Original Article

TIPS Trends in Pharmaceutical Sciences 2021: 7(2): 105-110.

Essential oil analysis of *Pistacia vera* L. hull samples from Iran

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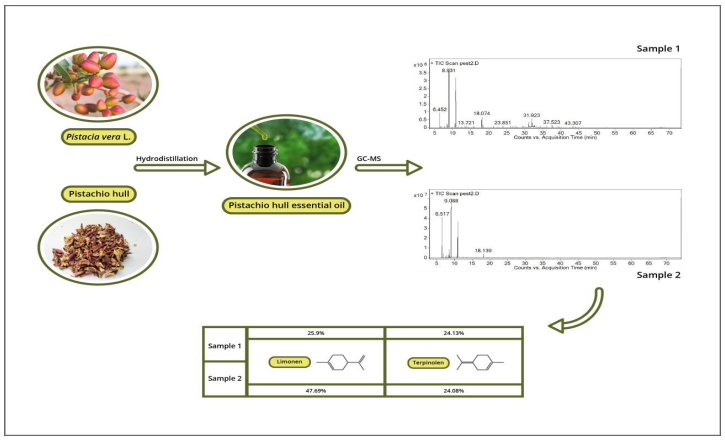
Graphical Abstract



Abstract

Pistachio hull (*Pistacia vera* from the Anacardiaceae family) is a by-product obtained after peeling pistachio fruits. Dried hull having numerous medical applications is sold in the herbal market of Iran. Antimicrobial, antioxidant, antimutagenic, cytoprotective, and antitumor activities of the hull have been proven. In the current study, two samples of pistachio hull were purchased from Shiraz, Iran. Hydrodistillation of samples and Gas chromatography–mass spectrometry (GC-MS) analysis of essential oils were carried out. Limonene and terpinolene were two major compounds of both essential oils although in previous studies, α-pinene has been reported as the major compound. The amount of limonene in samples 1 and 2 was 25.9% and 47.69% of total oils, respectively. The amount of terpinolene exceeded 24% of oils in both samples. Monoterpene hydrocarbons were predominant in both essential oils (57.65% and 94.86%). Because these compounds have numerous therapeutical effects, pistachio hull can be introduced as a valuable source for medicinal products.

Keywords: *Pistacia vera*, Pistachio hull, essential oil, GC-MS.

1. Introduction

Pistachio (*Pistacia vera* L.) from the Anacardiaceae family is a valuable fruit originating from Central Asia and the Middle East (1). The fruit is covered by a soft coat, then surrounded by a hard shell and a yellow-red fleshy covering or hull (1, 2). Iran, the United States, and Turkey

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Recieved: 08/03/2021; Accepted: 01/05/2021

are the largest producers of pistachio in the world (3). The share of Iran accounts for 44% of the world's pistachio production (4). The main areas under cultivation of this fruit in Iran are Kerman, Yazd, and Semnan provinces (5). The edible seeds are commercially important but the hull, which is separated from the shell at the harvest season, is routinely discarded to avoid staining of the shell (1).

The extract of *Pistacia vera* hull (PVH), rich in phenolic compounds as strong antioxidants, can be used as a substitute for synthetic antioxidants like BHA and BHT (3). Its scent is similar to Pinus resin because of the pinene content (2). Antimicrobial, antioxidant, antimutagenic, cytoprotective, and antitumor activities of PVH have been proven (3, 6, 7). Investigation on biologically active compounds of PVH can increase the value of this by-product in phytopharmaceutical industries (8).

In Traditional Iranian Medicine (TIM) manuscripts, pistachio is titled as Fostogh or Pesteh (9, 10). According to Al-Hawi-fi-Tibb (9th A.D.), Ekhtiarat e Badii (14th A.D.), and Tuhfat-al mu'minin (17th A.D.), the temperament of PVH is cold and dry, and it acts as a stomach and heart tonic. Its astringency leads to relieve diarrhea and strengthen teeth. PVH has been suggested for thirst, nausea, and halitosis (10-12).

