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Comparative Chemical Composition of *n*-Hexane and Ethanol Extractives from The Heartwood of Black Locust

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In this study, extractives from the heartwood of *Robinia pseudoacacia* L. (black locust), which was cultivated in the arid region of Iran, were obtained by two steps of extraction. In one step of extraction, fresh wood meal sample was inserted in a balloon and its extractives have been eliminated with *n*-hexane solvent. In the second step of extraction, the extracted wood meal residue was inserted in ethanol solvent at ambient temperature. The chemical compositions of two extractives were analyzed by gas chromatography-mass spectrometry (GC/MS). The comparison extraction extracts has shown that the major components in the *n*-hexane extract mainly to be contained the hexadecanoic acid, trimethylsilyl ester (13.39 %), (Z,Z)-9,12-octadecadienoic acid (10.10 %), tetradecane (6.88 %), *bis*(2-ethylhexyl)phthalate (6.21 %) and hexadecane (6.15 %), while the major components in the ethanol extract are resorcinol (51.96 %), (Z,Z,Z)-9,12,15-octadecatrien-1-ol, (6.27 %), hexadecanoic acid (6.06 %) and (Z,Z)-9,12-octadecadienoic acid (4.08 %). The same components present in the two different extracts also contained amounts of the octadecane, hexadecanoic acid, ethyl ester, (Z,Z)-9,12-octadecadienoic acid, linoleic acid ethyl ester and (Z,Z,Z)-9,12,15-octadecatrienoic acid.

Key Words: Heartwood extractives, Chemical composition, *Robinia pseudoacacia* L., Hexadecanoic acid, Trimethylsilyl ester, 1,3-Benzenediol.

INTRODUCTION

The extractives of trees are among the many classes of compounds known as secondary or special metabolites[1]. The concentrations of these metabolites vary between in softwood and hardwood and also among wood species from tree to tree and from season to season which can be classified according to their morphological site and function in the tree and according to their polarity and solubility in different solvents[2,3]. They present numerous challenges to utilization of trees and numerous opportunities for adding value to forest products such as adhesive, preservatives, food, perfume sector, pharmaceuticals and pharmacological applications[4].

The compounds within the extractives that play a main role in the protection of the tree against pathogens or other biotic attacks, which their presence is responsible for the natural durability of solid wood[5].

The genus *Robinia* of the family Legominoseae is considered as one of the most planted, fast-growing deciduous hardwood tree species which have been a kind of quality wood of multiple industrial purposes due to the valuable trait in stiffness, wear resisting and high basic density. This species is widely distributed throughout the temperate and Mediterranean zones of world.

Generally, the sapwood zone of black locust is quite narrow and most parts of the cross section area are heartwood. The colour of sapwood is yellowish and the heartwood is dark green or olive drab and both of them are inclined to colour variation during drying.

Black locust (*R. pseudoacacia* L.) is one such potential species, a nitrogen fixing tree[6,7], native to the south-eastern part of north America[8] and is a commonly used species for afforestation projects in arid and semi arid regions of Iran due to its soil rehabilitation capabilities. In Iran for instance, *ca.* 90 % of the total land area of the country is covered by arid or semi arid areas[9] and Afforestation projects are a frequent approach to control desertification in arid zones in many countries[10]. Plantations with N fixing trees can generally influence soil fertility but also improve the growth of associated trees positively by enriching N and organic matter including other nutrients such as phosphorus[11].

R. pseudoacacia L., which is a plant, was chosen for the extraction studies and this plant species contains a large amount of extract[12,13]. Among the extractive compounds tannins, flavonoids (*e.g.* robtein, butein, *etc.*), flavanones, flavanonols (*e.g.* dihydrorobinetin), flavonols (*e.g.* robinetin), stigmasterol, choline, syringenin, starch, simple sugars, water-soluble proteins and related metabolites have been identified[3].

Meszaros *et al.*[14] in the performed studies on the composition, thermal behaviour of extractive components of *R. pseudoacacia* L., have been used the thermogravimetry/mass spectrometry (TG/MS), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and methylation-gas chromatography/mass spectrometry (THM-GC/MS) methods and were compared the extraction procedures. They have shown that the quality of the solvent and the extraction time significantly influence the amount of extract eliminated from the plant sample and all the applied solvents (ethanol, acetone and dichloromethane) were capable of removing the phytosterols from the wood. However, fatty acid esters remained in the extracted wood samples in significant amounts even after successive extraction by dichloromethane, ethanol and water.

