Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (4) October-December, pp.265-270/Zhiani et al.
Research Article

CHEMICAL COMPOSITION OF THE OIL OF *PINUS PINEA L.* SEEDS FROMTWO LOCATIONS IN IRAN

[*]Zhiani R.[1], Abedi F.[1], Moradi M.[2], Emrani Sh.[3], Sharafkhani J.[3] and Taheri M.[3]
[1]Young Researchers and Elite Club, Neyshabur Branch, Islamic Azad University, Neyshabur, Iran
[2]Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, Iran
[3]Department of Chemistry, Faculty of Sciences, Neyshabur branch,
Islamic Azad University, Neyshabur, Iran
**Author for Correspondence*

ABSTRACT
Pine nuts contain healthy amounts of minerals like manganese, potassium, calcium, iron, magnesium, zinc and selenium. This paper presents a study of the chemical composition ofthe oils within pine seeds from two locations in Iran, extracted using the Soxhlet method and analyzed with gas chromatography and mass spectroscopy. A total of 38 compounds were identified with in the oils, and a relatively high variation in their contents was found. The main constituents of the pure oil were: Manool oxide, Germacrene D, γ-Cadinene, (E)-Caryophyllene, γ-Terpinene, α-Terpineol and Guaiol.

Keywords: *Pinus Pinea L, Chemical Composition, Oil, Gas Chromatography*

INTRODUCTION
Pine trees (family Pinaceae, genus Pinus). Several species of Pinus are known and they are grouped as soft pines (haploxylon) and hard pines (diploxylon). Pinus pinea is present in diploxylon pine groups and its seeds are the common pine nuts present in world markets (Mirov, 1967).
The composition of pine nuts shows variation among species and even some subspecies depending on geographical and climatic conditions (Sagrero-Nieves, 1992; Savage, 2001).
Recently there has been considerable interest in biologically active compounds from plants as sources of bio-pesticides. oils from aromatic plants are examples ofcompounds with potential to control pests; they are becoming more popular because many synthetic drugs are connected with unpleasant side effects. Volatile oils also represent an interesting alternative due to the emerging esistance of pests against synthetic agents (Singh *et al.*, 2003).
Certain treaties and laws negotiated by tribes in Nevada guarantee Native Americans' right to harvest pine nuts (Frazier, 2009). Pine nuts can cause taste disturbances, lasting between a few days to a few weeks after consumption. A bitter, metallic taste is described. Thoughunpleasant, there are no known lasting effects, with the FDA reporting that there are "no apparent adverse clinical side effects" (Mostin, 2001). This phenomenon was first described in a scientific paper in 2001 (Middleton, 2009) Publications have made reference to this phenomenon as "pine nut syndrome" or as "pine mouth" (The Great Pine Nut Mystery, 2011).
The Nestlé Research Centre has hypothesized that nuts from a particular species of pine occurring mostly in China, Pinus armandii, is the cause of the problem. The suspected species of pine nuts aresmaller, duller, and more rounded than typical pine nuts (Destaillats *et al.*, 2011). A 2011 study found results consistent with this hypothesis and also suggested that chemicals used in the shelling process might be responsible (Munk, 2010). Metallic taste disturbance, known as metallogeusia, is typically reported 1-3 days after ingestion, being worse on day 2 and lasting typically up to 2 weeks. Cases are self-limited and resolve without treatment (Moller, 2010). Moller has postulated a hypothesis that could explain why thebitter taste appears several days after ingestion and lasts for so long (US FDA). A well known physiological process known as enterohepatic recirculation (EHR) could play a key role in the development of pine nut syndrome, which is currently under investigation by the FDA (Hughes *et al.*, 2008). The objectives of this study were to investigate the chemical composition of the oils within Pinus pinea L. seeds from Iran.

