

## Comparative Survey on the Essential Oil Composition from the Leaves and Fruits of *Pistacia mutica* Fischer Kerman Province

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**Abstract:** The chemical composition of the essential oils of the leaves and the fruits of *Pistacia mutica* Fischer (Anacardiaceae) from Kerman province, Iran were obtained by hydrodistillation method and analyzed by gas chromatography and gas chromatography mass spectrometry. Twenty-one compounds, accounting for 82.95% of the total oil with 0.75% oil yield were identified in the essential oil of the leaves. The main constituents of the essential oil were  $\alpha$ -pinene (25.25%), myrcene (9.99%),  $\gamma$ -cadinene (8.37%) and trans-caryophyllene (8.08%). Twenty-six compounds, accounting for 93.21% of the total oil with 1.25% oil yield were identified in the essential oil of the fruits. The main constituents of the essential oil were  $\alpha$ -pinene (21.47%), myrcene (15.90%), terpinolene (11.48%) and limonene (7.76%).

**Key words:** *Pistacia mutica* Fischer •  $\alpha$ -pinene • Chemical composition • GC/MS

### INTRODUCTION

The pistachio tree is native to western Asia and Asia Minor, from Syria to the Caucasus and Afghanistan. Archaeological evidence in Turkey indicate the nuts were being used for food as early as 7,000 B.C. The pistachio was introduced to Italy from Syria early in the first century A.D. Subsequently its cultivation spread to other Mediterranean countries. The tree was first introduced into the United States in 1854 by Charles Mason, who distributed seed for experimental plantings in California, Texas and some southern states. In 1875 a few small pistachio trees, imported from France were planted in Sonoma, Calif. In the early 1900's the U.S. Dept. of Agriculture assembled a collection of *Pistacia* species and pistachio nut varieties at the Plant Introduction Station in Chico, Calif. Commercial production of pistachio nuts began in the late 1970's and rapidly expanded to a major operation in the San Joaquin Valley. Other major pistachio producing areas are Iran and Turkey and to a lesser extent, Syria, India, Greece, Pakistan and elsewhere. *Pistacia mutica* Fischer (*Pistacia atlantica* subsp. *mutica*) is a dominant native *pistacia* species throughout Iran plateau and as natural habitat in the wild and mountainous regions can be found. From a botanical point of view, the genus *Pistacia* belonging to the family Anacardiaceae comprises 11 European species [1].

The genus *Pistacia* includes many species widely distributed in the Mediterranean and Middle Eastern areas. Origin is Mediterranean basin, Middle East, east to Afghanistan. Growth Habits is Large broad deciduous or semi-evergreen tree, growing slowly to moderately fast, up to 40 to 60 feet tall and wide (12-18 m); odd-pinnately compound leaves with 7 to 11 glossy leaflets rounded at their tip. Watering Needs is Moderate to little water when established, needs good drainage. Propagation is Seeds and layering. *Pistacia mutica* is often used as a rootstock for pistachio nut trees. Blooming habits is dioecious, meaning that there are female trees and male trees. The fruits appear on female tree only if there are male trees in the vicinity. The fruits are pink, ripening to dark blue or purple. Iran is one of the most important pistachio producing countries, along with Turkey, India and Greece. Pistachios are commercially used as in-shell snacks, in confectionery, in ice creams, candies, bakery goods and as a flavouring [2]. The vast number of studies reported in the scientific literature has shown a considerable variability in terpene composition of essential oils; besides, monoterpene profiles are under strong genetic control and, therefore, we believe that they can be used as biochemical markers to characterize species, populations, provenances, hybrids, families and clones. *Pistacia* species are known for their medicinal properties since antiquity [3,4]. Various species of this genus are known

