

Using HPLC to Determination the Composition and Antioxidant Activity of *Berberis Vulgaris*

Parichehr Hanachi

*Women Research Centre, Biomedical Unite, Alzahra University
Tehran, Iran*

E-mail: hanachi_wrc@yahoo.com

Tel: +989125426316; Fax: +982177498112

Golkho SH

*Women Research Centre, Biomedical Unite, Alzahra University
Tehran, Iran*

Abstract

The *Berberis vulgaris* (BV) plant has been extensively used as a traditional medicine in some part of Asian region especially in Western Asia. The BV fruits is claimed also to have anti-viral activities, and as a treatment for chronic candidiasis, indigestion and parasites. The present study was conducted to evaluate the antioxidative activity, nutritional and antinutritional composition of *Berberis vulgaris* fruit. The antioxidant activity was determined by using thiobarbituric acid (TBA method). Absolute ethanol, absolute methanol and aqueous solution (distilled water) were used as a solvent in this study. Result shows significantly differences ($P < 0.05$). The antioxidant activity of ethanol extract was the highest (27.26 ± 1.07 %), followed by BHT (20.29 ± 0.23 %), methanol extract (16.80 ± 0.23 %), Vitamin E (6.68 ± 0.25 %) and the lowest was the aqueous extract (6.53 ± 0.29 %). Besides antioxidant activity, *Berberis vulgaris* also contains nutritional and antinutritional composition namely vitamin C, malic acid and Tannin. Vitamin C and malic acid also shows antioxidative properties. Vitamin C and malic acid also showed antioxidative properties and act synergistically to each other as antioxidants. The presence of antinutritional such as Tannin which can act as a medium to prevent nutritional composition from being absorbed by the body also has been detected. The amount of nutritional and antinutritional detected in 100g of berberry were $11102.81\mu\text{g} \pm 2.01$ of Vitamin C, $116.03\mu\text{g} \pm 1.12$ of Malic acid and $20.51\mu\text{g} \pm 0.59$ of Tannin.

Keywords: Antioxidant, *Berberis vulgaris*, Thiobarbituric acid, Vitamin C, Malic acid, Tannin, HPLC.

Introduction

Medicinal plant research includes much more than the discovery of new drugs. This field has been expanding to also include such diverse subjects as negotiation of power based on medicinal plant knowledge (Garro, 1986) and the co-evolution of humans and plants (Alcorn, 1981). The field also provides opportunities to study how human interaction with biological diversity is influenced by human psychology, cognition, and evolution. Therefore the identification of active plant chemicals is

an essential component of modern pharmacognosy and medical effects are not necessarily restricted to a single plant chemical. The biological activity and clinical value of the whole plant, as in medicinal herbalism, is also being pursued (Pasquale, 1984).

The importance of identification of active plant chemicals does not limit itself to local Malaysian plants but also sparks an inquisitive knowledge to foreign plants. This effort leads to study a non-native plant called *Berberis vulgaris* which predominantly found in Northern Asia and Europe (Chevallier, 2001).

The determination of nutritional and anti-nutritional compounds in *Berberis vulgaris* or berberry fruits is very important as this fruit is a part of daily diet of certain people. Their presence and relative ratio, in fact, can affect the chemical and sensorial characteristics of the matrix (e.g., pH, total acidity, microbial stability, sweetness, global acceptability) and can provide precious information on food wholesomeness or on how to optimize some selected technological processes (Romero, *et al* 1992). The nutritional values such as vitamin components, antioxidant compounds in this plant might be invaluable for treating diseases as more secrets within this plant are yet to be discovered. Hanachi *et al* 2005 reported that *Berberis vulgaris* exhibited vary degree of antioxidant properties Recently (Maznah, *et al*, 1999) had shown that antioxidant capacities varied according to the plant extracts and the comparison between different solvents for the extraction method could be done especially between water and organic solvents to investigate which extract had the highest antioxidant activities.

