.33	3.25	2.45	2.39
8.08	1,8-cineole	0.30	52.88	18.16	33.11	39.37
9.35	γ-Terpinene	0.09	0.56	0.27	0.54	0.49
9.59	*cis*-Ocimene	0.26	0.09	0.11	-	-
10.21	*o*-Cymene	0.26	1.99	0.46	0.67	0.80
10.58	α-Terpinolene	-	0.14	-	-	0.23
14.27	3-Hexen-1-ol	-	0.13	-	-	-
14.65	Fenchone	1.53	-	0.84	0.46	0.26
17.26	*trans*-Sabinene hydrate	-	0.28	0.24	0.31	0.58
20.45	*cis*-Sabinene hydrate	-	0.19	0.18	0.20	0.40
20.60	Linalool	-	0.79	0.52	0.75	1.34
21.03	α-Terpineol	-	0.12	0.91	1.92	1.99
21.15	Pinocarvone	-	0.13	0.06	0.06	-
21.62	Bornyl acetate	-	0.15	0.07	0.09	0.14
21.99	β-Elemene	-	0.58	0.15	0.19	0.28
22.12	β-Caryophyllene	-	0.92	0.07	0.10	0.17
22.54	Terpinen-4-ol	0.05	2.83	0.68	1.71	1.46
23.43	Myrtenal	-	0.20	-	-	-
23.55	Verbenol	-	0.28	0.19	0.20	0.36
24.31	β-Fenchol	-	0.55	0.21	-	0.37
24.46	*trans*-Pinocarveol	-	0.20	0.06	0.09	0.10
24.83	α-Humulene	-	0.09	-	-	-
25.23	Estragole	4.75	-	2.87	1.80	-
26.03	α-Terpineol acetate	-	11.77	4.38	6.95	9.44
26.30	Germacrene -D	-	0.29	-	-	-
26.64	β-Chamigrene	-	0.15	-	-	-
26.65	Aromadendrene	-	-	-	0.07	0.08
26.85	α-Selinene	-	0.11	-	-	-
27.22	γ-Elemene	-	0.38	0.07	0.09	-
27.26	Neryl acetate	-	-	-	-	0.17
27.38	Carvone	0.94	-	0.59	0.37	0.17
27.50	Limonene oxide	-	0.14	-	0.08	0.17
28.22	Germacrene A	-	0.56	-	-	-
28.84	α-Humulene	-	0.23	-	-	-
29.91	Linalyl acetate	-	-	0.08	0.14	-
29.91	Nerol	-	-	-	0.08	0.17
30.90	*trans*-Anethole	81.55	0.77	54.53	32.47	17.46
31.10	*trans*-Carveol	0.04	-	0.05	-	-
35.72	Caryophyllene oxide	-	0.28	0.09	0.11	0.18
37.24	Methyleugenol	-	0.71	0.46	0.54	1.05
37.51	*p*-Anisaldehyde	1.76	-	1.26	0.55	-
40.40	Spathulenol	-	0.13	0.08	0.15	0.2
41.49	Cinnamyl acetate	-	0.08	0.10	-	0.21
41.99	Eugenol	-	0.32	0.23	0.28	-
42.56	Methyl *trans*-Isoeugenol	-	-	0.1	0.06	0.16
43.56	β-Eudesmol	-	0.13	0.13	0.11	0.31
47.46	Dillapiole	0.06	-	0.07	-	-

RT: Retention times

When the essential oil mixtures obtained by the second method were examined (Table 2), major components were the same in all the essential oil mixtures. The main components of R¾D¼ were *trans*-anethole (64.83%) and 1,8-cineole (11.47%); the main components of R½D½ were *trans*-anethole (46.23%) and1,8-cineole (23.97%) and the main components of the R¼D¾ were 1,8-cineole (37.67%) and *trans*-anethole (24.93%).