MATERIALS AND METHODS

Samples of Pinus pinea L. seeds were collected from North and South Khorasan in Iran. To measure the amount of oil in a pine nut, the nut was powdered in an electric mill and the oil extracted using the Soxhlet method with a solvent of Hexan. After removing the solvent by evaporation, the amount of oil in the samples was calculated and expressed as a percentage of the overall sample weight before oil extraction (Horwitz, 2000).

Determination of Composition

To analyze samples Agilent's gas chromatography method was used. The gas chromatography machine used was the Agilent 7890 (Agilent Corporation of America) equipped with Mass Spectroscopy detector 5975C inert MSD.

chromatography conditions were according to AOAC method No. 963/22 (28) with capillary column of DB-35 MS of length 30 meters and diameter 0.25 mm. The thickness of Polar Silica was 0.25 micrometers. Chromatography temperature parameters were set as follows: injector at 250◦C, ion source at 200◦C. The oven was set as follows: initial temperature of 80◦C for 20s, then heated from 80◦C to 240◦C at a rate of 4◦C.min-1 , and finally 240◦C for 10 min. Carrier gas was helium; column flow was 1.4 ml.min-1 with split rate 1:30. A 1.0 mm3 sample was injected. The energy of the EI source of the Agilent mass spectrometer was 70eV.

RESULTS AND DISCUSSION

In the experiment, the oils, Pinus pinea L from South Khorasan constituted 34% of the chemical composition, while Pinus pinea L from North Khorasan constituted 42% of the chemical composition. Analysis revealed that the oil of Pinuspinea L from South Khorasan contained mainly Manool oxide (20.4%), Germacrene D (15.4%), γ-Cadinene(11.8%), (E)-Caryophyllene(7.8%), γ-Terpinene (3.6%) and α-Cadinol (3.4%). A total of 38 compounds amounting to 95.1% of the sample were identified from the oil of Pinus pinea L from North Khorasan. Its major components were Manool oxide (21.9%), Germacrene D (15.8%), γ-Cadinene (10.5%), (E)-Caryophyllene (6.4%), α-Cadinol (4.6%) and γ-Terpinene (4.4%) as shown in Table 1.

Table 1: Compounds obtained from GC/GC-MS analysis of *Pinus pinea L.* seed's oils*

Compound	**RI**	***South Khorasan (%)***	***North Khorasan (%)***
α-Pinene	940	1.5	0.6
Sabinene	977	1.4	2.7
β-Pinene	981	1.0	0.8
β-Myrcene	992	0.3	0.1
Limonene	1031	2.3	2.2
Ocimene	1044	0.5	0.3
γ-Terpinene	1060	3.6	4.4
p-Cresol	1074	0.7	-
Terpinolene	1088	1.2	0.8
Camphenone	1094	-	0.4
Linalool	1097	1.3	1.1
α-Terpineol	1189	1.7	1.4

Verbanol	1195	1.9	2.1
Linalool acetate	1257	1.4	1.3
Isobornyl acetate	1286	-	0.9
α-Copaene	1376	0.3	0.4
Longifolene	1387	0.8	0.1
β-Cubebene	1388	0.2	0.1
(E)-Caryophyllene	1420	7.8	6.4
α-Guaiene	1439	0.3	0.7
α-Humulene	1454	1.4	1.1
(E)-β-farnasene	1456	0.4	0.2
α-Muurolene	1477	0.6	0.9
α-amorphene	1484	2.3	2.8
Germacrene D	1485	15.4	15.8
α-muurolene	1500	2.1	1.3
α-Farnesene	1506	2.8	1.9
Germacrene A	1508	-	0.1
γ-Cadinene	1523	11.8	10.5
(E)-nerolidiol	1563	0.8	0.4
Caryophyllene oxide	1584	0.1	0.3
Guaiol	1595	1.4	1.3
α-Cadinol	1653	3.4	4.5
(*E.E*)-Farnesol	1722	1.1	2.8
(*Z.E*)-Farnesol	1743	0.9	0.8
(2E, 6E)-farnesyl acetate	1846	0.7	0.8
Manool oxide	1988	20.4	21.9
Thumbergol	2045	0.7	0.9
% Peaks identified		94.5	95.1
Total yield % (mL/100g)		34	42

**GC/ MS analysis of oil of Pinus pinea L. seeds*

Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (4) October-December, pp.265-270/Zhiani et al.
Research Article

The retention data of Pinus pineaL. seeds oil are presented in Fig. 1 and 2.The results from this study revealed that there is high variability between the chemical components of oils from two different locations in Iran.