Meszaros et al.[14] generally showed that the chemical composition in the ethanol and acetone extracts from the Py-GC/MS experiments have been include benzoic acid, methyl-inositol, neophitadiene, *n*-hexadecanoic acid, 9,12-octadecanoic acid, isomer of octadecanoic acid, 1-docosane, *n*-docosane, 1-tetracosane, *n*-tetracosane, 1-pentadecane, *n*-pentadecane, *n*-docosanoic acid, 1-hexacosane, hydrocarbon compound and *n*-nonacosane. The identified chemical composition in the wood extract from the THM-GC/MS experiments have been include *n*-dodecanoic acid methyl ester, nonanedioic acid dimethyl ester, *n*-tetradecanoic acid methyl ester, *n*-hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, *n*-octadecanoic acid methyl ester, 9,12-octadecadienoic acid methyl ester, 6,9-octadecadienoic acid methyl ester, 10,13-octadecadienoic acid methyl ester, *n*-nonadecenoic acid methyl ester, tridecandioic acid dimethyl ester, *n*-eicosanoic acid methyl ester, 9,12-octadecadienoic acid, hydrocarbon, *n*-docosanoic acid methyl ester, *n*-tricosanoic acid methyl ester, 3,3′,4,4′-tetramethoxy-stilbene, *n*-tetracosanoic acid methyl ester, *cis*-13-docosenoic acid, alkene, docosandioic acid dimethyl ester and *n*-hexacaosanoic acid methyl ester.

Magel *et al.*[15] determined three dominant heartwood extractives in the trunkwood of *R. pseudoacacia* L. and the results showed that all trunks exhibited the highest contents of non-structural storage carbohydrates (glucose, fructose, sucrose and starch) in the outermost sapwood zone and the levels of carbohydrates decreased with increasing depth of the trunk[15]. A very low amount of the flavanonol dihydrorobinetin (DHR) is present in the younger sapwood of *R. pseudoacacia* L. Higher amounts of DHR and the hydroxyl cinnamic acid derivative (HCA) were found in the sapwood-heartwood transi-tion zone. DHR increased within the heartwood. HCA increased towards the heartwood and decreased again in the inner heartwood parts. Flavonol robinetin (ROB) appeared in the innermost parts of the sapwood-heartwood transition zone and reached maximum values in older parts of the heartwood[15]. Early work has shown the presence of stigmasterol, choline, syringenin, starch and simple sugars and related metabolites.

Roux and Paulus[16] identified 14 flavonoids from the MeOH extract of the heartwood using paper chromatography, most notably robinetin (3,7,3′,4′,5′-pentahydroxy flavonol) and dihyrorobinetin (3,7,3′,4′,5′-pentahydroxydihydroflavonol). Besides, Smith *et al.*[17] claim that remarkable decay resistance of the heartwood of black locust is due to high flavonoid concentrations (6 % of dry weight), specifically the constituents, robtin (15,000 ppm), robinetin (20,000 to 80,000 ppm) and dihydrorobinetin (53,000 to 176,000 ppm) and other, flavonoids present in the heartwood are known butein, butin, fisetin, fustin and liquiritigenin[18].

In the recent decades, the black locust has been started to be cultivated in the arid part of Iran. So far, less[19] or no study has been done on the chemical composition of black locust cultivated in Iran. Therefore, in this study it was aimed at determining the chemical composition of the black locust cultivated in Iran.

EXPERIMENTAL

Robinia is planted in nearly all arid and semi-arid Afforestation projects of Iran. After pre-selection of available Robinia stand in location of Karaj, one tree of Robinia was chosen for this investigation. The selected study site was located in same climatic condition in terms of amount of precipitation, average temperature and elevation. Using the FAO[20], the Karaj study site is classified as arid area. According to Dawan and Famouri[21] soil in Karaj is characterized as calcareous Lithosol. It is supposed that this study site is representative for arid area in Iran. Climatic data of the study site was provided by the Iran Meteorological Organization for 40 years from 1965 to 2005 (Table-1).

