Identification of Wood and Bark Extractives in Indigenous Barberry (*Berberis vulgaris*)

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Members of the *Barberry* species belonging to the family Berbridaceae have many uses in traditional medicine. The aim of the present study was to investigate and compare the chemical composition of wood and inner bark extractives of *Berberis vulgaris*. The chemical composition of samples was obtained by GC-MS analysis. The yield of obtained extracts was measured to be 3% and 8% in wood and inner bark, respectively. Five compounds were identified in the acetone soluble wood extractives, of which the major compound identified was 4-vinylguaiacol (75.5%). In the inner bark, tetracosanoic acid, methyl ester (26.36%), phetalic acid, diisooctyl ester (20.93%), and 1,2-benzendicarboxylic acid, diisononyl ester (8.70%) were the main constituents. Phenolic compounds were the major class of compounds in the wood extract, while in the inner bark extract was predominated by waxes and volatile organic compounds such as fatty acid esters.

Keywords: Barberry wood and inner bark extractives; Berbridaceae; 4-Vinylguaiacol; Tetracosanoic acid, methyl ester

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INTRODUCTION

Barberry (*Berberis vulgaris* L.), which belongs to the Berberidaceae family, is well known in Iran and south Khorasan. It is a small shrub, various parts of which, including its root, bark, leaf, and fruit have been used as folk medicine. The different parts of *B. vulgaris* have several applications (Javadzadeh and Fallah 2012). The main bioactive compounds from *B. vulgaris* are alkaloids (berberine, berbamine, jatrorrhizine, columbamine, berberubine, oxicanthine, palmatine, vitamin C, resin, and tannins) and flavonoids (quercetin and kaempferol) (Aghbashlo *et al.* 2008; Fatehi *et al.* 2005; Ivanovska and Philipov 1996; Suau *et al.* 1998). Berberine has several favorable attributes such as non-toxic functions, specific medical actions, and environmentally friendly characteristics (Ali *et al.* 2009; Duke 2006). It is a cationic colorant present in the extract of *B. vulgaris* wood can be used as a natural dye for wool (Haji 2010). There is more colorant in the roots of this plant, but in some studies the woods were used to prevent from wasting of root of this plant (Haji 2010).

Based on previous studies, most of bioactive properties and the majority of the medical properties (such as drugs, food additives, odorant, and flavoring compounds, *etc.*) (Romberger 1997) of *B. vulgaris* are due to the overall antioxidant activity (Javadzadeh and Fallah 2012) generated by the presence of some flavonoid compounds,

alkaloids, or other compounds with phenolic hydroxyl groups (Srinivasan 2008) in the different parts of this plant.

In a previous study Javadzadeh and Fallah (2012) stated that the stem and root barks were already used in the Greek civilization by scientists as Galenus, Dave Securid and Pollini as a purgative, antibilious, laxative, tonic and appetizer. Also, in Europe and America, the bark of barberry stem and root have also been used as antiseptic, laxative, antipyretic, anti-vomiting, tonic, and as an antidepressant. They were also used to treat jaundice and stomachache. Flowers and bark of stems have been used to treat rheumatism as well (Javadzadeh and Fallah 2012).

In the recent decades the cultivation of the barberry has started in the arid part of Iran. Despite the widespread use and potential of barberry plant, little research has been focused on the determination of the chemical composition of barberry wood and inner bark from Iran. Therefore, in this study chemical composition of wood and inner bark of *Berberis vulgaris* L. collected from the north side of Iran was analyzed.

EXPERIMENTAL

Extraction of Extractives

The extracts were obtained from 2 g of fresh wood and inner stem bark samples from ground-line to top of plant (a length of 1 m) collected from the Siah Bisheh, Chalus, and Mazandaran, North of Iran, separately. The plant was collected during May, 2013. The wood and inner bark were first weighted, chopped to small pieces, and solvent extracted in a Soxhlet apparatus for 8 h in 200 ml acetone according to TAPPI standard (T204 om-88 (1988)). The extractives from the wood and inner bark acetone extracts 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 magnesium sulfate and stored at -18 ºC.