The identification of certain alkaloids and phenolic compounds in *Berberis vulgaris* somehow provide an alternative method for medicine and remedies as many studies have proved to alleviate certain ailments in treating liver cancer, gall bladder problems, kidney stones, menstrual pains and etc (Hanachi *et al*, 2008). All these problems have lead to the development of new drugs derived from plants, which is believed to be safer and more effective (Wargovich, 1999). The development of drugs from plants continues with the research of screening on herbs (Vickers and Zollman, 1999). This shows that herbal medicine has created a great deal of public interest. The objective of this study was to Using HPLC to determination the composition and antioxidant activity of *Berberis vulgaris*.

Material and Methods

The fruits of *Berberis vulgaris* were purchased from Iranian market in Kuala Lumpur. The fruits were bought in the dried form and were prepared to be extracted.

Preparation of Plant Extract

Barberry fruit extract was prepared according to method modified Shamsa *et al*. 310 g BV fruit that was free from fungus, bacteria and any other plant diseases were selected randomly. The fruits were dried in an oven (Memmert SLM 400, GmbH, Germany) for 3 days at a constant temperature of 65°C. The fruits were cut into small pieces and were grounded into fine powder using a dry grinder. The grounded samples were sieved to get uniform particle size, then were kept in air-tight container and stored for further extraction. The sample was extracted by boiling in water for 20-30 minutes at a temperature of 75°C. Water and ethanol were added in the ratio of 1:10 and stirred at 250 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co.KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No.1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of ethanolic extract was removed under reduced pressure at 50°C using a rotary evaporator (BÜCHI Rotavapor R-200, Germany) until thick syrup was collected. The thick syrup was evaporated completely using freeze drying system (FreeZone 77520, LABCONCO, USA) for the determination of total antioxidant activity and phenolic content.

Absolute Methanol and Aqueous Extractions

For absolute methanol and aqueous extractions, the extraction was done according to Endrini (2002) with slight modification. The extraction process was carried out by soaking about 100 g of dried powder in 1000 ml absolute methanol for 24 hours. The supernatant was filtered using Whatman filter paper no. 1. The supernatant was then evaporated at 45°C under reduced pressure and subsequently air-dried. The above steps were repeated to obtain aqueous extraction.

Determination of Antioxidant Activity

The method of Ottolenghi (1959) was used to determine the TBA values of the samples. In brief, a mixture of 4 mg of *Berberis vulgaris* extracts, BHT and α -tocopherol acetate (vitamin E) in 4 ml absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0), and 3.9 ml of distilled water was placed in a vial with a screw cap and then placed in a shaking water bath (100 rpm) at 40°C in the dark for 24 hours. A control was set up by replacing the sample with 4 ml ethanol. After the incubation period, 2 ml of 20% trichloroacetic acid (TCA) solution and 2 ml thiobarbituric acid (TBA) solution were added to 1 ml of sample solution. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was centrifuged at 3,000 rpm for 10 minutes and the absorbance of the supernatant was measured at 532 nm. The antioxidant activities were calculated as below:

$$\% \text{ Antioxidant Activity} = (\text{OD}_{\text{control}} - \text{OD}_{\text{sample}} / \text{OD}_{\text{control}}) \times 100 \%$$

In which $\text{OD}_{\text{sample}}$ is the optical density of sample and $\text{OD}_{\text{control}}$ is optical density of control.

Determination of Nutritional and Antinutritional Component

Standards of nutritional and anti-nutritional for ascorbic acid, malic acid and tannin (Sigma Chemicals, Co, USA) were used. The sample preparation techniques must be done before using the HPLC for the purpose of eliminating contamination and to get the best outcome of the experiment. Sample preparation of Vitamin C determination was prepared with a mixture of KH_2PO_4 , methanol and TBAOH with ratio of 97:3:0.05. The following sample preparation for Tannin was used. Samples of berberry fruits were dissolved in 100ml of a mixture of 100 volumes of 10% methanol and 0.1 volume of phosphoric acid. Different steps were used to prepare Malic acid. About 3 grams of berberry samples were diluted into 3 ml of distilled or purified water. Therefore the concentrations must be 50:50, v/v.