for their use in folk medicine and are used in eczema treatment, throat infections, renal stones, asthma and stomach ache and as a astringent, anti-inflammatory, antipyretic, antibacterial, antiviral, pectoral and stimulant [5]. Several members of the genus *Pistacia* have been chemically investigated. They are characterized mainly by the occurrence of flavonoids and flavonoid glycosides [6]. These plants have also been reported to contain phenolic compounds and triterpenoids [7,8]. The essential oil of *Pistacia* species has been widely studied in other countries but yet to now the composition of the essential oil of *Pistacia mutica* that grow in Kerman province haven't studied. In the present work we have studied the chemical composition of essential oils of the leaves and the fruits of *Pistacia mutica* that grow in Kerman province in Iran and then the results were compared with various origins and species in other countries.

## MATERIALS AND METHODES

**Plant Material and Isolation Procedure:** The leaves with fruits of *Pistacia mutica* plant were obtained from plants grown in a village in Kerman province, Iran at full ripening stage in September 2009. The samples were cleaned in shade condition to prevent hydrolyze of the existing materials and to keep the natural color of the sample fixed. Then they were dried in the temperature of the environment and were powdered and kept at appropriate conditions from the viewpoint of temperature and light until the essential oil taking stage. Afterwards, essential oil was taken from 150 grams of the powdered sample with using hydrodistillation method apparatus Clevenger set for three hours. Following the sample oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yield from leaves was calculated on a dry weight basis as 0.75%. The oil extracted from fruits was calculated on a dry weight basis as 1.25%.

**Gas Chromatography:** GC analysis was performed using a model HP-439 gas chromatograph equipped with column CP Sil. 5CB in 25 meters length, internal diameter of 0.25 mm and film thickness 0.39  $\mu\text{m}$ . Oven temperatures was from 60-220 centigrade at a rate of 7 centigrade slope per minute. Injector temperature was 280 centigrade and detector (FID) temperature was 270 centigrade and carrier gas was helium.

**Gas Chromatography/Mass Mass Spectrometry:** In order to analyze and identify the combinations forming the essential oil, the Chromatograph Gas set attached to a Mass Spectrometry, Model Hewlett Packard-5973 was

used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column HP 5MS in 60 meters length, internal diameter of 0.25 mm and layer thickness of 0.25 micro meter, thermal program of oven (3 minutes) in 60 centigrade, then 60-220 centigrade with a 6 centigrade slope per minute, then 3 minutes in 220 centigrade, the temperature of injector 280 centigrade, gas conveying helium, the speed at a rate of 1.0 milliliter per minute, the ratio of fission 1 to 43, the rate of injection 0.1 micro liter, temperature of the reservoir of ionization 230 centigrade, ionization mode EI, Ionization energy 70eV. The series of normal Alkane C<sub>8</sub>-C<sub>17</sub> were also injected to the set under the same conditions with that of essential oil injection to calculate Restrictive Index (RI) of components of essential oil. The Restrictive Index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil was identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 existing in Absolution software of GC/Ms set and calculation of standard Restrictive Index in accordance with C<sub>8</sub>-C<sub>17</sub> Alkane and comparing them with the existing standard figures in references [9].

## RESULTS AND DISCUSSION

The essential oil was extracted by the hydrodistillation of the dried parts from leaves and fruits of *Pistacia mutica* from village in Kerman province, Iran and their constituents were analyzed by GC/MS. The oil yields were calculated on a dry weight basis as 0.75% (w/w) from leaves and 1.25%(w/w) from fruits. The identified combinations in essential oil, restrictive index (RI) and quantitative percentage of the compounds from leaves and fruits are presented (Tables 1 and 2). Of the 21 compounds being identified in the essential oil from leaves of this plant with 82.95%. The combinations of  $\alpha$ -pinene (25.25%), myrcene (9.99%),  $\gamma$ -cadinene (8.37%) and Trans-caryophyllene (8.08%) with 51.69 percent constitute the highest percentage of essential oil. Of the 26 compounds being identified in the essential oil from fruits of this plant with 93.21%. The combinations of  $\alpha$ -pinene (21.47%), myrcene (15.90%), terpinolene (11.48%) and limonene (7.76%) with 56.61 percent constitute the highest percentage of essential oil. The quality and quantity of the materials forming *pistacia* species essential oil had some differences and similarities with the cases reported in other regions. The studies of the ingredients of the essential oil of botanical populations with ecological and genetic differences