GC/MS Analysis of Extractives

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

The acetone-soluble extractives were analyzed on an Agillent 5975B mass spectrometer coupled with a Hewlett-Packard GC-6890N series GC by using an HP-5MS (5 % phenyl methyl siloxane) equipped with a fused silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses) with Agilent 19091J-133 model number. Helium as carrier gas with 1 mL/min flow rate was used. 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. The identification of the components was compared based on their mass spectra with those of internal (computer) library, NIST libraries and some reference compounds (Stein 1999).

The Automated Mass Spectral Deconvolution and Identification System (AMDIS) (Stein 1999) from the National Institute of Standards and Technology (NIST) was used in this work. The system has excellent RI capabilities and is readily available for download (http://chemdata.nist.gov/mass-spc/amdis/). The software can read most manufacturers' data files and perform mass spectral deconvolution in order to 'clean up' the mass spectra prior to library searching. In addition, user library creation is simple and spectra can be searched against the NIST database (Table 1, www.hdscience.com). The AMDIS software has been applied to plant (Fiehn 2003) and urinary metabolites (Halket *et al.* 1999).

Table 1. A Selection of Major Commercially Available EI Libraries (Halket *et al.* 2004)

Database	Class	Spectra	www
Wiley	General	444 000	www.hdscience.com
NIST02	General	123 434	www.hdscience.com
Wiley/Hennerberg	General	40 000	www.hdscience.com
Wiley/Yarkov	Organic compounds	37 055	www.hdscience.com
Wiley/Zeist	Food volatiles	1620	www.hdscience.com
Wiley/Makin	Steroids	2500	www.hdscience.com
Wiley/Roesner	Designer drugs	1700	www.hdscience.com
Wiley/de Leeuw	Geochemical	1100	www.hdscience.com
Ehrenstorfer	Pesticides	1450	www.ehrenstorfer.com
Stan	Pesticides	300	www.chem.agilent.com
PMW	Drugs/toxicol.	6300	Instrument manufacturers
Adams	Fragrances	1606	www.allured.com

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 (Adams 1995, 2001; Julian and Konig 1988). Retention indices (*RI*) were determined with reference to a homologous series of normal alkanes, by using the following Formula (Kovats 1958),

$$\mathrm{RI}= 100\ \frac{(n+(N-n)\times \log t_{1R}(x)-\log t_{1R}(Cn)}{\log t_{1R}(CN)-\log t_{1R}(Cn)} \qquad (1)$$

where *RI* is the retention index of the interest compound, t_{1R} is the net retention time (t_R-t_0), t_0 is the retention time of the solvent (dead time), t_R is the retention time of the interest compound, *Cn* and *CN* are the number of carbon atoms in the *n*-alkanes eluting immediately before and after the interest compound, and *N* and *n* are the number of carbon atoms in the *n*-alkane eluting immediately before and after the interest compound.

RESULTS AND DISCUSSION

Wood Extractives

Acetone soluble extractives of the fresh wood of *Berberis vulgaris* gave rise to a yellowish extract with pleasant aroma and yield of 3% (v/w). According to Table 2, the

major components in the wood extract were 4-vinylguaiacol (75.5%), D-mannoheptulose (8.83%), benzothiazole, 2-methyl (6.17%), and tetradecanoic acid (5.60%).

Inner Bark Extractives

Acetone soluble extractives of the fresh inner bark of *Berberis vulgaris* allowed isolation of a yellowish extract with pleasant aroma and a yield of 3% (v/w). According to Table 2, the major components in the inner bark extract were: tetracosanoic acid, methyl ester (26.36%), 1,2-benzenedicarboxylic acid, diisooctyl ester (20.93%), and 1,2-benzenedicarboxylic acid, diisononyl ester (8.70%).