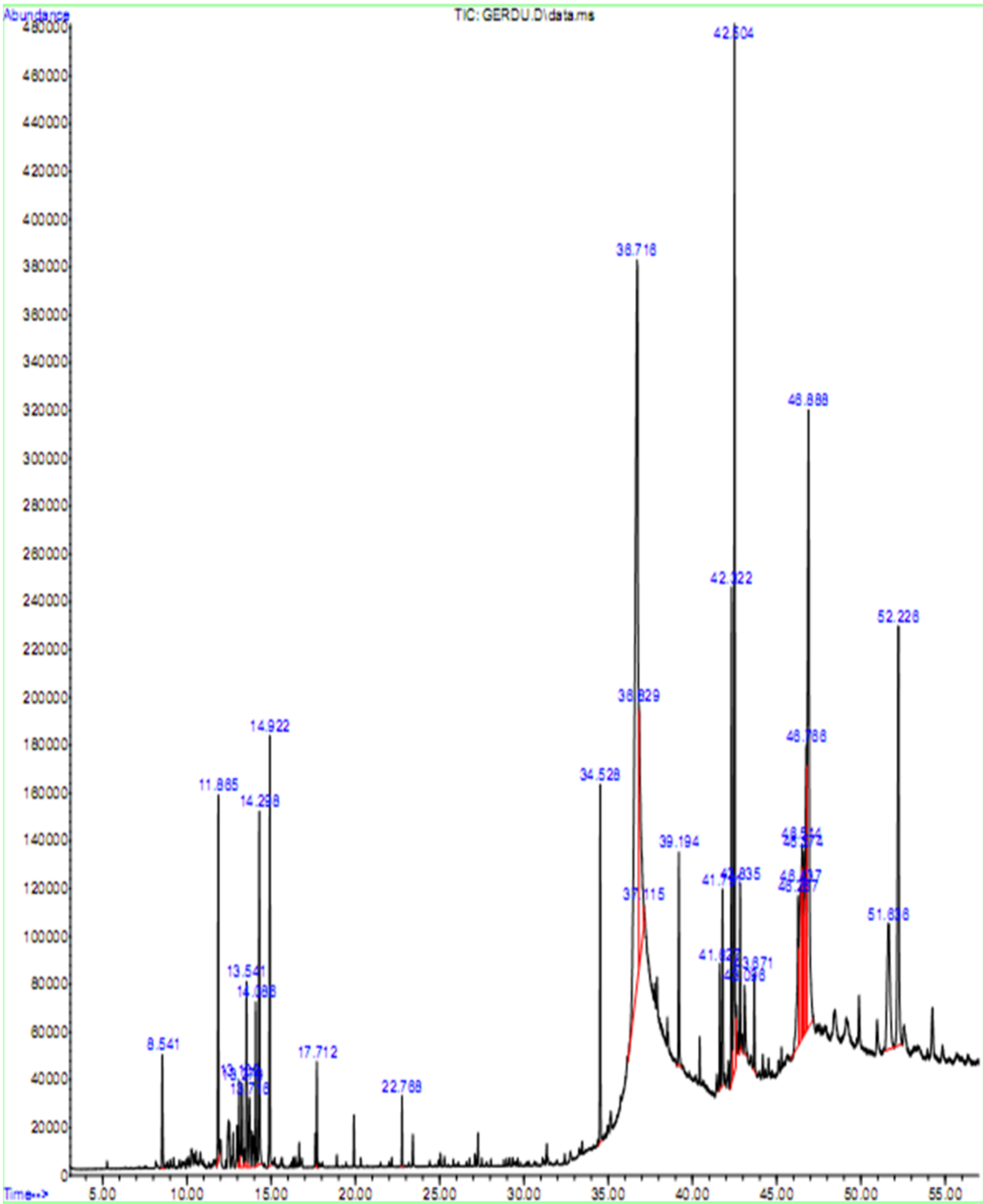

Figure 1: Chromatogram ofPinus pinea L.seeds oil of plants collected from the northwest region of Khorasan, Iran