Determination of Vitamin C, Malic acid and Tannin

Hewlett Packard series 1100 consist of Hewlett Packard 1049A programmable electrochemical detector, degasser G1322A, UV/ visible detector was used to detect Vitamin C, Malic acid and Tannin. The method of Abdulnabi *et al*, 1997 with a slight modification was used to determine Vitamin C using HPLC. The content of organic acid of Malic acid can be determined by using method from Rebecca, (2000). Meanwhile the presence of Tannin is adopted from Hagerman *et al.*, 1992 with slight changes. This method is particularly useful for separating the constituents of tannic acid in a simple isocratic system. Better resolution can be obtained with reversed phase system especially if gradient HPLC is available. Typical run takes about 30 minutes to ensure elution of all peaks although longer runs could also be necessary if a sample had very high molecular weight Tannin. The conditions for Vitamin C, Malic acid and Tannin are listed in Table 1

Statistical Analysis

The results obtained were analyzed using one-way ANOVA for mean differences. The Statistical Package for Social Science for windows version (12.0) was used to analyze the data. The ($p < 0.05$) was considered significant and data from detection of nutritional and antinutritional were expressed as mean \pm standard deviation (SD).

Results

Determination of Antioxidant Activity of *Berberis vulgaris*

The results of determination of antioxidant activity, Table 2 shows ethanol extract of BV was the highest among the other extracts which was about (27.26 ± 1.07 %) and this percentage also exceeds the percentage of antioxidant activity of BHT and Vitamin E followed by BHT (20.29 ± 0.23 %), methanol extract (16.80 ± 0.23 %), Vitamin E (6.68 ± 0.25 %) and the lowest was the aqueous extract (6.53 ± 0.29 %).

Determination of Nutritional and antinutritional components of *Berberis vulgaris*

The presence of Vitamin C in the berberry fruits was detected at 225 nm at 3.096 min. Comparisons were made between the standard chromatograph and the samples chromatograph. All the retention time lie at the same time. The standard chromatograph had a bigger area compared to the sample area. Larger area indicates that the standard had a larger amount of Vitamin C compared to the samples. Little interferences were detected in the chromatograph due to the contaminants and interferences. The HPLC profile of aqueous *Berberis vulgaris* showed significant presence of Vitamin C

Retention time for standard Malic acid was similar to the retention time in the sample. Both standard and sample detected at 14.416 min. Few interferences were detected in the chromatograph due to the contaminants and interferences. The presence of Malic acid was detected at 225 nm.

Antinutritional composition of Tannin was detected at 350 nm and the retention time of 16.527 min. Both the standard and sample chromatograph did not show any sign of interference.

Nutritional and antinutritional quantification were calculated from the curve generated by plotting the peak area of each standard versus sample concentration. The highest amount was Vitamin C $11102.81 \mu\text{g} \pm 2.01$, followed by Malic acid $116.03 \mu\text{g} \pm 1.12$ and Tannin with $20.51 \mu\text{g} \pm 0.59$ (Table. 3).

Discussion

Antioxidant Activity of *Berberis vulgaris*

All the extractions were significantly different in terms of antioxidant activity. Both the BHT and natural antioxidant Vitamin E act as comparison standard. The methanol extraction showed second highest and aqueous extraction came in last with values of 16.80 ± 0.23 % and 6.53 ± 0.29 % respectively. Based on the results obtained, it was highly possible that several compounds of different polarity may contribute to the total phenolic content of the extracts. Methanol extracts may include phenolic and hydroxy-phenolic compounds with acids, alcohols, sugars or glycosides, as reported by Kim and Pratt (1993). Many researchers had shown that high total phenols content increases antioxidant activity and there was a linear correlation between phenolic content and antioxidant activity (Motaleb et al, 2005).

The antioxidant activity of phenolics was mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Marja *et al.*, 1999).