Table 2. Comparison of Chemical Composition of Wood and Inner Bark Extractives of *B. vulgaris*

Component	Component Type	RT* (min)	RI*	Area %	
				Wood	Inner bark
4-Vinylguaiacol	Phenolic	21.304	1352	75.5	-
D-Mannoheptulose	Sugar	26.821	1573	8.83	-
1-Amino-2-(hydroxymethyl)anthraquinone	Aromatic Amine	31.460	1783	-	5.57
Benzothiazole, 2-methyl	Organic	33.245	1870	6.17	-
Benzaldehyde, 2-{(1-(2-{[1-(2aminophenyl)methylidene] amino}phenyl)methylidene]amino}	Aromatic Amine	33.575	1886	-	2.79
Tetradecanoic acid	Fatty Acid	35.147	1967	5.60	-
Di-(2-ethylhexyl)phthalate	Phthalate	44.992	2545	3.16	-
1,2-Benzenedicarboxylic acid, diisooctyl ester	Fatty Acid ester	45.024	2547	-	20.9
Tetracosanoic acid, methyl ester	Fatty Acid ester	48.278	2726	-	26.4
2-(4-Methylphenyl)-3-(1-hydroxyhexyl)oxetane	Oxetane	48.388	2731	-	6.80
2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	Benzoxepine Derivative	48.964	2756	-	7.04
Phenyl(I-P-diphenylmethylphenyl-3,3-dimethyl)butyl ketone	Ketone	49.436	2777	-	6.34
1,2-Benzenedicarboxylic acid, diisononyl ester	Fatty Acid ester	49.585	2784	-	8.70
Epi-ligulyl oxide	Sesquiterpene	50.568	2821	-	5.22

*RT and RI are mean retention time (min) and retention indices respectively.

Analyses of the two extracts showed that they were predominantly phenolic compounds and volatile organic compounds in the form of fatty acid esters. As a result, a basic qualitative difference between the extract from the wood and the inner bark was apparent, as the wood and inner bark extracts showed no similarity in their chemical composition. All the components of the wood extract were absent in the inner bark extract and *vice versa*.

Jeong and Jeong (2010) reported that 2-methoxy-4-vinylphenol is a naturally occurring phenolic compound used as a flavoring agent. This compound has been found in the stem bark extracts of *Zanthoxylum tetraspermum* as a new compound (Ravikumar *et al.* 2012). It has been found in buckwheat, apple, peanut, clove, and curry as well, and

there is little information about the functional and pharmacological effects of 2-methoxy-4-vinylphenol in experimental carcinogenesis.

Benzothiazole, 2-methyl is one of the benzothiazole derivatives: where among these compounds, benzothiazole-2-thiol have exhibited interesting biological activities (Quiroga *et al.* 2002; Kok *et al.* 2006, 2007) and attracted continuing interest for further molecular exploration as useful anticancer agents (Kok *et al.* 2008; Song *et al.* 2008). Furthermore, a review of the recent literature revealed that many effective antimicrobial agents showed a heterocyclic moiety within their structure (Daidone *et al.* 1990) and, in particular, that substituted benzimidazole, benzoxazole, and benzothiazole derivatives exhibit different biological properties such as chemotherapeutical, antibacterial, antifungal, and antiviral activities, with a low toxicity for the antimicrobial therapeutic use in man (Haugwitz *et al.* 1982; De Wever *et al.* 1997).

CONCLUSIONS

1. Acetone extract of the wood contained the following suggestible main chemical compound by GC/MS: 4-vinylguaiacol.
2. The suggestible major components identified in the acetone extract of inner bark by GC/MS were tetracosanoic acid, methyl ester, phthalic acid, diisooctyl ester, and 1,2-benzenedicarboxylic acid, diisononyl ester.
3. It can be concluded that most of the identified compounds within the extracts from the wood of *Berberis vulgaris* are phenolic and most of the identified compounds within the extracts from the inner bark are fatty acids ester.

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