The need for standardization of herbal products is globally increasing (13), therefore, studies related to the chemical composition of medicinal plants can be helpful for industries, specifically in pre-production phases.

In the present study, GC-MS analysis was performed to determine the chemical composition of the essential oils of two PVH samples from Iran. Moreover, the composition of oils was compared with those in similar studies.

2. Materials and methods

2.1. Plant material

Dried samples (PVH1 and PVH2) were purchased from the herbal market of Shiraz, Iran. The samples were authenticated by a botanist as *Pistacia vera* L. hull, and stored under voucher numbers PM-1330 and PM-1346 (PVH1 and PVH2, respectively) at the herbarium of the traditional pharmacy department, Shiraz Faculty of Pharmacy.

2.2. Extracting essential oil

For this purpose, the hydrodistillation method using a Clevenger apparatus was employed. Briefly, powdered PVH1 and PVH2 were immersed in water, followed by collecting the volatile oil after four hours. Next, the essential oil was dried over anhydrous sodium sulfate and kept at -20 °C.

2.3. GC-MS analysis of pistachio hull essential oil

Agilent Technologies (7693) apparatus was equipped with a DB-1MS column (25 m length × 0.25 mm i.d.; film thickness 0.25 µm) connected to a mass spectrometer. Helium was selected as the carrier gas with a flow rate of 1.2 ml/min, the split ratio was 1:10. The mass spectrometer was acquired in EI mode (70 eV) in a mass range of 30-300 m/z. Injector temperature was 250 °C, and detector temperature was 280 °C, while column temperature was linearly programmed from 70 to 280 °C (at the rate of 3 °C/min) and then held for 4 min at 280 °C. The whole run time was 74 minutes. Chromatograms were based on position 1.

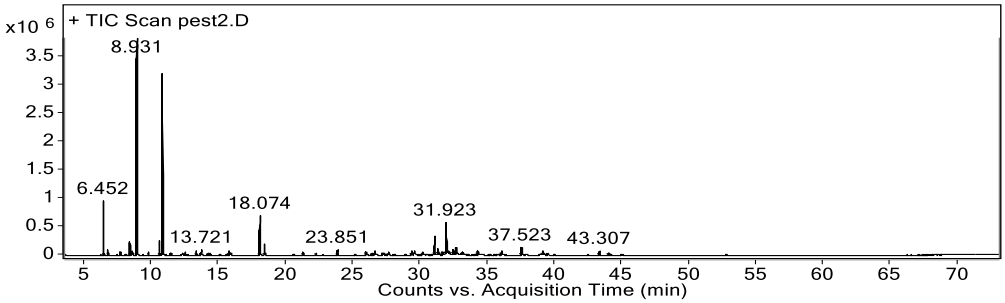

Figure 1. Gas chromatogram of PVH1 essential oil.

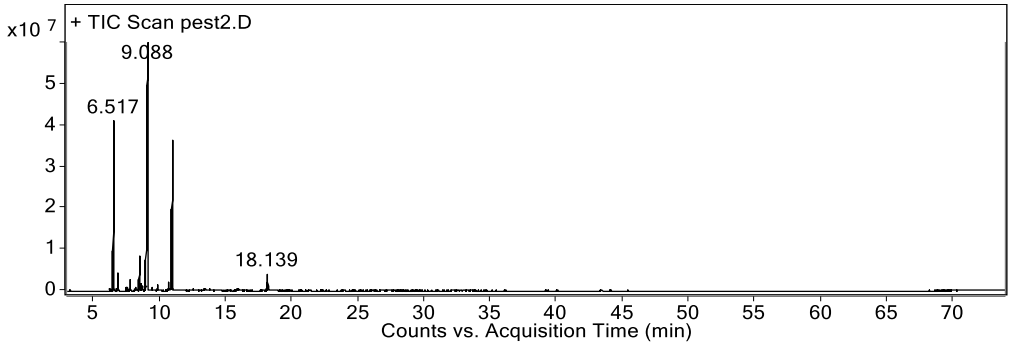

Figure 2. Gas chromatogram of PVH2 essential oil.

