



Comparative Chemical Analysis of the Extractives Constituents in the Bark and Heartwood of *Juglans regia* L. from North of Iran

Seyyed Khalil Hosseinihashemi*

*Department of Wood Science and Paper Technology, Karaj Branch, Islamic Azad University, Karaj, Iran.

Abstract: Exhaustive ethanol-toluene of the bark and heartwood of *Juglans regia* L. (Juglandaceae) afforded pale red colored extractives 10 and 12% yields, respectively. Detailed chemical evaluation of these extracts using GC/MS revealed the major components in the bark and heartwood extractives to be the 3,7-dioxa-2,8- disilanonane,2,2,8,8-tetramethyl (25.17%), while the major heartwood extractives constituent was benzoic acid, 3,4,5-tri(hydroxyl)/Gallic acid (44.57%). The same components of the bark and the heartwood also contained amounts of the gallic acid, 3,7-dioxa-2,8-disilanonane, 2,2,8,8- tetramethyl, and d-glucose,2,3,4,5,6- pentakis-o-(hydroxyl). The most toxic components in the heartwood were juglone (5.15%) and 2,7-dimethylphenanthrene (5.81%).

Keywords: Heartwood, Bark, Juglone, Benmary bath.

1. Introduction

The genus *Juglans* of the family Juglandaceae is comprised of more than 25 species distributed throughout the old world, new world and temperate region of the world. *Juglans regia* is native to the mountain ranges of Central Asia extending from Xinjiang province of western China, parts of Kazakhstan, Uzbekistan and Southern Kirghizia and from mountains of Nepal, Tibet, Northern India and Pakistan through Afghanistan, Turkmenistan and Iran to portions of Azerbaijan, Armenia, Georgia, and eastern Turkey (McGranahan and Leslie, 1991). Detailed phytochemical and pharmacological studies have shown the chemical constituent to consist mostly of juglone that can cause blackening, blistering, and peeling of the skin and is a tranquilizer and sedative (Bhargava, 1967; Westfall *et al.*, 1961); has antitumor activity (Bhargava and Westfall, 1968); is fungitoxic and an antibiotic; and is allelopathic (Soderquist, 1973). The durability of walnut has been related to the presence of phenolic compounds such as flavonoides, naphthoquinones and hydrolysable tannins (Gupta *et al.*, 1972). The wood and the bark of black walnut have

not been found to contain tannins. However, the wood contains appreciable amounts of gallic acid as well as ellagic acid, glucose and a dark violet polymer (Gupta *et al.*, 1972; Seshadri, 1973). Ellagic acid is a sedative and tranquilizer (Bhargava and Westfall, 1968) and has antitumor activity (Bhargava and Westfall, 1968). As part of an ongoing research program to identify and document the chemical constituent in the extractives from all parts of endemic Persian walnut were reported herein a complete analysis of extractives obtained from the bark and heartwood of *J. Regia* from in this location.

2. Materials and Methods

2.1 Raw material preparation

The bark and heartwood of *J. regia* were collected from Ramsar forests, north of Iran, in March 2005. The fresh bark and heartwood were milled to a very fine homogenous composition and ground to a fine powdery mixture. The individual plant parts were exhaustively ethanol-toluene (2:1 by v/v) over a 6-8 hour period in a soxhlet standard distillation set-up. The pure extractives obtained were separated and dried by nitrogen gas to

give pale red colored extracts in 10 and 12% yields from the bark and heartwood, respectively. In order to identify of extract, about 1mg solid extracts obtained, mixed with 30 microliters BSTFA + 1% TMCS reagent and about 15 microliter pyridine inside tube test. Afterward was placed into the benmry bath with 70°C temperature.

The analyses of the extracts were done using GC/MS on an Agilent 6890 Gas Chromatograph, equipped with a split/splitless injector and a 7963 Mass Selective Detector (MSD). The column oven was programmed as follows: Chromatography was performed on an HP-5MS capillary column (SGE, 30m, 0.25mm), kind of carrier gas, helium with 1ml/min speed and temperature program between 60-260°C, increase temperature 6°C/min.

2.2 Identification of extract components

The extractives samples were injected into GC/MS apparatus injector portion. The individual compounds in the extractives were identified by their retention time relative to known compounds and further by

comparison of their mass spectra with either the known compounds or published spectral data.

