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Myrtus communis L.: Characterisation of Essential Oil of Leaves and Fatty Acids of Seeds Using Gas Chromatography-Mass Spectrometry (GC/MSD)

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Abstract: In this study, the chemical contents of essential oil acquired from *Myrtus communis* leaves using hydrodistillation and seed oil obtained using cold press method analyzed with gas chromatography-mass spectrometry (GC/MSD). According to the analyse result, forty-five component were identified and 1,8-cineole (21.68%), alpha-pinene (18.02%), linalool (14.12%) and alpha-terpinyl acetate (10.40%) were detected as major compounds in the *M. communis* leaf essential oil. On the other hand, seven different fatty acids were determined in seed oil. Linoleic acid (77.59%) and palmitic acid (10.36%) were detected to be major fatty acids in the *M. communis* cold-pressed seed oil. The present study has shown that the nutritional and other industrial use of *M. communis* leaves and seeds are possible due to their rich phytochemical contents of essential oil and seed oil.

Myrtus communis L.: Yaprak Uçucu Yağının ve Tohum Yağ Asitlerinin Gaz Kromatografisi-Kütle Spektrometresi (GC/MSD) ile Karakterizasyonu

Anahtar Kelimeler
Myrtus communis,
Uçucu yağ,
Tohum yağı,
1,8-sineol,
GC/MSD

Özet: Bu çalışmada, *Myrtus communis* yapraklarından hidrodistilasyon yoluyla elde edilen uçucu yağın ve tohumlarından soğuk-pres yöntemiyle elde edilen tohum yağının kimyasal kompozisyonu gaz kromatografisi-kütle spektrometresi (GC/MSD) ile analiz edilmiştir. Analiz sonuçlarına göre kırkbeş bileşen tanımlanmış ve 1,8-sineol (%21.68), alfa-pinen (%18.02), linalol (%14.12) ve alfa-terpinil asetat (10.40%) *M. communis* yaprak uçucu yağının majör bileşenleri olarak tespit edilmiştir. Diğer taraftan tohum yağında yedi farklı yağ asidi tanımlanmıştır. Linoleik asit (%77.59) ve palmitik asit (%10.36) *M. communis* soğuk-pres tohum yağında majör yağ asitleri olarak tespit edilmiştir. Yapılan çalışma, *M. communis* yaprak ve tohumlarının, sahip oldukları zengin fitokimyasal içerik ile gıda ve diğer endüstriyel alanlarda kullanım potansiyeline sahip olduğunu göstermektedir.

1. Introduction

Essential oils are secondary plant metabolites that found in various portions of plants such as leaves, flowers, roots, seeds, fruit and wood [1]. Essential oils, also known as etheric oils by people, can contain terpenic hydrocarbons and their oxygenated derivatives as well as alcohols, organic acids, phenols and ketones [2]. The basic components of essential oils are commonly mono and sesquiterpenes [3, 4] and the main plant families which essential oils derived *Asteraceae, Myrtaceae, Lauraceae, Lamiaceae, Rutaceae and Zingiberaceae* [5].

Myrtus communis, one of the main sources essential oils, is a member of the *Myrtaceae* family and naturally distributed in the Mediterranean region as an evergreen shrub with bright green leaves and edible fruits in black and white [6]. *M. communis* is an evergreen shrub, common in Mediterranean woodlands, maquis and garrigue [7, 8]. *Myrtus communis* (myrtle) is a common endozoochorous shrub species of forest patches in lowland agricultural Mediterranean areas [9]. It is also called "mersin" or "murt" by the people in Turkey. *M. communis* has been used for medicinal and nutritional purposes since ancient times. The leaves and fruits of *M. communis* are traditionally used as

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antiseptic, disinfectant, and hypoglycemic agents [10, 11]. In the previous literatures, Elfellah et al. were studied on anti-hyperglycaemic effect of an extract of *Myrtus communis* in Libya [12], and Al-Hindawi et al. were described an anti-inflammatory activity of some Iraq plants using intact rats [13].

The leaves of *M. communis* are widely known for the presence of essential oils, and their composition determines the specific aroma and flavor of the plant. The various applications of this oil are generally valued for kitchen purposes. Fresh and/or dried leaf oils are used in cosmetics, and beverage industries [11]. Some of the known biological activities of *M. communis* leaf essential oil include antioxidant activity and anti-mutagenic activity [11], antimicrobial activity, antibacterial activity and antifungal activity [14].