Table 1: Combinations identified in the essential oil of the leaves of *Pistacia mutica* Fischer from Kerman

Compound No.	Compound Name	Restrictive Index (RI)	Percentage (%)
1	Octane	800	3.55
2	Tricyclene	927	1.48
3	$\alpha$ -pinene	939	25.25
4	Camphene	954	5.06
5	Myrcene	991	9.99
6	Decane	1004	0.84
7	$\delta$ -3-carene	1031	0.45
8	Limonene	1029	1.36
9	Terpinolene	1089	3.21
10	Linalool	1097	0.61
11	$\alpha$ -terpineol	1189	1.54
12	Bornyl acetate	1289	3.25
13	$\beta$ -elemene	1391	1.8
14	Trans-caryophyllene	1419	8.08
15	$\alpha$ -gurjunene	1440	1.01
16	$\alpha$ -humulene	1455	4.38
17	Allo-aromadendrene	1460	1.02
18	$\alpha$ -amorphene	1485	0.79
19	$\alpha$ -muurolene	1500	0.72
20	$\gamma$ -cadinene	1514	8.37
21	$\delta$ -cadinene	1523	0.19
Total			82.95

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C<sub>8</sub>-C<sub>17</sub>) to HP-5MS column

Table 2: Combinations identified in the essential oil of the fruits of *Pistacia mutica* Fischer from Kerman

Compound No.	Compound Name	Restrictive Index (RI)	Percentage (%)
1	Tricyclene	927	2.63
2	$\alpha$ -pinene	939	21.47
3	Camphene	954	6.23
4	Sabinene	975	0.33
5	Myrcene	991	15.9
6	$\delta$ -2-carene	1002	0.32
7	A-phellandrene	1003	4.44
8	$\alpha$ -terpinene	1017	1.74
9	Unknown	1025	0.1
10	Limonene	1029	7.76
11	B-phellandrene	1030	4.88
12	Trans- $\beta$ -ocimene	1050	2.55
13	$\gamma$ -terpinene	1060	0.79
14	Terpinolene	1089	11.48
15	Allo-ocimene	1132	0.2
16	Camphor	1146	0.27
17	Borneol	1169	0.65
18	$\alpha$ -terpineol	1189	1.34
19	Bornyl acetate	1289	2.19
20	$\delta$ -elemene	1338	0.21
21	$\beta$ -elemene	1391	0.74
22	Trans-caryophyllene	1419	1.98
23	$\alpha$ -humulene	1455	0.89
24	$\alpha$ -amorphene	1485	0.38
25	$\alpha$ -muurolene	1500	0.3
26	$\gamma$ -cadinene	1514	3.25
27	$\delta$ -cadinene	1523	0.19
Total			93.21

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C<sub>8</sub>-C<sub>17</sub>) to HP-5MS column.

