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Heartwood Extractives of *Robinia pseudoacacia* Wood

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Abstract: In this study, extracts from the heartwood of black locust (*Robinia pseudoacacia* L.) were obtained with ethanol solvent before and after extraction with *n*-hexane. Chemical composition were analyzed and compared by GC-MS. The results showed that the major components in the heartwood ethanol extract before extraction with *n*-hexane solvent to be the (23S)-ethylcholest-5-en-3.beta.-ol (18.33%), while the major heartwood ethanol extracts constituents after extraction with n-hexane solvent was resorcinol (51.96%). The same components of the heartwood ethanol extracts before and after extraction with *n*-hexane solvent also contained amounts of the Hexadecanoic acid and 9,12-Octadecadienoic acid (Z,Z)-. The other main components of the ethanol extract mainly contained about 13.75% stigmasterol, 9.35% 9,19- Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-, 7.11% Lup-20(29)-en-3- one, 6.27% 9,12,15-Octadecatrien-1- ol, (Z,Z,Z)-, 6.06% Hexadecanoic acid 4.54% Ergost-5-en-3-ol, (3.beta.)-, and 3.53% campesterol.

1. Introduction

The extractives of trees are among the many classes of compounds known as secondary or special metabolites (Gottlieb, 1990). The compounds within the extractives where play the 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 (Pereira *et al*., 2003). It was reported that the black locust bark has exceptional resistance to biodegradation, which is attributed to the concentration of robinetine and dihydrobine (Rudman, 1963). Also, the results from the research showed that three compounds identified in ethanol extracts from the leaves of black locust, consisting of robinetin, myricetin and quercetin have allelopathic properties and inhibit the growth of plants (Fujii *et al*., 2005). Essentially the same flavonoids were detected in the heartwood and the sapwood, but the heartwood contained considerably larger quantities. The flavonoids found include the chalcones robtein (2′,3,4,4′,5pentahydroxychalcone), butein (2′,3,4,4′-tetrahydroxychalcone) and 2′,4,4′-trihydroxychalcone; the flavanones *L*-robtin [(2*S*)-3′,4′,5′,7-tetrahydroxyflavanone], *L*-butin [(2*S*)-3′,4′,7-trihydroxyflavanone] and liquiritigenin [(2*S*)-4′,7-dihydroxyflavanone]; the flavanonols *D*-dihydrorobinetin [(2*R*, 3*R*)- 3,3′,4′,5′,7-pentahydroxyflavanone], which is the major component (4-5 pct) found in the heartwood, and *D*-fustin; the flavonols robinetin (3,3′,4′,5′,7-pentahydroxyflavone) and fisetin; the flavan-3-ol *L*-robinetinidol (3,3′,4′,5′,7-pentahydroxy-2,3 -*trans*-flavan); and the flavan- 3,4-diols leucorobinetinidin and *D*-3,3′,4,4′,7-pentahydroxy-2,3- *trans*-3,4-*cis*-flavan. In addition, the heartwood contained β-resorcylic acid and methyl β-resorcylate (Kubota and Hase, 1966).

As part of an ongoing research program to identify and document the chemical constituents in the extractives from one part of non-indigenous black locust, this is a report on a complete analysis of ethanol extractives obtained from the heartwood of *R. pseudoacacia* L. from this location.

2. Materials and Methods

2.1 Preparation of Extractives

In this study, the extractives of black locust were obtained from the fresh black locust (*Robinia pseudoacacia* L.) heartwood, which was milled to a very fine homogenous composition and ground to a fine powdery mixture. Wood flour (10mg) was inserted into a 200mL balloon and extractives successively extracted

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with ethanol and *n*-hexane solvents (150mL) separately, and then the extracted wood flour from *n*-hexane was inserted in ethanol solvent again, and by a long-term (15 days) maceration technique at ambient temperature, and its chemical composition was analyzed and compared with GC-MS. Extracts from the ethanol were dried by evaporating the solvent at the 40°C until a viscous deposit was left in the flask. Two ethanol extracts were dried over anhydrous sodium sulfate (Na_2SO_4) and stored at -18°C.