3. Result and Discussion

Comparative data on the different extractives constituent in the bark and heartwood of *J. regia* is presented in Table-1, Fig. 1, and 2. The results indicate that gallic acid, 3,7-dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl, and D-glucose,2,3,4,5,6-pentakis-o-(hydroxyl), present in similar amounts in the bark and heartwood and gallic acid found in the bark extractives in trace amounts. This 2,7-dimethylphenanthrene is an important compound extractive, whose presence in the heartwood has not been reported previously, but 2,7-dimethylphenanthrene-like compounds (naphthalene) has been reported in the fruit extracts of *J. regia* (Muller and Leistner, 1978; Talapatra *et al.*, 1988). The heartwood extractives also contained small amounts of α -pinene and juglone, which were not found in the bark extractives and cholesterol benzoate and pentacosane are the main constituents of the bark extractives of *J. regia* whose has not been reported previously and are absent in the heartwood extractives.

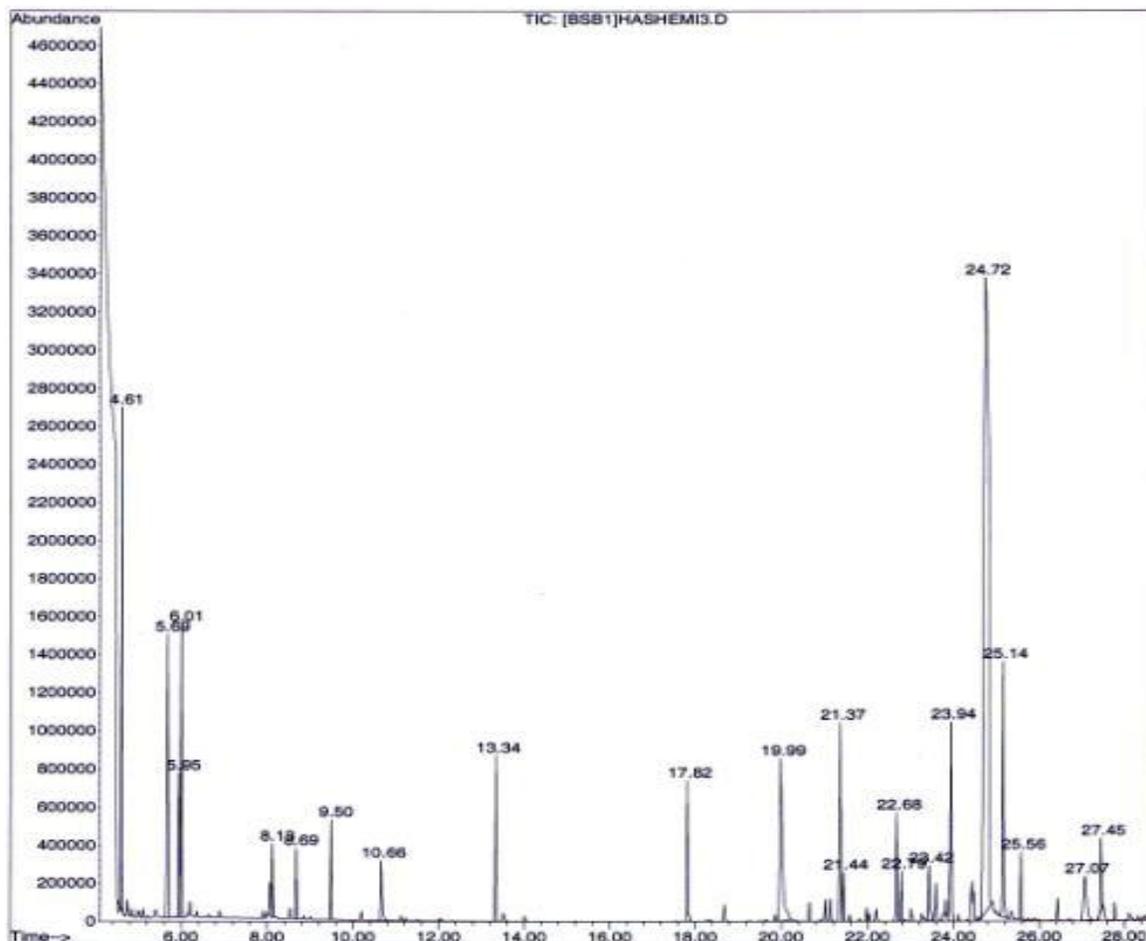
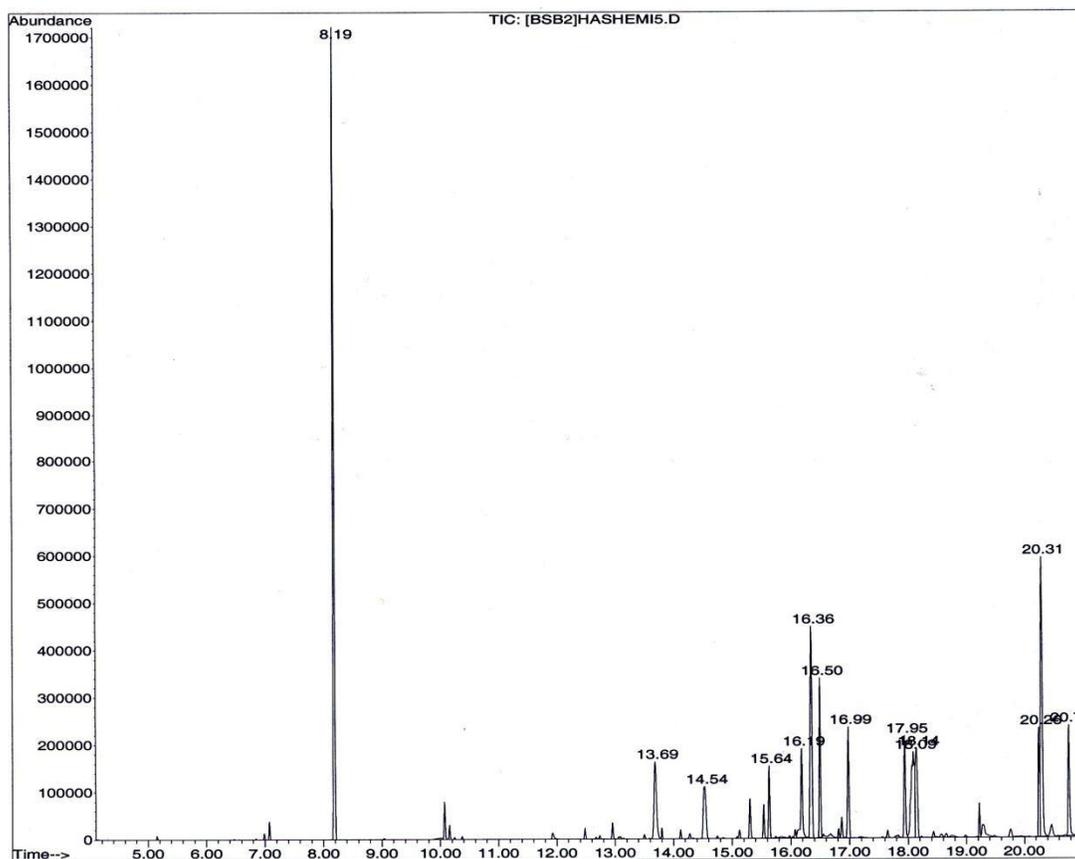


Fig. 1. GC-MS Chromatogram of ethanol-toluene extractives *Juglans regia* heartwood.

Table 1. A comparative analysis of the composition of the chemical constituent (area %) in the bark and heartwood extractives of *J. regia*.