can be of great importance in identifying the variety of essential oil inside the population of specie. It seems that the geographical origin of *pistacia* species greatly influences the oil quality. The essential oil of *pistacia* species has been studied in Iran and in the world. The studies have reported the chemical composition of the essential oil from the fresh husk of *Pistacia vera* L. (Anacardiaceae) fruit from Iran was analyzed by GC/FID and GC/MS. The oil yield obtained by hydrodistillation was 0.25% + 0.05 (v/w). Twenty-six compounds, accounting for 98.1% of the oil, were identified. The main class of the compounds was found to be hydrocarbon monoterpenes (96.0%). The major constituents were limonene (35.1%),  $\alpha$ -pinene (28.7%) and  $\alpha$ -terpinolene (21.1%) [10]. A scientific study of the chemical composition of the essential oil obtained by hydrodistillation of *Pistacia vera* L. gum was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Twenty compounds were identified, representing 99.5% of the total components.  $\alpha$ -Pinene,  $\beta$ -pinene and  $\alpha$ -thujene were found to be the major constituents. The bacteriostatic activity of the essential oil against 12 clinical isolates of *Helicobacter pylori* was determined using the hole-plate method. All isolates were sensitive to the essential oil and the Minimum Inhibition Concentration (MIC) was 1.55 mg/ml for all isolates using the agar dilution method [11]. The oleoresin of *Pistacia atlantica* var. *mutica*, growing in different regions of Iran, is a popular naturally occurring chewing gum and has been used traditionally in the treatment of peptic ulcer. The GC-MS analysis of the essential oil, obtained from steam distillation of the oleoresin of *P. Atlantica* var. *mutica*, has led to the identification and quantification of eleven terpenoids,  $\alpha$ -pinene (70%),  $\beta$ -pinene (1.94%), 3-carene (0.2%), carveol (2.18%), epoxy-pinene (2.15%), limonene oxide (9%), myrtenol (5.31%), limonene (0.62%), citral (5.72%),  $\alpha$ -phellandrene (0.2%) and  $\beta$ -myrcene (0.3%). The total amount of essential oil obtained was 2.2% v/w which is higher than any other species of the genus *Pistacia* [12]. In this research samples were collected on July 1998 from Khojeer park which were in east of Tehran and the essential oils from fresh leaves and fruit obtained by steam distillation method sample by hydrodistillation method. The percentage of oils from female leaf was 0.04% and for male was 0.07% and for fruit was 0.58% calculated on the fresh weight. The essential oils were analysed by capillary GC and GC/MS on DB-1 column. The major constituents for female leaf were  $\alpha$ -pinene (21.7%),  $\gamma$ -cadinene (19.3%) and myrcene (4.7%) and for male leaf were  $\alpha$ -pinene (36.2%),

$\gamma$ -cadinene (17.9%) and  $\beta$ -pinene (6.3%) and for fruit were  $\alpha$ -pinene (73.6%),  $\beta$ -pinene (5.3%), myrcene (3.3%) and camphene (2.3%) [13]. The main and important ingredient studied in different regions of the world in Greece on the study essential oils of the fruits and the leaves of pistachio (*Pistacia vera* L.) were analyzed by GC and GC/MS. Fresh unripe pistachio fruits were richer in essential oil (0.5 %, w/w) than the leaves (0.1 %, w/w). Twenty one compounds were identified in the essential oil of the fruits and the major components were (+)- $\alpha$ -pinene (54.6 %) and terpinolene (31.2 %). The enantiomeric ratio of the major constituents of the essential oil of the fruits was determined using chiral GC/MS and it was found that the (+)/(-)- $\alpha$ -pinene ratio was 99.5:0.5, (+)/(-)-limonene 80:20, (+)/(-)- $\beta$ -pinene 96:4 and (+)/(-)- $\alpha$ -terpineol 0:100. Thirty three compounds were identified in the essential oil of the leaves and the major components were found to be  $\alpha$ -pinene (30.0 %), terpinolene (17.6 %) and bornyl acetate (11.3 %) [14]. Such as on the chemical composition of the essential oils, obtained by separate distillation of the leaves, branches and fruits of *pistacia lentiscus* from Tuscany (Italy), was analyzed and identified by GC and GC/MS. The leaf oil contained  $\alpha$ -Pinene (16.1-25.3%), limonene (6.6-12.3%), terpinene-4-ol (7.6-12.7%) and germacrene D (9.6-14.3%) as major components. The branch oil contained  $\alpha$ -Pinene (34.4-46.2%), myrcene (6.3-11.6%) and limonene (8.1-13.0%), while the fruit oil contained  $\alpha$ -Pinene (7.5-11.2%), myrcene (68.2-71.0%) and limonene (9.6-19.7%) as major constituents [15]. The comparative essential oil composition of various parts of the *Pistacia terebinthus* L. growing wild in Turkey showed major component as limonene (34.2%) [16]. Chemical composition of the essential oils of *Pistacia atlantica* Desf. Bornyl acetate (21.5%) has been reported as the main constituent [17]. The composition of the hydrodistilled essential oil of leaves of *Pistacia lentiscus* L. (Anacardiaceae) was analysed by GC-MS. Twenty-seven compounds were identified, representing 58% of the total oil.  $\alpha$ -Pinene (17%),  $\gamma$ -terpinene (9%) and terpinen-4-ol (12%) were characterized as the main constituents [18]. A scientific study of the chemical composition of essential oils obtained from the leaves of *Pistacia vera*, *Pistacia terebinthus*, *Pistacia lentiscus* and the resin of *Pistacia lentiscus* were analyzed by GC and GC-MS.  $\alpha$ -Pinene,  $\beta$ -pinene, limonene, terpinen-4-ol and  $\alpha$ -terpineol were found to be the major components [19]. Results of GC and GC-MS analyses of the essential oils of leaves, galls produced by *Baizongia pistacia* and ripe and unripe fruits of *Pistacia palaestina* Boiss. collected in Jordan are reported. Both qualitative and