Research Article

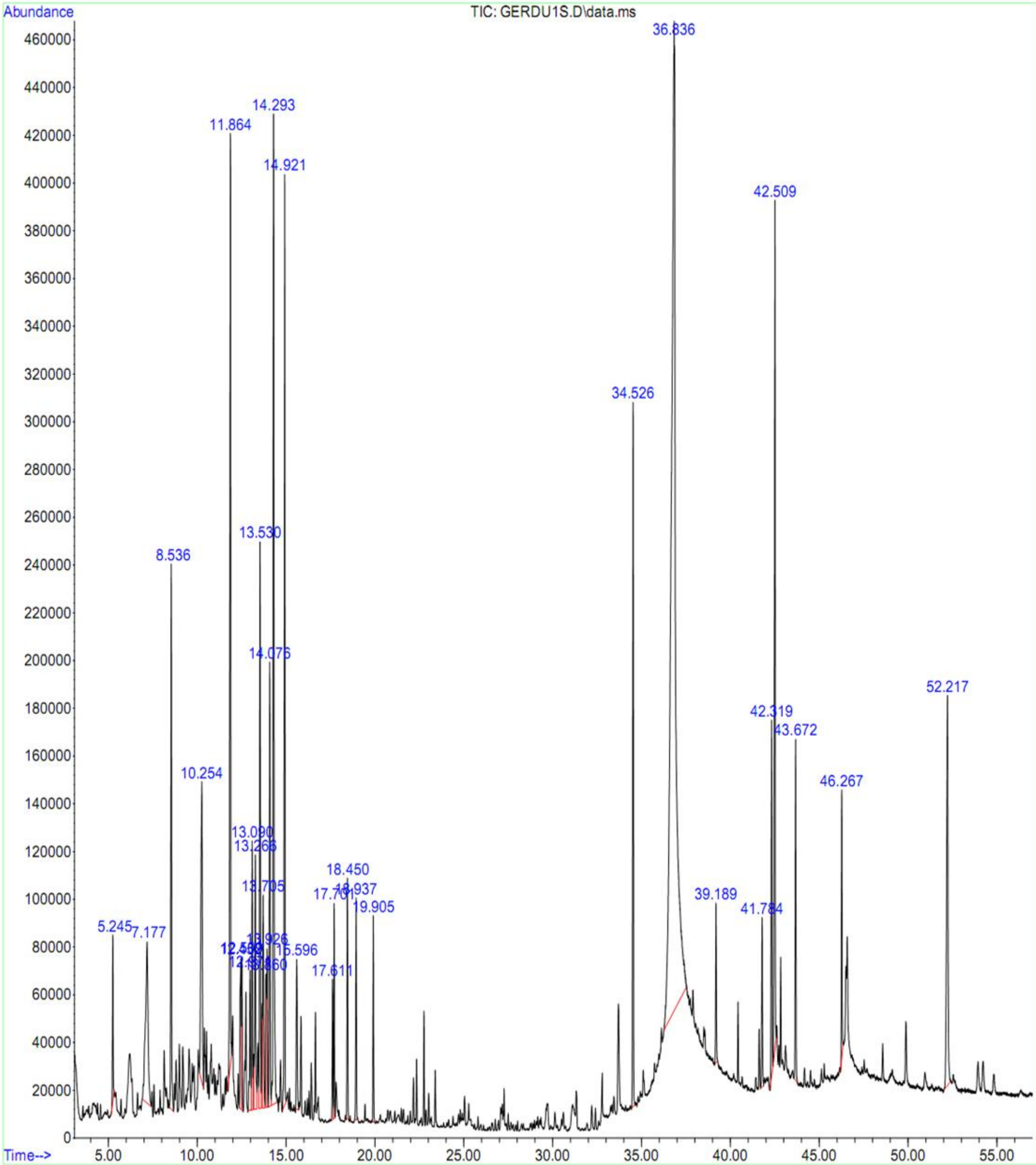

Figure 2: Chromatogram ofPinus pineaL.seeds oil of plants collected from the southern region of Khorasan, Iran

Each organic compound from the oil samples was identified based on their retention time indices in the resulting gas chromatograph with reference to homologues series of n-alkanes. The obtained mass spectrum of each analyte was also compared with the mass fragmentation from library pattern number NIST 08. L database / ChemStation data system, and also was compared with other results from previous researches. Table 1 presents the results of the analyses carried out in this study.

Conclusion

In conclusion, this study shows that the difference in seed oils between the two locations in Iran was relatively small, which has not been previously reported. The results in this paper also confirm that the seeds are a rich source of many important nutrients that appear to have a very positive effect on human health.

REFRRENCES

Destaillats F, Cruz-Hernandez C, Giuffrida F, Dionisi F, Mostin MO and Verstegen G (2011). Identification of the Botanical Origin of Commercial Pine Nuts Responsible for Dysgeusia by Gas-Liquid Chromatography Analysis of Fatty Acid Profile. *Journal of Toxicology* 1–7.

Frazier P (2009). Pine Nuts, Politics and Public Lands, *Raw Foods News Magazine*.

Horwitz W (2000). *Official Methods of Analysis of the AOAC*, 17th edition, AOAC. *Iternational*, MD, USA **49** 1-28.