2.4. GC-MS data evaluation

A homologous series of n-alkanes C9-C30 was injected to GC/MS for identification and determination of the components via calculating the Kovats indices (KI). Identification of components was based on a comparison of their mass spectra with NIST and Adams' library of spectra 2017 as well as with those reported in previous studies (14). For calculating KI, the equation 1 for temperature-programmed columns was applied:

$$KI=100z+100\ (tRi-tRz)/(tR(z+1)-tRz) \quad \text{(Eq. 1)}$$

Where, z, i, and z+1 stand for number of previous carbon atoms in reference n-alkanes, substance for which the index shall be calculated, and number of next carbon atoms in reference n-alkanes, respectively.

3. Results

Gas chromatograms of PVH1 and PVH2 essential oils have been presented in Figure 1 and 2. Chemical constituents of PVH1 and PVH2 essential oils are shown in Tables 1 and 2. The main components of PVH1 essential oil were limonene (25.9%) and terpinolene (24.13%). Similarly, the main components of PVH2 essential oil were limonene (47.69%) and terpinolene (24.08%). Forty-three compounds and fifteen compounds were detected in PVH1 and PVH2 essential oils. Monoterpene hydrocarbons with high percentages of limonene (25.9%) and terpinolene (24.13%) had the highest amount in PVH1 essential oil (57.65%). These compounds were more predominant (94.86%) in PVH2 essential oil, with limonene (47.69%) and terpinolene (24.08%) as main components.

4. Discussion

Traditionally, PVH was applied as a stomach and heart tonic. Moreover, it has been suggested for thirst, diarrhea, nausea, and halitosis. Essential oils of PVH1 and PVH2 were characterized by the predominance of limonene and terpinolene although α-pinene was reported as a major compound in most previous studies with the amount

Table 1. Chemical constituents of PVH1 essential oil.

Peak	Structure name	%	RT	KIC	KIa
1	α-pinene	5.4	6.5	938	932
2	Camphene	0.5	6.8	949	946
3	Myrcene	0.4	7.7	982	988
4	δ-3-Carene	1.7	8.4	1006	1008
5	α-terpinene	0.46	8.5	1009	1014
6	o-cymene	0.48	8.6	1012	1022
7	Limonene	25.9	8.9	1021	1024
8	Gamma-terpinene	0.5	9.8	1047	1054
9	m-cymenene	2.11	10.6	1072	1082
10	Terpinolene	24.13	10.8	1079	1086
11	Thujone <cis>	0.55	11.5	1098	1101

12	Menthatriene <1,3,8-p->	0.36	12.5	1125	1108
13	Thujanol<3->	0.69	13.4	1147	1164
14	Acetophenone (para-methyl)	0.86	13.7	1157	1179
15	UN	0.38	14.2	1170	-
16	Methyl chavicol	0.26	14.4	1173	1195
17	Cuminaldehyde	0.62	15.8	1210	1238
18	Carvone	0.28	15.9	1213	1239
19	UN	7.14	18.1	1267	-
20	Thymol	2.08	18.4	127	1289
21	α-longipinene	0.52	21.3	1347	1350
22	Cubebene<alpha>	0.39	22.3	1372	1345
23	Caryophyllene (Z)	0.94	23.9	1412	1408
24	Curcumene<ar>	0.47	26	1467	1479
25	Curzerene	0.38	26.4	1479	1499
26	Sesquisabinene hydrate<cis>	0.74	26.6	1484	1542
27	γ -cadinene	0.87	27.6	1513	1513
28	γ-vetivenene	0.72	29.3	1556	1531
29	UN	0.92	29.5	1562	-
30	Spathulenol	0.62	30.2	1579	1577
31	UN	3.1	31.1	1602	-
32	UN	0.87	31.3	1609	-
33	Guaiene<cis-beta>	0.69	31.7	1619	1492
34	Aromadendrene epoxide <allo->	5.47	32	1627	1639
35	UN	0.79	32.4	1641	-
36	Cadalene	1.48	32.7	1647	1675
37	Cedrenal <1,7-diepi-α->	0.81	34.3	1693	1639
38	UN	0.95	36.1	1744	1772
39	Laurenene	1.75	37.5	1788	1879
40	Acorone<iso>	0.79	39.1	1836	1812
41	Longifolol acetate<iso>	0.38	39.5	1848	1819
42	UN	0.72	43.3	1969	-
43	Rimuene <tetrahydro>	0.31	44	1959	1960
	Total identified (%)	84.61%			
	Monoterpene hydrocarbon	57.65%			
	Oxygenated Monoterpene	3.6%			
	Oxygenated sesquiterpene	2.14%			
	Sesquiterpene hydrocarbon	5.36%			
	Sesquiterpene hydrocarbon	5.36%			