The TBA test is a colorimetric technique in which the absorbance of a red chromogen formed between TBA and malondialdehyde is measured (Rhee, 1978). The first step in lipid oxidation is the abstraction of a hydrogen atom from a fatty acid and subsequent oxygen involvement gives a peroxy

radical. Peroxide then gradually decomposes to lower molecular compounds; mainly malondialdehyde (Ledwozyw *et al.*, 1986) during oxidation process and malondialdehyde can be determined by TBA method. At low pH and high temperature, malondialdehyde binds TBA to form red complex that can be measured at 532 nm (Ottolenghi, 1959). Generally, the antioxidants suppress the hydrogen atom abstraction from a fatty acid, which leads to the decrease of hydroperoxide formation. It is well known that phenolic compounds act as hydrogen donors in that reaction mixture and therefore, the formation of hydroperoxides were decreased (Farag *et al.*, 1989).

From the result, the antioxidant activity of ethanol extraction of BV was the highest among all the extracts, also maybe due to its phenolic content. Recently, Farag *et al.* (2003) reported that, addition of extracted phenolic compound from olive oil, to sunflower oil at 400-ppm level, exhibited remarkable antioxidant activity and were superior to that of BHT in retarding sunflower oil oxidative rancidity. The results also had indicated that different solvents used may have different effects on antioxidant activities.

Nutritional and Antinutritional Components of *Berberis vulgaris*

In this studies, only fully ripen fruits were used for the detection of Vitamin C, Malic acid and Tannin. This because during the ripening process many fruits undergo characteristic changes that, in general lead to an increase in nutritive value. However, some important nutrients tend to decrease when fruits become fully ripe. This depends upon the mechanism of the ripening process such as climate, temperature, sunlight and many more. These factors must be taken into account because the ripening can affect the nutritional and antinutritional composition. For example the advanced ripeness caused the Vitamin C content to decrease, most due to its antioxidant function when ripening absorb high amounts of oxygen as a result of increase rate of cell respiration, the physiologic change in climatic fruits (Tünk *et al.*, 1993).

The presence of nutritional compositions such as Vitamin C and Malic acid and also antinutritional composition of Tannin has been detected. The highest amount was Vitamin C $11102.81\mu\text{g} \pm 2.01$, followed by Malic acid $116.03 \mu\text{g} \pm 1.12$ and Tannin with $20.51 \mu\text{g} \pm 0.59$. The results of Vitamin C and Malic acid had higher mean and standard deviation because common processing techniques, like dry and especially wet heating, extracting with water, and etc sometimes have unintended adverse effects on the nutritional quality of the fruits for example the heat treatment reportedly alters the chemical nature and decreases the nutritional quality of berberry fruits.

HPLC is a highly sensitive method of detection and quantification of any chemicals in a particular sample using ultraviolet and visible absorbance. However this method suffers from interferences. For example contaminants in the Vitamin C sample chromatogram indicate the presence of contaminants that can interfere with the peaks displayed by the chromatograph, which lead to high standard deviation. All in all, a slightest presence of foreign materials or contaminants can affect the outcome of experiment. Other factors that can cause error in detection and quantification are with the selection of mobile phase and solvents. Only selected mobile phase and solvents with the right amounts have been recommended because they yield higher recoveries. For example Vitamin C is highly sensitive to factors such as light, heat, and pH. A slight change in mobile phase, solvents and temperature during the detection can give false result to the chromatograph that would lead to change in retention time. Further more the differences in solvent refractive index cause an unstable chromatographic baseline. Selected solvents such as methanol and acetonitrile were used because they were found to be very stable and compatible with the reversed-phase HPLC.

Conclusion

From these results it can be concluded that, BV has an antioxidant activity at different degrees in different extraction solvents The antioxidant activity of absolute ethanol extraction was significantly higher and exceeds standard antioxidant such as BHT and Vitamin E. The presences of nutritional and

antinutritional composition were also detected using high performance liquid chromatography (HPLC). From the result it can be concluded that BV fruits contain rich amount of Vitamin C, followed by Malic acid and Tannin. The amount of Vitamin C provides a bulk characteristic of antioxidant activity in the fruit. Meanwhile Malic acid act synergistically with Vitamin C to increase the antioxidant activity. The nutritional composition of Tannin was detected. The rich amount of Vitamin C makes *Berberis vulgaris* fruit a good source of Vitamin C for daily consumptions.

Table 1: Condition for HPLC separation of Vitamin C, Malic acid and Tannin.