Hughes GM, Boyland EJ, Williams NJ,Mennen L, Scott C, Kirkham TC, Harrold JA, Keizer HG and Halford JC (2008). The effect of Korean pine nut oil (PinnoThin™) on food intake, feeding behaviour and appetite: A double-blind placebo-controlled trial. *Lipids in Health and Disease* **7** 6.

Middleton C (2009). Pine mouth puzzle: Why do these nuts leave you with a bitter taste?, Daily Mail, <http://www.dailymail.co.uk/health/article-1184261/Pine-mouth-puzzle-Why-nuts-leave-bitter-taste.html>, (Retrieved 2009-09-01).

Mirov NT (1967). *The Genius Pinus* (The Ronald press company) New York 602.

Moller G (2010). The Curious Case of the Epicurean Nut. *Food Technology Magazine* **64** 5.

Mostin M (2001). Taste disturbances after pine nut ingestion. *European Journal of Emergency Medicin* **8** 76.

Munk MD (2010). Pine Mouth Syndrome: Cacogeusia Following Ingestion of Pine Nuts (Genus: Pinus). *Journal of Medical Technology* **2** 158-159.

Sagrero-Nieves L (1992). Fatty acid composition of mexican pine nut (Pinus cembroides) oil from three seed coat phenotypes. *Journal of the Science of Food and Agriculture* **59** 413–414.

Savage GP (2001). Chemical composition of wallnuts (Juglans regia L.) grown in New Zealand. *Plant Foods for Human Nutrition* **56** 75–82.

Singh HP, Batish DR and Kohli RK (2003). Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. *Critical Reviews in Plant Sciences* **22** 239–311.

The Great Pine Nut Mystery (2011). U.S Food and Drug Administration. 'Pine Mouth' and Consumption of Pine Nuts. Availble: http://pinenutsyndrome.wordpress.com.

