



International Journal of Herbal Medicine

Isolation of Berberine from *Berberis vulgaris* Linn. and Standardization of Aqueous extract by RP-HPLC

Deepak Pradhan ^{1*}, Prativa Biswasroy ², Dr. K. A. Suri³

1. Research Chemist., R & D Dept., Ixoreal bio-med Pvt Ltd., Hyderabad, India.
[E-mail: hi_honey.com@rediffmail.com]
2. Research Chemist., R & D Dept., Ixoreal bio-med Pvt Ltd., Hyderabad, India.
3. Scientist-G, Ex- Director Grade Scientist, Indian Institute of Integrative Medicine (IIIM), Jammu, India.

Berberis vulgaris L. belongs to family Berberidaceae is native to Europe and the British Isles in Iran. Barberries is an important production of South Khorasan; biggest producer of barberries in Iran. It is a deciduous shrub having yellow flowers and scarlet colored fruit in the form of berries. Twenty two alkaloids have been reported so far from root, stem leaves and fruit of this plant, which are of medicinal importance. From preliminary Phytochemical analysis showed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, saponin, tannin and flavonoid. One of the major Isoquinoline alkaloid is Berberine. From the present investigation an attempt has been made to standardize aqueous extract of *Berberis vulgaris* on the basis %age Berberine content by RP-HPLC.

Keyword: *Berberis vulgaris* L., Berberine, Aqueous extract, RP-HPLC.

1. Introduction

Medicinal herbs are the reservoir of therapeutically bio-active phytoconstituents. The amazing structural diversity among them makes a useful source of novel therapeutic compounds^[1]. In the current scenario natural product chemists with interest in natural products have intensified their efforts towards scientific evaluation of traditional medicines^[2]. *Berberis vulgaris* Linn which is commonly known as Barberry belongs to the family Berberidaceae. Berberis is the genus of spiny deciduous evergreen shrubs, with yellow wood and yellow flowers, and comprises 190 species^[3,4]. Anthropologists believe in a ritual practice or sacred object, especially by Native Americans that it works as a supernatural power or as preventive or remedy of illness^[4,5]. In traditional

medicine the plant is therapeutically active against gastrointestinal diseases, hemorrhages, gum inflammation, sore throat, biliary fevers, malaria, leishmaniasis, hepatitis, inflammation, diarrhea, and high blood cholesterol^[6-9]. Berberine, an Isoquinoline alkaloid and the major ingredient of this plant, has been used for treating diarrhea and gastrointestinal disorders for a long time Indian Ayurveda practices^[10-14]. It has multiple pharmacological effects including; antimicrobial activity against microorganisms, inhibition of intestinal ion secretion and smooth muscle contraction, inhibition of ventricular tachyarrhythmia, reduction of inflammation, stimulation of bile secretion and bilirubin discharge^[15-18]. Standardization is one of the measures designed to ensure consistency in the quality

and quantity of the active principle in the herbal extract and herbal formulations. Standardization is a basic prerequisite of quality. Irrespective of the question of whether the active ingredient of an herbal drug are known or not, every manufacturing process should be subjected to standardization. The extract can be standardized to a specified percentage of active principle by appropriate dilution with adjuvant carrier materials. In practice most botanicals owe their activity to a group of structurally related active compounds rather than to a single compound and the extract may be standardized according to its total content of active ingredients. The presumption is that when the extract is properly standardized to a key constituent, the other constituents in the extract which are responsible for the activity are also present in sufficient quantity. In these cases the constituent in the question is a marker substance; while practical experiences have shown that extracts containing the marker compound at a specified percentage have the desired activity. Standardization of botanical extract necessary because strong standardized extracts may cause stomach upset and should be used for no more than two weeks continuously^{19,20}. So the present work is carried out for preliminary Phytochemical screening, isolation of phytomarker and standardization of aqueous extract of *Berberis vulgaris*.