M. communis is an important plant not only because of the essential oils of leaves, but also because of the fatty acids contained in the fruits. Together with the increasing interest of consumers in natural products, seed oils of plants have found application field in food, medicine and cosmetic industries. Linoleic acid-rich oils are used as raw materials in the production of conjugated linoleic acid, a therapeutic nutrient with antioxidant and anti-tumor characters [15, 16]. Certain polyunsaturated fatty, vitamin F, are essential for skin growth and guard [17].

Fatty acid composition of plants is widely affected by the geographical origin of the plant [18-20]. Most of the studies on *M. communis* fruit has focused on it's volatile components and phenolics. However, few work has been done on the fatty acid contents of the *M. communis* fruits and seeds. *M. communis* fruits have a significant oil content about 15.40% and are rich in polyunsaturated linoleic acid [21, 22]. Messaoud et al. were identified approximately 79.10% of the oil studied that the major constituents were α-pinene and 1,8-cineole in Tunisia [Messaoud]. Plants rich in linoleic acid are being cultivated commonly and their oils are extensively used [23]. However, except for a few studies, it is difficult to find data in the literature on *M. communis* seed oil composition, especially from Turkey [22, 24].

In this study, chemical contents of *M. communis* leaf essential oil and seeds oil were investigated from Gökova (Muğla), Turkey to reveal potential use of *M. communis* essential oil and seed oil in food, industrial, and pharmacological applications.

2. Material and Method

2.1. Plant material

M. communis leaves and fruits were gathered from plants growing wild in November 2016 in Gökova (Muğla), Turkey. Leaves of *M. communis* have been air-dried in room temperature (25 °C) for seven days. Seeds were cleaned manually to remove mesocarp and other materials, then allowed to air-dry for ten days in ambient temperature (25 °C).

2.2. Isolation of essential oil and seed oil

Air-dried *M. communis* leaves were subjected to hydrodistillation for 3h to obtain essential oil while the seed oil of was obtained by cold press of air-dried seeds. The resulting oils were dried with anhydrous sodium sulfate, filtered and stored in a brown glass pot at +4 °C until analysis by Gas Chromatography-Mass Spectrometry (GC/MSD).

2.3. Analytical procedure for essential oil

M. communis leaf essential oil was weighed (30 mg) into a volumetric flask, dissolved in hexane (2 mL) for injection to the gas chromatography mass spectrometry instrument. GC/MS analyses were carried out using GC equipped with a Multi Mode Inlet (MMI) (280 °C). The carrier gas was He (2.1 mL/min), and the oven temperature was held at 60 °C for 5 min, then increased up to 220 °C at a rate of 2 °C/min and held at this temperature for 10 min. The injected volume was 2 µl and the split ratio was 40:1. The library search was carried out using NIST and Wiley 2008 GC/MS libraries.

2.4. Analytical procedure for seed oil

100 µL of seed oil was weighed into a volumetric flask and 9.80 mL of hexane was added. This mixture was vortexed for 5 min and then 100 µL of 2N KOH (dissolved in methanol) solution was added. The lidded tube was vortexed for 1 min followed by this addition, centrifuged at 4000 rpm for 10 min, the supernatant was removed for injection.

A quadrupole mass spectrometer (MS) and a J&W 112-88A7, HP-88 (60 m x 250 µm x 0.25 µm) column were used for the gas chromatography. For GC/MS detection, an EI system with ionization energy of 70 eV was used. 1 µL of sample was injected automatically in the split mode (50:1). Mass range was from m/z 50 to 650 amu. The library search was carried out using NIST and Wiley 2008 GC/MS libraries.

3. Results

In this study, chemical compounds of essential oil and seed oil of *M. communis* were examined using GC/MS. Forty-five components include a*lpha*-pinene, 1,8-cineol, linalool, *alpha*-terpinyl acetate were determined in the essential oil of *M. communis* leaves. All results were given in Table 1. Gas chromatography-mass spectrometry examination of *M. communis* leaf essential oil showed 1,8-cineol (21.68%), *alpha*-pinene (18.02%), linalool (14.12%), *alpha*-terpinyl acetate (10.40%), myrtenol (8.59%) and *alpha*-limonene (4.92%) as major compounds among forty-five components.