Table 2. Essential Oil Composition of Fennel-Laurel Mixtures (R¾D¼, R½D½, R¼D¾) Mixed By Hand (Mix 2)

				Mix-2		
RT	Compound Name	R	D	R¾D¼	R½D½	R¼D¾
3.87	α-Pinene	0.53	5.32	1.49	2.62	3.93
4.64	Camphene	-	0.22	0.10	0.09	0.16
5.49	β-Pinene	0.16	3.65	1.02	1.59	2.64
5.80	Sabinene	0.20	8.05	1.82	3.55	5.78
6.91	β-Myrcene	-	0.67	-	0.32	0.52
7.33	α-Terpinene	-	0.35	0.11	0.13	0.25
7.59	Dehydro-1,8-cineole	-	0.29	0.10	0.12	0.20
7.88	Limonene	5.88	1.33	4.97	3.87	2.72
8.08	1,8-cineole	0.30	52.88	11.47	23.97	37.67
8.16	β-Phellandrene	0.05	-	-	-	0.41
9.35	γ-Terpinene	0.09	0.56	0.22	0.26	0.41
9.59	*cis*-Ocimene	0.26	0.09	0.22	0.14	0.10
10.21	*o*-Cymene	0.26	1.99	0.63	1.03	1.52
10.58	α-Terpinolene	-	0.14	-	0.05	0.11
14.27	3-Hexen-1-ol	-	0.13	-	0.04	0.12
14.65	Fenchone	1.53	-	1.19	0.87	0.45
17.26	*trans*-Sabinene hydrate	-	0.28	0.08	0.10	0.20
20.45	*cis*-Sabinene hydrate	-	0.19	-	-	0.13
20.60	Linalool	-	0.79	0.19	0.37	0.58
21.03	α-Terpineol	-	0.12	0.6	-	0.08
21.15	Pinocarvone	-	0.13	-	-	0.09
21.62	Bornyl acetate	-	0.15	-	0.06	0.11
21.99	β-Elemene	-	0.58	0.16	0.25	0.41
22.12	β-Caryophyllene	-	0.92	0.18	0.42	0.64
22.54	Terpinen-4-ol	0.05	2.83		1.23	1.96
23.43	Myrtenal	-	0.20	0.07		0.12
23.55	Verbenol	-	0.28	0.11	0.14	0.19
24.31	β-Fenchol	-	0.55	0.14	0.25	0.41
24.46	*trans*-Pinocarveol	-	0.20	0.07	0.08	0.18
25.23	Estragole	4.75		3.8	2.81	1.57
26.03	α-Terpineol acetate	-	11.77	2.43	5.56	8.28
26.30	Germacrene -D	-	0.29	0.08	0.17	0.22
26.64	β-Chamigrene	-	0.15	0.06	-	-
26.85	α-Selinene	-	0.11	-	-	0.07
27.22	γ-Elemene	-	0.38	-	0.16	0.23
27.38	Carvone	0.94		0.73	0.51	0.25
28.22	Germacrene A	-	0.56	-	0.29	-
28.84	α-Humulene	-	0.23	0.07	0.12	0.17
29.51	Myrtenol	-	0.13	-	0.08	0.10
30.90	*trans*-Anethole	81.55	0.77	64.83	46.23	24.93
35.72	Caryophyllene oxide	-	0.28	-	0.13	0.19
37.24	Methyleugenol	-	0.71	0.16	0.34	0.5
37.51	*p*-Anisaldehyde	1.76	-	1.38	0.97	0.5
40.40	Spathulenol	-	0.13	-	0.06	0.09
41.99	Eugenol	-	0.32	-	-	0.23
43.56	β-Eudesmol	-	0.13	-	-	0.07

RT: Retention times

Conclusions

In conclusion, our investigation demonstrates the changing of the mix essential oil composition. It is clear that the main components of essential oils do not change with the new mixtures obtained, whereas the area of the main components varied according to the essential oil. If we want to obtain a mixture with a high 1,8-cineole ratio, R¼D¾ combination (Mix-1) can be selected from the mixes used. On the other hand, in order to obtain a mixture with a high *trans*-anethole ratio, R¾D¼ combination (Mix-2) can be chosen. Mixtures of essential oils and compositions can vary in desired proportions and more effective product can be obtained with less material. In addition, it has been proved that it is possible to produce a more economical and effective essential oil by mixing an expensive essential oil with a cheap essential oil. However, more studies are needed to elucidate effects of fennel and laurel essential oil mixtures.

Conflict of interest

There is no conflict of interest between the authors.

Contribution statement of the authors

The authors were equally solid in the study.

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