2.2 Chemical Analysis

In order to identify components of extracts, trimethylsilylation was achieved by heating 1mg of sample at 70°C for 1h with 30µL bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 10µL of trimethylchlorosilane and 30µL pyridine (Holmbom, 1999). Two ethanol extracts were analyzed on an Agilent 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 (30m x 0.25mm i.d., 0.25µm film thicknesses) with Agilent 19091J-133 model number. Helium (He), having a flow rate of 1mL/min, was used as carrier gas. The GC oven temperature was kept at 50ºC for 5 min. and programmed to 250ºC at a rate of 20ºC/min and then kept at 250ºC. The injector temperature was 250ºC. The amount of injection was 1µL. The carrier gas was delivered at a constant pressure of 7.35 psi. MS spectra were taken at E1 ion source of 70eV. Identification of the components was based on comparison of their mass spectra with those of internal (computer) library, NIST libraries and some reference compounds.

Table 1. *Robinia pseudoacacia* extractives in ethanol before extraction with *n*-hexane.

Chemical component	Retention Time (min.)	Total (%)
Hexadecanoic acid	35.234	0.31
9,12-Octadecadienoic acid (Z,Z)-	38.417	0.47
(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene	38.533	0.62
Campesterol	46.652	3.53
Ergost-5-en-3-ol, (3.beta.)-	46.742	4.54
Stigmasterol	49.08	13.75
Silane, [[(3.beta.,24R)-ergost-5-en-3-yl]oxy]trimethyl-	49.964	2.88
Stigmasterol trimethylsilyl ether	52.409	4.00
(23S)-ethylcholest-5-en-3.beta.-ol	53.288	18.33
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, (3.beta.,4.alpha.,5.alpha.)-	55.701	6.22
Lup-20(29)-en-3-one	56.393	7.11
2,6,6,9,2',6',6',9'-Octamethyl-[8,8']bi[tricyclo[5.4.0.0(2,9)]undecyl]	56.458	2.69
Silane, trimethyl[[(3.beta.)-stigmast-5-en-3-yl]oxy]-	57.118	5.86
9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	58.735	3.16
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	64.350	9.35