quantitative differences between different parts of the plant were observed. The oil was rich in monoterpenes and the main constituents were  $\alpha$ -Pinene (63.1%) and myrcene (13.3%) in the leaves and  $\alpha$ -Pinene (49.4%), sabinene (22.8%) and limonene (8.1%) in the galls. (E)-Ocimene (33.8-41.3%), sabinene (20.3-24.1%) and (Z)-ocimene (3.8-13.0%) were the main ones in both unripe and ripe fruits. Sesquiterpenes have been detected in small quantities in leaves and fruits and in trace amounts in galls [20]. The essential oil from the gum of Pistachio (*Pistacia vera* L. (Anacardiaceae)) grown in Turkey was obtained by the hydro-distillation method and its chemical composition was analyzed by GC and GC-MS. Moreover, the antimicrobial activities of the oil against the growth of 13 bacteria and 3 pathogenic yeasts were evaluated using the agar-disk diffusion and minimum inhibitory concentration (MIC) methods. The results showed that the essential oil contained about 89.67% monoterpenes, 8.1% oxygenated monoterpenes and 1.2% diterpenes.  $\alpha$ -Pinene (75.6%),  $\beta$ -pinene (9.5%), trans-verbenol (3.0%), camphene (1.4%), trans-pinocarveol (about 1.20%) and limonene (1.0%) were the major components. The antimicrobial results showed that the oil inhibited nine bacteria and all the yeasts studied and the activities were considerably dependent upon concentration and its bioactive compounds such as carvacrol, camphene and limonene. Moreover, the essential oil of the gum was found to be more effective yeastcide than Nystatin, synthetic yeastcide. Furthermore, the antibacterial activities of the oil were lower than those of standard antibiotics, ampicillin sodium and streptomycin sulfate under the conditions studied [21]. The essential oil and gum of *Pistacia lentiscus* var. *chia*, commonly known as the mastic tree, are natural antimicrobial agents that have found extensive uses in medicine in recent years. In this work, the chemical composition of mastic oil and gum was studied by GC-MS and the majority of their components was identified.  $\alpha$ -Pinene,  $\beta$ -myrcene,  $\beta$ -pinene, limonene and  $\beta$ -caryophyllene were found to be the major components. The antibacterial activity of 12 components of mastic oil and the oil itself was evaluated using the disk diffusion method. Furthermore, attempts were made to separate the essential oil into different fractions in order to have a better picture of the components responsible for its antibacterial activity. Several trace components that appear to contribute significantly to the antibacterial activity of mastic oil have been identified: verbenone,  $\alpha$ -terpineol and linalool. The sensitivity to these compounds was different for different bacteria