2. Materials and Methods

2.1 Plant Material and Extract Preparation

Fresh root of *Berberis vulgaris* L. were collected from Jammu, in the month of November. Roots were cleaned with running tap water were chopped into pieces. They were dried under shade at ambient temperature for 5 days and the air-dried roots were then ground to powder for extraction. The aqueous extract was

prepared by cold maceration of 1.5Kg of powdered root in 5lts of distilled water for 48 hr. Then the extract was filtered, concentrated, dried in vacuum (yield 142gm).

2.2 Preliminary Phytochemical Investigation

Preliminary Phytochemical analysis was performed through standard official procedure for the identification of different classes of components present in extract²¹.

2.3 Isolation of Berberine

The aqueous extract was dissolved in 1% HCl. The solution was filtered, alkalinized with a concentrated NH_4OH to pH 8 and extracted with chloroform from which tertiary alkaloids were obtained after evaporation of the solvent (21.22 gm). The pure phytoconstituents from the chloroform fraction isolated by column chromatography on silica gel 100-200 mesh and eluted with chloroform and gradient with methanol ($\text{CHCl}_3:\text{MeOH}$, 9:1; 8:2) to isolated a yellow needles shape crystal compound (1.021gm) which was identified by TLC, ^1H NMR, ^{13}C NMR and compared with the spectral data from literature values²².

2.4 Standardization of Aqueous Extracts by RP-HPLC

2.4.1 Preparation of Standard Solution

A stock solution of 10mg/ml is prepared by taking 100mg accurately weight pure standard Berberine and transferred to a 10 ml volumetric flask. 7ml of hplc grade methanol was added and sonicated for 10 min. Final volume is made up to 10ml of hplc grade methanol to a concentration of 10mg/ml. The working standard solution of concentration 400, 800, 1200, 1600, 2000ppm are prepared by transferring 0.4, 0.8, 1.2, 1.8, 2ml solution from stock solution and volume made up to 10ml with hplc grade methanol.

2.4.2 Preparation of Working Solution

7mg of powder aqueous extract is weight accurately and transferred to a 10ml volumetric flask. To this 7ml of hplc grade methanol is added and sonicated for 15 min. to get completely dissolved. Finally the solution volume made up to 10ml with hplc grade methanol.

2.4.3 Chromatographic Conditions

The HPLC analysis was carried out using a WATER RP-HPLC system with EMPOWER 2.0 software. The separation was carried out on a Sunfire column RP-C18, (250 × 4.6 mm ID, 5 μm). Detection

was performed at 254nm. Optimum efficiency of separation was obtained using isocratic mode 1% ammonium chloride (solvent A) and Acetonitrile (solvent B) with a flow rate 0.8ml/min. Other parameters adopted were as follows: injection volume 20 μL, column temperature 30°C, detection wavelength 254nm.

3. Results

The %age yield of the aqueous extract found to be 9.46. Organoleptic study revealed that the aqueous extract is brown color and bitter test. Preliminary Phytochemical analysis of aqueous extract showed in the table no. 1.

Sl. No.	Constituents	Result
1.	Alkaloids	+ve
2.	Carbohydrates	+ve
3.	Amino acids	+ve
4.	Tannins	+ve
5.	Flavonoid	-ve
6.	Tri terpenoids	-ve
7.	Phytosterol	-ve
8.	Glycoside	+ve
9.	Saponin	+ve

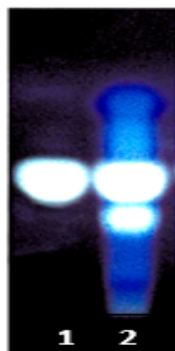


Fig 1: TLC of Berberine¹¹ and Aqueous extract²¹ at 365nm.

The yellow color compound isolated from the chloroform fraction has Melting point 264.6°C, Rf 0.73 with the mobile phase n-Propanol: Formic acid: Water (90 :10 : 5) and the TLC plate is visualized with 5% sulfuric acid in Methanol. The Compound

shows Rt of 30.75 min. at wavelength 254nm. A standard calibration curve is plotted [calibration equation $y = 5969.867x + C$ ($C=0$) and regression coefficient $r^2 = 0.999$].