Preparation of extractives: In this study, the extractives of black locust (*Robinia pseudoacacia* L.), which was cultivated in the arid region of Iran, were obtained from the fresh heartwood sample. The heartwood sample was milled to a very fine homogenous composition and ground to a fine powdery mixture. The extractives of wood meal sample were extracted by two steps of extraction (Maceration technique). In first step, fresh heartwood meal sample (10 mg) was inserted in a 200 mL balloon and its extractives have been eliminated with *n*-hexane solvent (150 mL). In the second step, the extracted heartwood meal residue (Slag) was inserted in ethanol solvent (150 mL). Every one of steps of extraction to take a 15 days long time at ambient temperature and its chemical compositions were analyzed by GC-MS. Extracts from the *n*-hexane and ethanol were dried by evaporating the solvent at 40 ºC until a viscous deposit was left in the flask, then the extracts were dried over anhydrous sodium sulfate and stored at -18 ºC.

Chemical analysis: In order to identify components of extracts, trimethylsilylation was achieved by heating 1 mg of sample at 70 ºC for 1 h with 30 µL *bis*(trimethylsilyl)trifluoroacetamide (BSTFA) with 10 µL of trimethylchlorosilane and 30 µL pyridine.

TABLE-1
BASIC SITE CONDITIONS FOR SELECTED AREA

Study site	Elevation (m)	Latitude	Longitude	Annual temperature (ºC)			Annual precipitation (mm)
				Min.	Max.	Average	
Karaj	1275	35º44'N	51º10'E	8.7	21.2	15.7	243.8

The *n*-hexane and ethanol extractives were analyzed on an Agillent 5975B mass spectrometer coupled with a Hewlett-Packard GC-6890N series GC by using a HP-5MS (5 % phenyl methyl siloxane) fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thicknesses) with Agilent 19091J-133 model number. Helium having a flow rate of 1 mL/min, was used as carrier gas. The GC oven temperature was kept at 50 ºC for 5 min and programmed to 250 ºC at a rate of 20 ºC/min and then kept at 250 ºC. The injector temperature was 250 ºC. The amount of injection was 1 μL. The carrier gas was delivered at a constant pressure of 7.35 psi. MS spectra were taken at E1 ion source of 70 eV. Identification of the components was based on comparison of their mass spectra with those of internal (computer) library, NIST libraries and some reference compounds. The identification of the chemical constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature[22-24]. Retention indices (RI) were determined with reference to a homologous series of normal alkanes, by using the following formula[25].

$$RI = 100\,[(n + (N-n) \times \log t1R\,(x) - \log t1R\,(Cn)/\log t1R\,(CN) - \log t1R\,(Cn)] \quad (1)$$

where RI is the retention index of the compound of interest, t1R is the net retention time (tR-t0), t0 is the retention time of solvent (dead time), tR is the retention time of the compound of interest, Cn and CN are No. of carbons in the *n*-alkanes eluting immediately before and after the compound of interest, N and n are the number of carbon atoms in the *n*-alkane eluting immediately before and after the compound of interest.

RESULTS AND DISCUSSION

The total ion chromatograms of the *n*-hexane and ethanol extractives by GC/MS are shown in Figs. 1 and 2, respectively. Relative content of each component was counted by area normalization. The MS data and the NIST standard MS map were analyzed by computer, open-published books and papers. It was also shown that in the obtained peaks from the *n*-hexane and ethanol extractives (Figs. 1 and 2) were found 28 and 14 compounds which 90.20% and 85.65% of the total 39 and 28 peaks areas were identified (Tables 2 and 3).

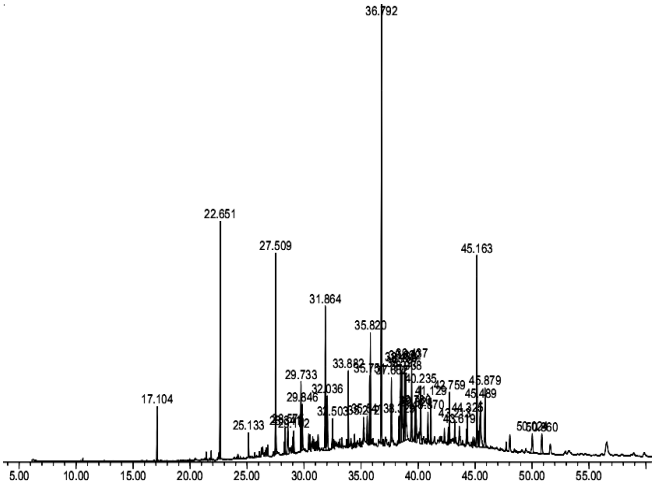

Fig. 1. Total ion chromatogram of *n*-hexane extractive of black locust heartwood by GC/MS