Table 1. Essential oil composition of *Myrtus communis* leaves.

No	RT	Compound	Conc.(%)	No	RT	Compound	Conc.(%)
1	5.121	Isobutyl isobutyrate	0.48	24	17.545	Estragole	0.19
2	5.707	*aplha*-Thujene	0.26	25	17.749	Myrtenol	8.59
3	5.924	*alpha*-Pinene	18.02	26	19.437	4-(1-methylethyl)benzaldehyde	0.22
4	6.260	Camphene	0.06	27	21.579	*trans*-Geraniol	1.12
5	7.101	Sabinene	0.02	28	21.958	Linalyl acetate	4.71
6	7.196	*beta*-Pinene	0.44	29	22.101	Geranial	0.08
7	7.898	*beta*-Myrcene	0.12	30	23.854	*trans*-Pinocarvyl acetate	0.38
8	8.623	3-Carene	0.44	31	24.368	Carvacrol	0.11
9	8.972	p-Cymene	2.64	32	25.507	*alpha*-Terpinyl acetate	10.40
10	9.331	1,8-Cineol	21.68	33	25.648	Methyl geranate	0.32
11	9.410	*alpha*-Limonene	4.92	34	27.120	Unknown	0.12
12	9.869	*trans-beta*-Ocimene	0.03	35	28.229	Neryl acetate	0.15
13	10.366	*cis-beta*-Ocimene	0.06	36	28.759	*trans*-Myrtanyl acetate	0.08
14	10.726	*gamma*-Terpinene	0.10	37	29.351	Geranyl acetate	1.31
15	11.188	*trans*-Linalool oxide	0.05	38	29.737	Methyl eugenol	0.80
16	11.887	Unknown	0.12	39	31.394	*beta*-Caryophyllene	0.25
17	12.825	Linalool	14.12	40	33.301	*alpha*-Caryophyllene	0.33
18	14.308	*trans*-Pinocarveol	0.14	41	33.701	Alloaromadendrene	0.13
19	16.088	4-ısopropylcyclohex-2-en-1-one	0.18	42	33.937	Unknown	0.25
20	16.503	1,8-Menthadien-4-ol	0.06	43	35.870	*alpha*-Selinene	0.25
21	16.595	Terpinen-4-ol	0.41	44	39.706	Spathulenol	1.91
22	16.851	Myrtenal	0.39	45	39.852	Caryophyllene oxide	0.49
23	17.326	*alpha*-Terpineol	3.09				

Conc.: Concentration, RT: Retention time

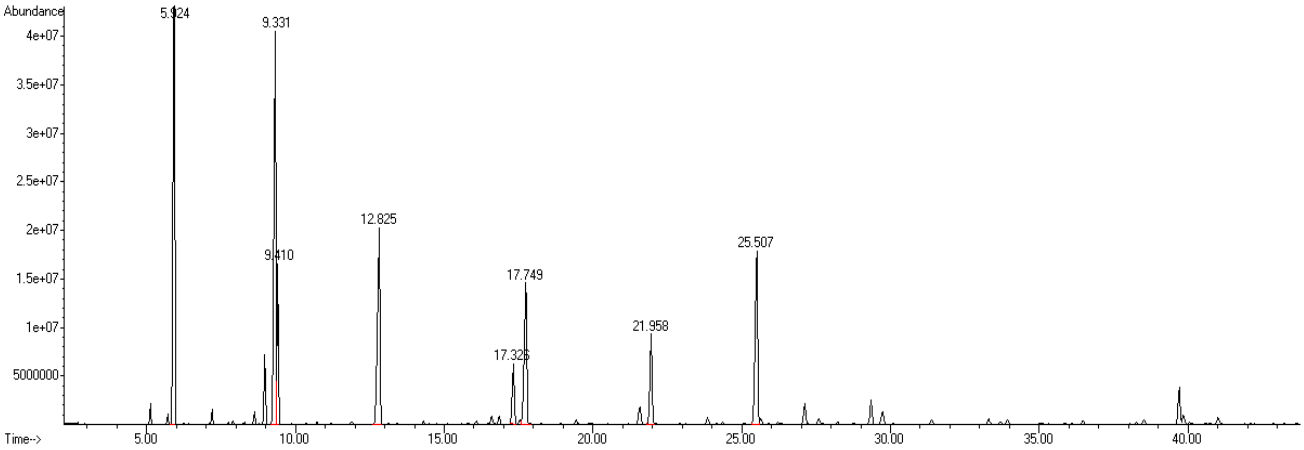

Figure 1. Total ion chromatogram of *M. communis* essential oil major compounds. (Retention times of the components above the concentration of 3% are given). alpha-Pinene (5.924), 1,8-cineole (9.331), alpha-limonene (9.410), linalool (12.825), alpha-terpineol (17.326), myrtenol (17.749), linalyl acetate (21.958), alpha-terpinyl acetate (25.507).