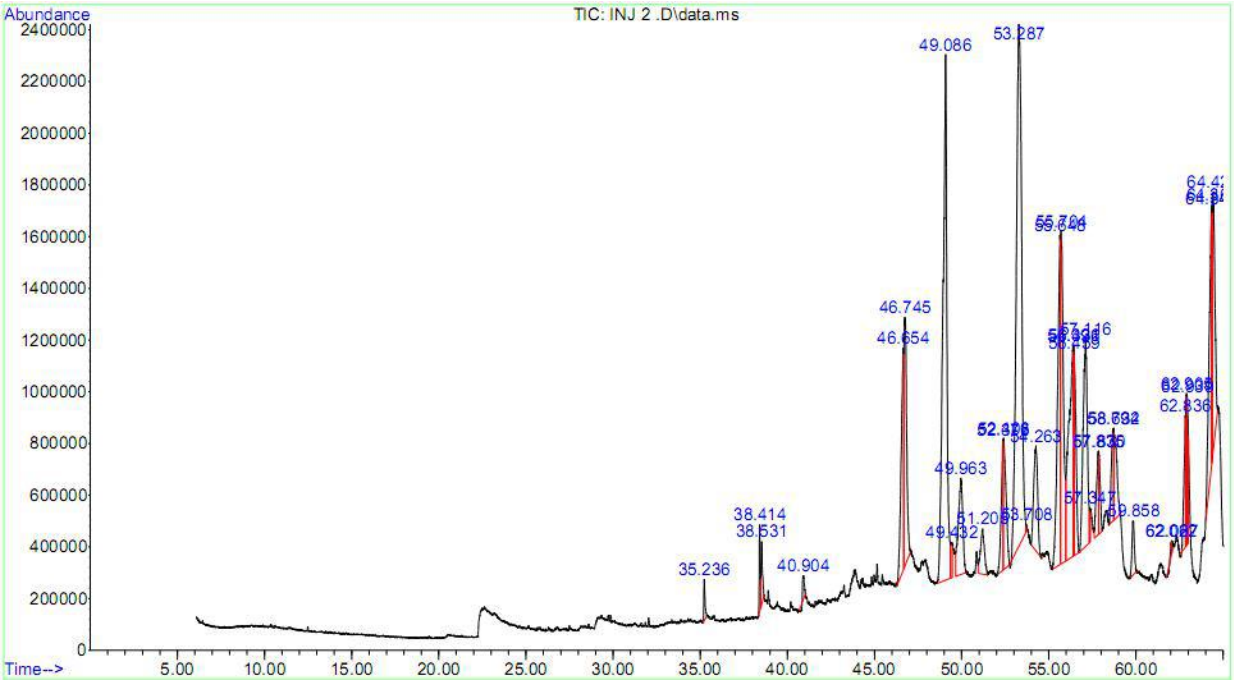

Fig. 1. GC-MS Chromatogram of ethanol extractives of *Robinia pseudoacacia* heartwood before extraction with *n*-hexane.

Table 2. *Robinia pseudoacacia* extractives in ethanol after extraction with *n*-hexane.

Chemical component	Retention Time (min)	Total (%)
Phenol, 5-methyl-2-(1-methylethyl)-	20.405	1.47
Resorcinol	21.752	51.96
Ethanone, 1-(2,4-dihydroxyphenyl)-	27.279	2.87
9-Methylnonadecane	27.485	1.63
Octadecane	31.847	0.39
Methyldibenzothiophene	33.030	0.36
Hexadecanoic acid	35.256	6.06
Hexadecanoic acid, ethyl ester	35.740	1.05
9,12-Octadecadienoic acid (Z,Z)-	38.424	4.08
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	38.545	6.27
9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	38.921	5.27
Bis(2-ethylhexyl) phthalate	45.144	3.40
2-Methoxy-5-(2',3'-dimethoxyphenyl) cyclohepta-2,4,6-trien-1-one	47.288	0.84