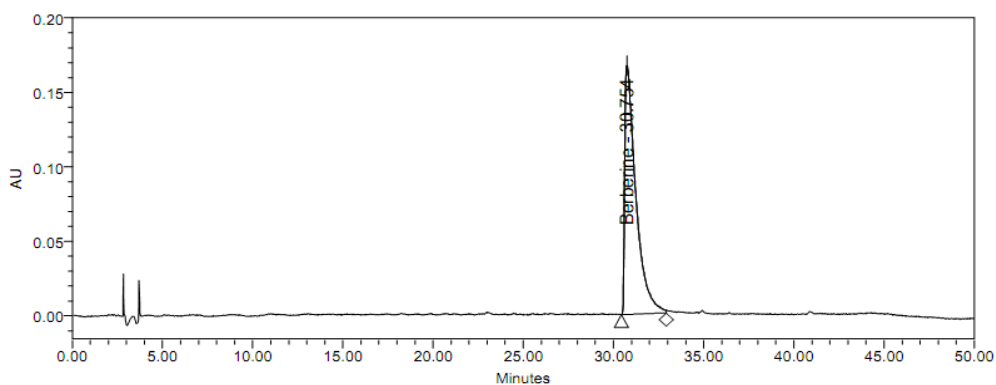


Fig 2: Chromatogram of pure Berberine (HPLC) at λ_{max} 254nm

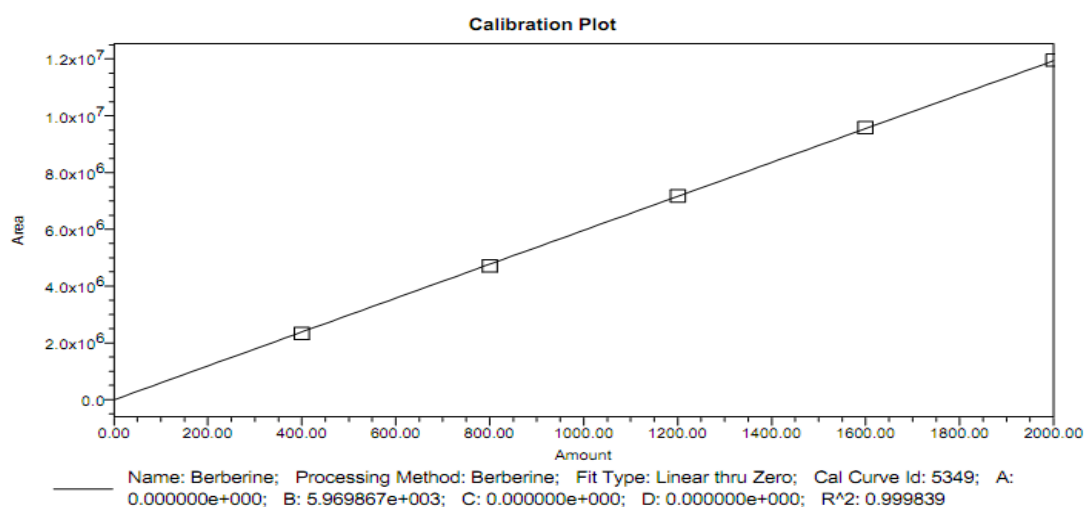


Fig 3: Standard calibration of Pure Berberine (HPLC) at λ_{max} 254nm

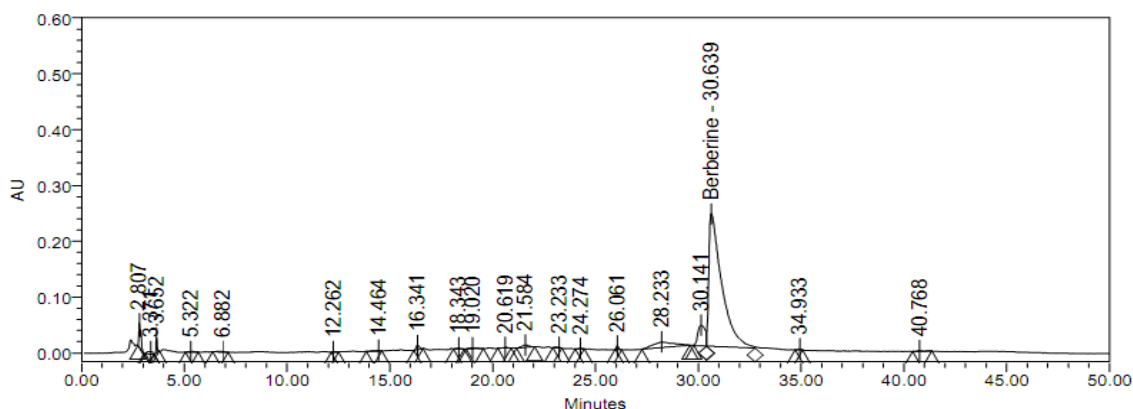


Fig 4: Chromatogram of Aqueous extract (HPLC) at λ_{max} 254nm

4. Discussion

From the preliminary Phytochemical analysis it evidence that Aqueous extract *Berberis vulgaris* L. is a broad spectrum extract which includes Alkaloids, Glycoside,

Amino acids, Tannin, Saponin. By comparing melting point, TLC profile, HPLC with the literature values it concluded that yellow color compound is Berberine.

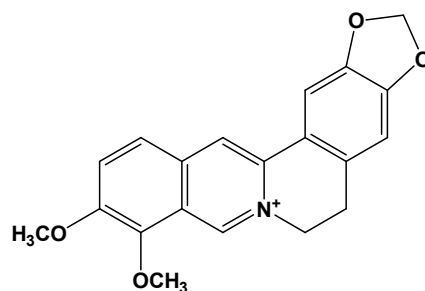


Fig 5: Structure of Berberine

It is calculated that the Aqueous extract contain 9.83% with the help of standard calibration curve.

5. Future prospective

In recent years, complications such as drug tolerance, drug resistance, multi drug resistance, critical side effects, intercal treatment of diseases in modern practice of medicine, enforce research to moves towards natural product chemistry to isolate and standardization of the botanical extract. According to Hippocrates “*Let food be thy medicine and medicine be thy food*”. The Aqueous extract of *Berberis vulgaris* is a broad full spectrum extract, which has rich history medicinal use against cardiovascular disorders such as, hypertension, Ischemic induced ventricular tachyarrhythmia, liver and biliary diseases, gastrointestinal diseases, hemorrhages, gum inflammation, sore throat, malaria, leishmaniasis, hepatitis, inflammation, diarrhea, and high blood cholesterol. From clinical trial profile for this aqueous extract has great potency in increase the cardiac contractility, it reduces the blood pressure by lowering the peripheral vascular resistance. Berberine increases the function of m-RNA, protein and hepatic low density lipoprotein receptors expression which improved clearance of plasma LDL-c through receptor mediated endocytosis leads to inhibiting the cellular cholesterol biosynthesis. Hence forth Berberine may be a novel drug for the monotherapy treatment of hypercholesterolemia. Due to presence of citric acid, malic acid, citric acid and ascorbic acid in the aqueous extract,

contribute its beneficial effect towards inflammatory fever. So the aqueous extract of *Berberis vulgaris* L. has a tremendous potency to come as *Future green medicine*.

6. Conflict of Interest Statement

The authors report no conflict of interest.