(RT: Retention time; KIC: Calculated Kovats index; KIa: Kovats index from Adams' library; UN: unknown).

range of 8.8-54.4% (2, 15-19). The characteristic scent of fresh PVH due to α-pinene content was absent in dried samples. The amount of α-pinene was 5.4% in PVH1, but this compound was not detected in PVH2 essential oil.

PVH2 essential oil had the least number of compounds compared to PVH1 and samples reported in other studies. The composition of PVH2 essential oil might have been affected by the low-quality storage conditions or the oldness of the sample. Regarding the reported amount of terpinolene in other studies (2.7-34.15%), terpinolene

Table 2. Chemical constituents of PVH2 essential oil.

Peak	Structure name	%	RT	KIC	KI Adam's
1	γ-terpinene	0.25	6.2	922	1054
2	Carene <delta 2>	16.4	6.5	933	1002
3	Camphene	1.47	6.8	945	946
4	Sabinene	0.31	7.5	972	969
5	Beta-pinene	0.94	7.7	982	974
6	UN	0.42	8.2	998	-
7	Carene <delta 3>	3.49	8.5	1007	1011
8	UN	0.62	8.6	1011	-
9	O-cymene	0.22	8.7	1013	1022
10	Limonene	47.69	9.1	1026	1024
11	Ocimene <E-beta>	0.25	9.4	1037	1044
12	UN	0.6	9.8	1049	-
13	m-cymenene	1.07	10.7	1074	1082
14	Terpinolene	24.08	11	1083	1086
15	UN	2.18	18.1	1268	-
	Total identified	96.17 %			
	Monoterpene hydrocarbon	94.86 %			
	Oxygenated monoterpene	-			
	Sesquiterpene hydrocarbon	-			
	Oxygenated sesquiterpene	-			
	Other constituents	5.36 %			

(RT: Retention time; KIC: Calculated Kovats index; KIa: Kovats index from Adams' library; UN: unknown).

in both samples was nearly the same (24.13% in PVH1 and 24.08% in PVH2). The percentage of limonene in PVH2 (47.69%) was almost twice that of PVH1 (25.9%). However, previous studies reported percentages of 1.4-6.62% for limonene in PVH essential oils (2, 6, 15-17).

The biological activities of the compounds in essential oils have been studied i.e. α-pinene has biological effect against bacteria, fungi, and insects. Myrcene has shown antifungal and insecticide effects. Limonene acts against fungi and insects (17). It is worth extracting essential oil from PVH, as a waste product, because of the high-value of volatile compounds which are used in pharmaceutical and cosmetic industries.

5. Conclusion

In this study, limonene was detected as the major compound of PVH1 and PVH2 essential oils. In both samples, monoterpene hydrocarbons were predominant with high percentages of limonene and terpinolene (more than 24% each). Therefore, this composition valorizes PVH as a source of bioactive molecules and aroma compounds used in industries. The number of compounds in PVH2 essential oil was less than that in PVH1 and those in literature, suggesting the potential effect of storage conditions and oldness of samples on the composition of essential oils.

Acknowledgement

This study was a part of Pharm. D thesis by Seyede Zahra Ayatollahi, supported by Shiraz University of Medical Sciences under grant no. 99-7844. The authors would like to thank Ms. Sedigheh Khademian for the authentication of herbal samples and Ms. Aida Iraji for instrumental assistance.

Conflict of Interest

None declared.

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