Linalyl acetate (4.71%), *alpha*-terpineol (3.09%) and p-cymene (2.64%) were determined as secondary major components. The other compounds such as spathulenol (1.91%) and geranyl acetate (1.31%) were present in minor percentages. All results were summarized in Table 1 and total ion chromatograms with retention times of the components above the concentration of 3% were given in Figure 1. Seven fatty acid methyl esters include palmitic acid methyl ester, oleic acid methyl ester and linoleic acid methyl ester were detected in the seed oil obtained by cold press method from the seeds (Table 2).

Linoleic acid methyl ester (77.59%), palmitic acid methyl ester (10.36%) and oleic acid methyl ester (8.26%) were detected as major components in the seed oil obtained from M. communis. Myristic acid methyl ester (0.03%), Stearic acid methyl ester (2.81%), elaidic acid methyl ester (0.91%) and cis-11-Eicosenoic acid methyl ester (0.04%) were found to be minor components in seed oil.

Table 2. Fatty acid composition of *M. communis* seeds.

No	Fatty Acid Methyl Esters	RT	Conc. (%)
1	C4:0	3.291	nd
2	C6:0	3.611	nd
3	C8:0	4.208	nd
4	C10:0	5.176	nd
5	C11:0	5.786	nd
6	C12:0	6.455	nd
7	C13:0	7.177	nd
8	C14:0	7.991	0.03
9	C14:1	8.658	nd
10	C15:0	8.917	nd
11	C15:1	9.713	nd
12	C16:0	10.022	10.36
13	C16:1	10.799	nd
14	C17:0	11.340	nd
15	C17:1	12.316	nd
16	C18:0	13.006	2.81
17	C18:1n9t	13.689	0.91
18	C18:1n9c	14.053	8.26
19	C18:2n6t	14.998	nd
20	C18:2n6c	15.938	77.59
21	C18:3n6	17.430	nd
22	C20:0	17.681	nd
23	C18:3n3	18.353	nd
24	C20:1	18.880	0.04
25	C21:0	19.949	nd
26	C20:2	20.628	nd
27	C20:3n6	21.744	nd
28	C22:0	21.923	nd
29	C20:3n3	22.464	nd
30	C20:4n6	22.505	nd
31	C22:1n9	22.823	nd
32	C23:0	23.616	nd
33	C22:2	24.220	nd
34	C20:5n3	24.220	nd
35	C24:0	25.357	nd
36	C24:1	26.345	nd
37	C22:6n3	29.267	nd

nd: Not detected, Conc.: Concentration, RT: Retention time

4. Discussion and Conclusion

Although the results vary depending on factors such as the oil extraction techniques, analysis conditions, geographical factors, in previous researches [25] it was determined that basic constituents of this essential oil were 1,8-cineole, *alpha*-pinene, linalool, bornyl acetate, *alpha*-terpineol, linalyl acetate and limonene. In another study [26], *alpha*-pinene and 1,8-cineol were reported to be present in high proportions and agreed on our results.

Linoleic acid, as a major fatty acid methyl ester component, has been shown in previous studies with its properties as skin moisturizing and skin healthiness activity in addition to high nutritional properties [3, 27].

Results given in this work shown that leaf essential oil of naturaly grown *M. communis* in Gökova (Muğla), Turkey is rich in 1,8-cineole, *alpha*-pinene, linalool and poor in sabinene, camphene and ocimene. Also seed oil of *M. communis* is rich in linoleic acid, palmitic acid and oleic acid.

In conclusion, essential oil and seed oil of M. communis may be considered as a natural raw material source for food, pharmaceuticals and cosmetic products. GC/MSD analyse results verified nutritional and industrial usage of *M. communis* has great advantages due to its significant chemical composition. Essential oil and seed oil of *M. communis* worth studying further.

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