TABLE-2
COMPONENTS OF THE *n*-HEXANE EXTRACT FROM *R. pseudoacacia* L. HEARTWOOD BY GC/MS ANALYSIS

No.	Compound	m.w.	m.f.	RT[a]	KI[b]	Area (%)
1	Dodecane	170	$C_{12}H_{26}$	17.104	1199	1.39
2	Tetradecane	198	$C_{14}H_{30}$	22.651	1401	6.88
3	Pentadecane	212	$C_{15}H_{32}$	25.133	1500	0.66
4	Hexadecane	226	$C_{16}H_{34}$	27.509	1601	6.15
5	5-Phenyl-undecane	232	$C_{17}H_{28}$	28.339	1639	0.70
6	4-Phenyl-undecane	232	$C_{17}H_{28}$	28.572	1650	1.64
7	Hexadecamethyl-cyclooctasiloxane	593	$C_{16}H_{48}O_8Si_8$	29.102	1673	0.72
8	Heptadecane	240	$C_{17}H_{36}$	29.733	1701	1.93
9	2,6,10,14-Tetramethyl pentadecane	268	$C_{19}H_{40}$	29.846	1706	1.78
10	Octadecane	254	$C_{18}H_{38}$	31.864	1801	4.26
11	2,6,10,14-Tetramethyl-hexadecane	282	$C_{20}H_{42}$	32.036	1810	2.69
12	Nonadecane	268	$C_{19}H_{40}$	33.882	1901	3.16
13	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	35.752	1998	2.08
14	Eicosane	282	$C_{20}H_{42}$	35.820	2002	3.08
15	Hexadecanoic acid, trimethylsilyl ester	328	$C_{19}H_{40}O_2Si$	36.792	2055	13.39
16	Heneicosane	296	$C_{21}H_{44}$	37.660	2101	2.10
17	(Z,Z)-9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	38.488	2149	10.10
18	Linolenic acid, ethyl ester	308	$C_{20}H_{36}O_2$	38.825	2168	2.84
19	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, ethyl ester	306	$C_{20}H_{34}O_2$	38.935	2174	3.10
20	Docosane	310	$C_{22}H_{46}$	39.439	4189	2.99
21	Octadecanoic acid, trimethylsilyl ester	356	$C_{21}H_{44}O_2Si$	40.235	2250	2.73
22	Octadecamethyl-cyclononasiloxane	667	$C_{18}H_{54}O_9Si_9$	40.869	2287	0.95
23	Tricosane	324	$C_{23}H_{48}$	41.129	2302	1.37
24	Tetracosane	338	$C_{24}H_{50}$	42.759	2402	1.66
25	DehydroAbietic acid	314	$C_{20}H_{28}O_2$	43.619	2457	1.17
26	Bis(2-ethylhexyl) phthalate	340	$C_{24}H_{38}O_4$	45.163	2556	6.21
27	Docosanoic acid	340	$C_{22}H_{44}O_2$	45.489	2577	3.30
28	(2,6,10,15,19,23-Hexamethyl,2,6,10,14,18,22-tetracosahexaene)squalene	410	$C_{30}H_{50}$	50.863	2832	1.17

[a]Retention time (min), [b]Kovat's index relative to *n*-alkanes (C_7-C_{31}) on a HP-5MS column (5% phenyl methyl siloxane).