7. References

1. Arora S, Kaur K, Kaur S. Indian medicinal plants as a reservoir of protective phytochemicals, Teratog Carcinog Mutagen 2003; 1:295-300.
2. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test), International Journal of Applied Science and Engineering 2006; 4(2): 115-125.
3. Mahdi S, Saied J, Fallah R. Therapeutic application of different parts *Berberis vulgaris*, International Journal of Agriculture and Crop Sciences 2012; 4 (7): 404-408.
4. Arayne MS, Sultana N, Bahadur SS. The berberis story: *berberis vulgaris* in therapeutics, Pak. J. Pharm. Sci. 2007; 20(1): 83-92.
5. Chandler R, Frank LF, Shirley NH. Herbal Remedies of the Maritime Indians, Journal of Ethnopharmacology 1979; 1: 49-68.
6. Golzarand M, Ebrahimi MM, Arefhosseini SR, Ali AA. Effect of processed *Berberis vulgaris* in apple vinegar on blood pressure and inflammatory markers in type 2 diabetic patients, Iranian J. Diabetes and Lipid Disorders 2008; 8: 15-20.

7. Motalleb G, Hanachi P, Kua S, Fauziah O, Asmah R. Evaluation of phenolic content and total antioxidant activity in *Berberis vulgaris* fruit extract. *J. Biological Sci.* 2005; 5(5): 648-653.
8. Naffisi A, Food and Drinks' properties. Isfahan University Press, Isfahan, 1990; pp-150
9. Aynehchi Y, Pharmacognosy and Medicinal Plants of Iran, Tehran University Press, Tehran 1986; p: 1041
10. Freile ML, Giannini F, Pucci G, Sturniolo A, Rodero L, et al. Antimicrobial activity of aqueous extracts and of berberine isolated from *Berberis heterophylla*, *Fitoterapia* 2003; 74: 702-705.
11. Kuo CL, Chi CW, Liu TY. The anti-inflammatory potential of berberine in vitro and in vivo. *Cancer Lett.* 2004; 203: 127-137.
12. Lee S, Lim HJ, Park HY, Lee KS, Park JH, et al. Berberine inhibits rat vascular smooth muscle cell proliferation and migration in vitro and improves neointima formation after balloon injury in vivo: Berberine improves neointima formation in a rat model, *Atherosclerosis* 2006; 186: 29-37.
13. Peng PL, Hsieh YS, Wang CJ, Hsu JL, Chou FP. Inhibitory effect of berberine on the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2 Pei-Ling, *Toxicol Appl Pharmacol* 2006; 214: 8-15.
14. Yamamoto K, Takase H, Abe K, Saito Y, Suzuki A. Pharmacological studies on antidiarrheal effect of a preparation containing berberine and geranii herba, *NipponYakurigaku Zasshi* 1993; 101: 169-175.
15. Kuo CL, Chou CC, Benjamin YM. Berberine complexes with DNA in the berberine-induced apoptosis in human leukemic HL-60 cells, *Cancer Lett* 1995; 93: 193-200.
16. Wang YX, Yao XJ, Tan YH. Effects of berberine on delayed after depolarizations in ventricular muscles in vitro and in vivo, *J Cardiovasc Pharmacol* 1994; 23: 716-722.
17. Joshi V, Joshi RP. Some Plants used in Ayurvedic and Homoeopathic Medicine, *Journal of Pharmacognosy and Phytochemistry*, 2013; 2(1): 269-275.
18. Kunwar RM, Nepal BK, Kshhetri HB, Rai SK, Bussmann RW. Ethnomedicine in Himalaya: a case study from Dolpa, Humla, Jumla and Mustang districts of Nepal, *Journal of Ethnobiology and Ethnomedicine* 2006; 2(27).
19. Patel DK, Patel K, Dhanabal SP. Standardization of *Berberis aristata* extract through conventional and modern HPTLC techniques, *Asian Pacific Journal of Tropical Disease*, 2012; S136-S140.
20. Bairwa R, Jain H, Shrivastav B. Standardization and phytochemical investigation of *Berberis aristata*, *Asian J. Pharm. Ana.* 2012; 2(3): 81-84.
21. Quality Control Methods of Medicinal Plant Materials. Geneva: World Health Organization (WHO). 1998.
22. Jewers K, Manchanda A H. *J.C.S.Perkin II*, 1972; 1393-6.