Chemical component	Retention Time (min.)	Bark	Heartwood
α -pinene	5.69	-	5.28
Juglone	6.02	-	5.15
3,7-dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl	8.19	25.17	-
Propanoic acid, 2-(trimethylsilyloxy)-, trimethyl	8.69	-	1.07
Silane, trimethyl(phenylmethoxy)	10.66	-	1.33
3,7-dioxa-2,8-disilanonane, 2,2,8,8-tetramethyl	13.34	-	2.40
6-amino-3-cyano-5-methylpyrazine-2-carboxamide	13.69	5.09	-
Cis-9-oxabicyclo [6.1.0] non-2-yne	14.54	3.88	-
D-glucose, 2,3,4,5,6-pentakis-o-(hydroxyl)	15.63	2.40	-
Benzoic acid, 3,4,5-tri(hydroxyl)/Gallic acid	16.19	4.53	-
2-butenic acid, 2-methyl-, 2-propenyl ester	16.36	10.28	-
Mannose, 2,3,4,5,6,-pentakis-o-(hydroxyl)	16.51	5.21	-
2,6,10-timethylundecane-(5z)-2,5,9-trien-4-one	16.98	4.25	-
3,8-dioxa-2,9-disiladecane, 2,2,9,9-tetramethyl	17.81	-	2.15
Butanoic acid, 2,4-bis(hydroxyl)-, trim	17.95	4.06	-
Dimethyl 8-(tert-butyl)-5,6,10-trim	18.09	7.77	-
Cholesteryl benzoate	18.15	5.37	-
2,7-dimethylphenanthrene	19.99	-	5.81
Pentacosane	20.26	3.72	-
1,2-benzenedicarboxylic acid, diisooctyl ester	20.31	13.71	-
Xylitol 5-TMS	21.37	-	3.26
Benzoic acid, 3,4-bis(hydroxyl)	22.67	-	2.06
D-fructose, 1,3,4,5,6-pentakis-o-(hydroxyl)	22.87	-	0.79
D-glucose, 2,3,4,5,6-pentakis-o-(hydroxyl)	23.94	-	4
Benzoic acid, 3,4,5-tri(hydroxyl)/Gallic acid	24.72	-	44.57

**Fig. 2. GC-MS Chromatogram of ethanol-toluene extractives *Juglans regia* bark.**

4. Conclusion

The tree *Juglans regia* (Persian walnut) (Juglandaceae) is a species endemic island of Iran, from which the gallic acid could be obtained from either common walnut. A comparative study of the bark and heartwood extractives indicates the bark and heartwood extractives to be the main natural sources of the gallic acid and 3,7-dioxa-2,8-disilanonane,2,2,8,8-tetramethyl. The juglone and 2,7-dimethylphenanthrene were found to occur as minor constituents in the heartwood extractives. The bark extractives, on the other hand, were found to be composed partially of cholesterol benzoate and pentacosane.

References

- [1]. Bhargava, U.C. (1967). Ph.D. Thesis, Pharmacology of ellagic acid from black walnut. University of Missouri, 670 p.
- [2]. Bhargava, U.C. & Westfall, B.A. (1968). Antitumor activity of *Juglans nigra* (black walnut) extractives. *Journal of Pharmaceutical Sciences*, 57 (10):1674-1677.
- [3]. Gupta, S.R., Ravindranath, B. & Seshadri, T.R. (1972). Juglandaceae: Polyphenols of *Juglans nigra*. *Photochem.*, 11: 2634-2636.
- [4]. McGranahan, G. and Leslie, C. (1991). Walnuts (*Juglans L.*). In Genetic Resources of Temperate Fruit and Nut Crops. J.N. Moore and J.R. Ballington (eds). International Society for Horticultural Science, Wageningen, pp. 907 – 951.
- [5]. Muller, W.U. & Leistner, E. (1978). Aglycones and glycosides of oxygenated Naphthalenes and a glycosyltransferase from *Juglans*. *Phytochemistry*, 17(10): 1739-1742.
- [6]. Seshadri, T.R. (1973). An investigation of the phenolic constituents of certain woods and barks, including North American species and representatives of genera common to North America and India, (J.W Rowe, USDA PL-480 Proj. FG-In-425 Forest Prod. Lab. Madison Wis.).
- [7]. Soderquist, C.J. (1973). Juglone and allelopathy. *Journal of Chemical Education*, 50 (11): pg. 782.
- [8]. Talapatra, S.K., Karmacharya, B. & Talapatra, B. (1988). Levoregiolone an alpha-tetralone from *Juglans regia*; Structure, Stereochemistry and confirmation. *Phytochemistry*, pg. 3929.
- [9]. Westfall, B.A., Russell, R.L. & Auyong, T.K. (1961). Depressant agent from walnut hulls. *Science*, 134: 1617.