TABLE-3
COMPONENTS OF THE ETHANOL EXTRACT FROM *R. pseudoacacia* L. HEARTWOOD BY GC/MS ANALYSIS

No.	Compound	m.w.	m.f.	RT[a]	KI[d]	Area (%)
1	5-Methyl-2-(1-methylethyl)phene	150	$C_{10}H_{14}O$	20.405	1317	1.47
2	Resorcinol	110	$C_6H_6O_2$	21.118	1345	51.96
3	1-(2,4-Dihydroxyphenyl)ethanone	152	$C_8H_8O_3$	27.279	1592	2.87
4	9-Methylnonadecane	282	$C_{20}H_{42}$	27.485	1600	1.63
5	Octadecane	254	$C_{18}H_{38}$	31.847	1800	0.39
6	Methyldibenzothiophene	198	$C_{13}H_{10}S$	33.030	1860	0.36
7	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	35.256	1973	6.06
8	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	35.740	1997	1.05
9	(Z,Z)-9,12-Octadecadienoic acid	280	$C_{18}H_{32}O_2$	38.424	2145	4.08
10	(Z,Z,Z)-9,12,15-Octadecatrien-1-ol	264	$C_{18}H_{32}O$	38.545	2152	6.27
11	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	38.810	2167	2.29
12	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, ethyl ester	306	$C_{20}H_{34}O_2$	38.921	2173	2.98
13	Bis(2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$	45.144	2555	3.40
14	2-Methoxy-5-(2',3'-dimethoxyphenyl)cyclohepta-2,4,6-trien-1-one	272	$C_{16}H_{16}O_4$	47.228	2675	0.84

[a]Retention time (min), [b]Kovat's index relative to *n*-alkanes (C_7-C_{31}) on a HP-5MS column (5 % phenyl methyl siloxane)

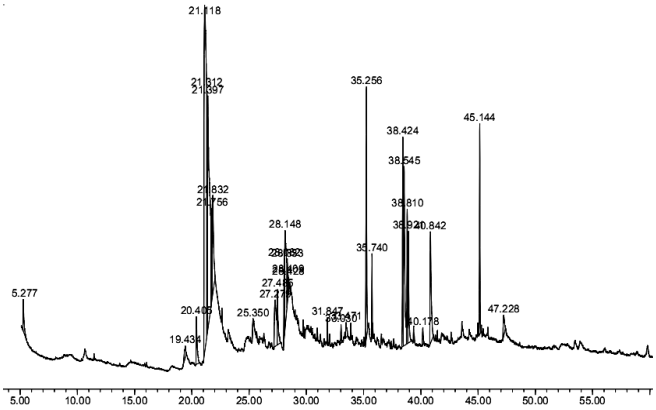

Fig. 2. Total ion chromatogram of ethanol extractive of black locust heartwood by GC/MS

Generally the chemical compositions present in the *n*-hexane and ethanol extracts have been including volatile compounds such as: acids, fatty acids, aliphatic hydrocarbons, aromatic hydrocarbons, esters, fatty acids ester, pure hydrocarbon oils or mineral oils[26,27], alcohol aliphatic, *etc*.

Since present results report all of the values; however some of these components that also are very important were first reported in this study.

In previous studies, was found the same components in the extractives of black locust (*Robinia pesudoacacia* L.)[14], but some of components such as tannins, flavonoids (*e.g.* robtein, butein, *etc*.), flavanones, flavanonols (*e.g.* dihydrorobinetin), flavonols (*e.g.* robinetin), stigmasterol, choline, syringenin, starch, simple sugars, water-soluble proteins and related metabolites that previously were reported[3,15-18] were not found in the *n*-hexane and ethanol extracts. It can be claim that wood species, provenance conditions, type of extraction solvent, process, technique and time is remarkably important to remove these components.

Since our results reported all of the components; however some of these components that also are very important were first time reported in this study.

Conclusion

The chemical composition of black locust heartwood extractives provided from the Karaj site in Iran was investigated. *n*-hexane and ethanol extracts from black locust (*Robinia pseudoacacia* L.) cultivated in the arid region of Iran, was obtained from maceration extraction method and its chemical composition was determined by GC-MS.

The principal and valuable components of the *n*-hexane and ethanol extractives of black locust (*Robinia pseudoacacia* L.) heartwood by GC/MS analysis were hexadecanoic acid, trimethylsilyl ester (13.39 %), 9,12-octadecadienoic acid (Z,Z)- (10.10 %), tetradecane (6.88 %), *bis*(2-ethylhexyl) phthalate (6.21 %), hexadecane (6.15 %), resorcinol (1,3-benzendiol) (51.96 %), 9,12,15-octadecatrien-1-ol, (Z,Z,Z)- (6.27 %), hexadecanoic acid (6.06 %) and 9,12-octadecadienoic acid (Z,Z)- (4.08 %).

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