tested (*Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*), which suggests that the antibacterial efficacy of mastic oil is due to a number of its components working synergistically. The establishment of a correlation between the antibacterial activity of mastic oil and its components was the main purpose of this research. Mastic gum was also examined, but it proved to be more difficult to handle compared to the essential oil [22]. The Anacardiaceae family is known for its species producing essential oil, such as *Pistacia* genus. As for pistachio tree (*Pistacia vera*), its fruit is composed by a fleshy and odorant envelope or hull (mesocarp + epicarp) covering the hard shell (endocarp) containing an edible seed. We aimed to characterize the oils of pistachio hulls (*Pistacia vera* L.) during fruit development. Fruits were sampled in the region of Kairouan (Middle of Tunisia). Yields generally increased and ranged between 0.1–0.4% on a dry weight basis. Monoterpene hydrocarbons, mainly represented by  $\alpha$ -pinene (15.0–47.4%) and terpinolene (32.2–51.1%) were prominent during all fruit development, reaching 90.3% of the oil at full ripeness [23]. The essential oils of *Pistacia lentiscus* L. leaves from two Algerian populations have been analyzed by GC and GC/MS. Approximately 95% of the components were identified among which terpinen-4-ol (17.3-34.7%),  $\alpha$ -terpineol (10.4-11.0%) and germacrene D (8.4-15.8%) were the major constituents [24]. Essential oils of leaves of *Pistacia khinjuk* Stocks, *P. chinensis* Bunge and *P. lentiscus* L., prepared by hydrodistillation and studied by GC and GC-MS, showed qualitative and quantitative differences. All three were found to be rich in monoterpene hydrocarbons. In *P. lentiscus* 4% sesquiterpene alcohols were found and no monoterpene alcohols, whereas in *P. khinjuk* and *P. chinensis* 16% and 8% monoterpene alcohols respectively were detected and no sesquiterpene alcohols [25]. Air-dried aerial parts of *Pistacia lentiscus* L. collected from different regions of Morocco (Mehdia, Oulmes and Chaouen) were water-distilled to produce oil in 0.2% yields. The chemical composition of *P. lentiscus* oils changes from a region to another. The variations in chemical composition are important between plant populations. A total of 45 constituents were identified. The major oil components of *P. lentiscus* from Oulmes were  $\alpha$ -pinene (16.5-38.5%),  $\beta$ -myrcene (10.2-11.5%) and limonene (6.8-9.8%), while terpinen-4-ol (32.7-43.8%),  $\alpha$ -pinene (7.1-13.5%) and bornyl acetate (6.8-10.3%) were the main constituents of Chaouen oil. For *P. lentiscus* from Mehdia, terpinen-4-ol (14.5-19.3%), caryophyllene oxide (6.5-10.3%) and limonene (6.7-8.1%) were the major components. The effect of harvesting time on the oil production and

chemical composition was also examined at different vegetative stages (December-June). For the three locations, the best oil content was obtained during the flowering period March-June [26]. The essential oil of *Pistacia* species has been widely in other countries studied but yet to now the composition of the essential oil of *Pistacia mutica* Fischer that grow in Kerman province haven't studied. In present study results showed the major oil components of the leaves and the fruits of *Pistacia mutica* from Kerman province, Iran were  $\alpha$ -pinene and myrcene that reported as the main constituents in many other *Pistacia* species, but in this study, in addition to terpinolene and  $\gamma$ -cadinene, we also identified Trans-caryophyllene and limonene as the major compound. The results of this study could be of interest for further phytochemical and biological investigation of *Pistacia mutica* taking into account that  $\alpha$ -pinene oil showed marked antimicrobial activity.  $\alpha$ -pinene was reported as an important constituents of *Pistacia* species.

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