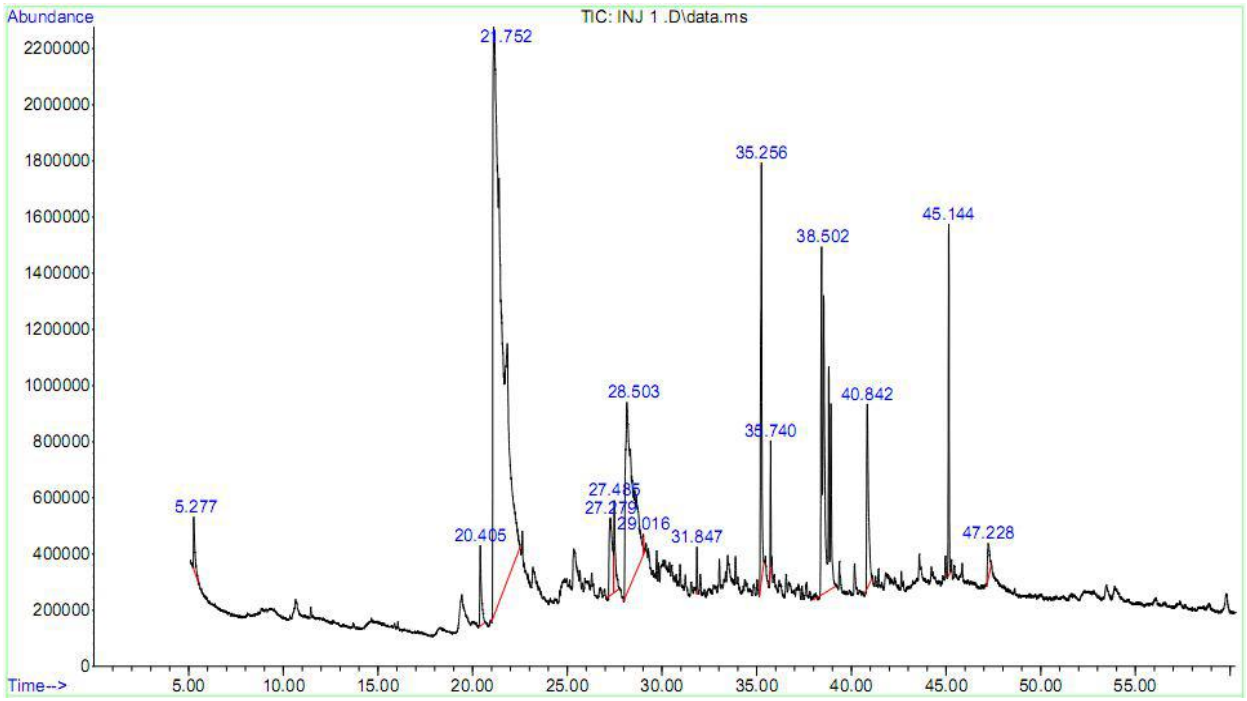

Fig. 2. GC-MS Chromatogram of ethanol extractives of *Robinia pseudoacacia* heartwood after extraction with *n*-hexane.

3. Results and Discussion

Table-1 and 2 represent the chemical composition of the ethanol extracts from the heartwood of black locust before and after extraction with *n*-hexane, respectively. As can be seen from these tables, 15 compounds only for ethanol extract before extraction with *n*-hexane and 13 compounds for ethanol extract after extraction with *n*-hexane, representing about 82.82% and 85.65% of the ethanol extracts from black locust, respectively were characterized. The major components are as follows: resorcinol (51.96%), (23S)-ethylcholest-5-en-3.beta.-ol (18.33%), and stigmasterol (13.75%). The chemical composition of black locust heartwood extractives provided from the Karaj site in Iran was investigated. Ethanol extracts from black locust (*Robinia pseudoacacia* L.) cultivated in the arid region of Iran, was obtained from the maceration extraction method before extraction with *n*-hexane, and its chemical composition was determined by GC-MS. The findings indicated that (Table-1 and Fig. 1, and Table-2 and Fig. 2) the ethanol extracts before and after extraction with *n*-hexane had the following approximate levels: resorcinol (51.96%), (23S)-ethylcholest-5-en-3.beta.-ol (18.33%), stigmasterol (13.75%), 9,19-Cyclolanostan- 3-ol, 24-methylene-, (3.beta.)- (9.35%), Lup-20(29)-en-3-one (7.11%), 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- (6.27%), Hexadecanoic acid (6.06%), Ergost-5-en-3-ol, (3.beta.)- (4.54 %) and campesterol (3.53%).

4. Conclusions

Generally, the chemical composition contents in the ethanol extracts after extraction with *n*-hexane solvent were higher than before extraction with *n*-

hexane solvent, but the number of chemical constituents in ethanol extracts after extraction with *n*-hexane solvent was lower than before extraction with *n*-hexane solvent. The results showed that some chemical components such as Hexadecanoic acid and 9,12-Octadecadienoic acid (Z,Z)- are extractable not only with ethanol solvent before extraction with *n*-hexane but also after extraction with *n*-hexane solvent.

The result showed that ethanol extracts of *Robinia pseudoacacia* heartwood contain high amount of resorcinol (about 52%) as a phenolic compound. Resorcinol used in the manufacture of resins, plastics, dyes, medicine, and numerous other organic chemical compounds. In medicine, it is used externally in ointments and lotions as an antifungal. Resorcinol is also used as a chemical intermediate for the synthesis of pharmaceuticals and other organic compounds. It is used in the production of diazo dyes and plasticizers and as a UV absorber in resins (Sadeghifar *et al.*, 2011).

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