Parameters	Vitamin C	Malic acid	Vitamin E
Column	Genesis C18, 5 μ m, 4.6 X 150mm	Allure Organic Acids, 5 μ m 4.6 X 300mm	Merck Hibar, lichrosolv RP 18 5 μ
Mobile Phase	0.1M KH ₂ PO ₄ / methanol/ TBAOH (97:3:0.05)	1000mM phosphate buffer, pH 2.5	A 0.2% H ₃ PO ₄ in water; B: 0.2% H ₃ PO ₄ in methanol
Flow rate	1.0 mL/min	0.5 mL/min	1.0 mL/min
Detection	UV 225nm	UV 225nm	UV 350nm

Table 2: Antioxidant activity of *Berberis vulgaris* extracts, BHT and Vitamin E using Thiobarbituric acid method (TBA).

Sample	Antioxidant Activities (%) (Mean \pm SD)
Absolute Ethanol extract	27.26 \pm 1.07 ^a
Methanol extract	16.80 \pm 0.23 ^a
Aqueous extract	6.53 \pm 0.29 ^a
BHT	20.29 \pm 0.23 ^a
Vitamin E	6.68 \pm 0.25 ^a

Values with the same letter (a) were significantly ($p < 0.05$) different between the samples.

Figure 1: Antioxidant activity of methanol and ethanol of extractions of *Berberis vulgaris* as measured by the TBA method. Absorbance value represents triplicates of different samples analyzed. Values with same letter are significantly different ($p < 0.05$) between samples.

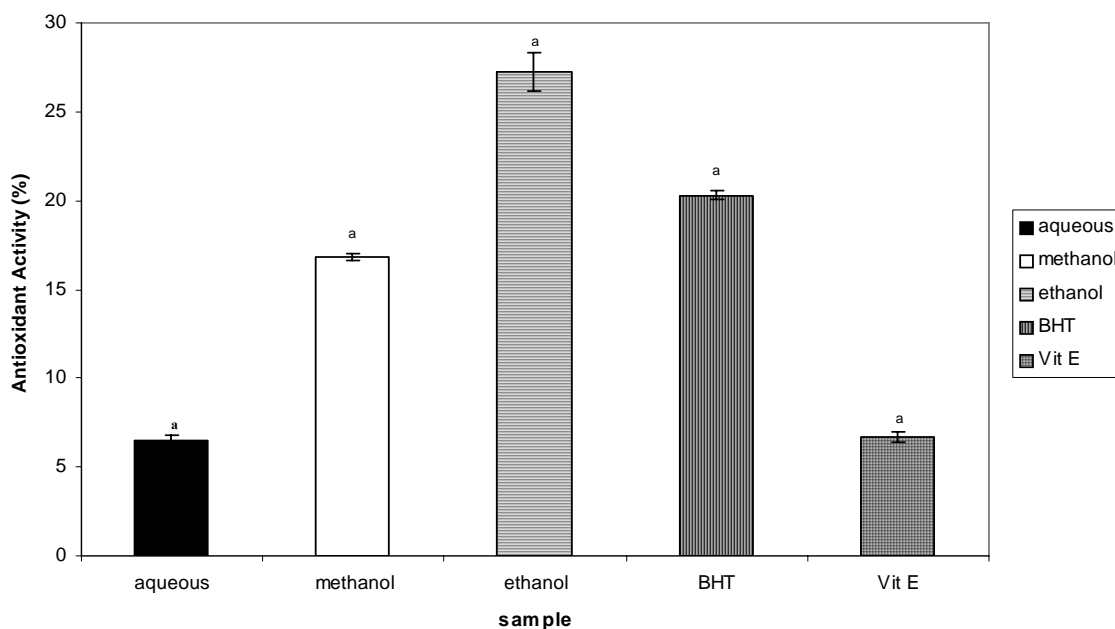


Table 3: Composition of nutritional and antinutritional in 100g of *Berberis vulgaris* fruits

Sample	Amount in $\mu\text{g}/100\text{g}$ (%) (Mean \pm SD)
Vitamin C	11102.81 \pm 2.01
Malic acid	116.03 \pm 1.12
Tannin	20.51 \pm 0.59

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