INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

DEVELOPMENT OF QUALITY STANDARDS OF *BERBERIS ARISTATA* STEM BARK

Javed Ahamad*, Showkat R. Mir, Kamran J Naquvi
Phytochemistry Research Laboratory, Department of Pharmacognosy & Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi- 110062, India

Article Received on: 02/11/11 Revised on: 20/01/12 Approved for publication: 11/02/12

*E-mail: jas.hamdard@gmail.com

ABSTRACT
Berberis aristata is an important medicinal plant of family Berberidaceae. It is commonly known as *Zarishk* and *Daruhaldi*. It is mainly used for the treatment piles, liver diseases and diabetes. As the herb is used widely in the Indian traditional systems of medicine, it was thought worthwhile to develop the quality standards for its stem bark. The results of Pharmacognostic standardization of stem bark of *B. aristata* are very helpful in determination of quality and purity of the crude drug and its marketed formulation.

Keywords: *B. aristata*, Berberidaceae, Zarishk, Standardization.

INTRODUCTION

Berberis aristata DC (Berberidaceae) is an erect, spinous, deciduous shrub, growing in dry hot places of Himalayas from Garhwal to Bhutan up to altitude of 1800-3000 m, usually 1.8-3.6 meter in height.[1] Stem barks of *B. aristata* have astringent, febrifuge and stomachic effect[2,3,4] and are used to treat piles, spleen and diarrhoea, malaria, menorrhagia, diabetes.[5,6,7,8] *B. aristata* contains isoquinoline alkaloids mainly berberine and other phytoconstituents of it includes oxyberberine, berbamine, aromaline, kakrachine, palmatine, oxyacanthine, taxilamine and jatrorrhizine, some of the alkaloids are reported as being found in the chloride salt form.[9] The present paper deals with the preliminary standardization of stem bark of *B. aristata*

MATERIAL AND METHODS

Plant Material

The stem bark of *Berberis aristata DC*. was procured from the Khari Baoli market of Delhi and identified by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard. New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard.

Preparation of the Hot Methanolic Extract

The stem bark of *B. aristata* were cut into small pieces and air dried for 12-14 days in the shade. 500g of powdered aerial parts were extracted with 1.5 L of methanol using Soxhlet extraction apparatus for 4 h. The extraction was filtered and the filtrate was evaporated to dryness under reduced pressure at 50 °C (yield 12.5% *w/w* dry weight basis) and stored at 4 °C until use.

Preliminary Phytochemical Screening of *B. aristata* Extracts

The qualitative chemical tests were performed for hot methanolic extract according to the methods described by Farnsworth[10]; with some modifications.

Presence/Absence of Polyphenolic Compounds

Two to three drops of 1% $FeCl_3$ solution was added to 2 ml portions (1%) of each extract. Phenolic compounds produce a deep violet color with ferric ions.

Presence/Absence of Saponins

The extract is taken in test tube with small amount of water and shaken vigorously for one minute and observed for formation of rich lather, which is stable for more than ten minutes.

Presence/Absence of Flavonoids

The extract was dissolved in methanol (50 %, 1-2 mL) by heating. Then metal magnesium and 5-6 drops of conc. hydrochloride acid (HCl) were added. The solution turns red when flavonoids are present.

Presence/Absence of Steroid Glycosides

The extract was dissolved in equal volumes of acetic anhydride and $CHCl_3$. The mixture was transferred to a dry test tube and conc. H_2SO_4 acid was added at the bottom of the tube. Formation of a reddish brown or violet brown ring at the interface of the 2 liquids indicates presence of steroids.

Presence/Absence of Alkaloids

The alkaloids were extracted by refluxing the sample with sufficient amount of water for about 2 hr. The extract was concentrated on a rotor vapor, basified with NH_4OH and was extracted with $CHCl_3$ (three times). Then the content was concentrated and 2 drops were spotted separately on a thin layer chromatography (TLC) plate. After development the plate was dried, Dragendorff's reagent was sprayed onto them. Alkaloids give an orange color with Dragendorffs reagent.

TLC Fingerprint Profile

Solvent systems were developed for establishing the HPTLC pattern for the methanolic extract of *B. aristata*. Various visualization techniques were used to come up with the best HPTLC fingerprint, like UV 254, UV 366, iodinisation and spray reagents like anisaldehyde, vanillin and sulphuric acid were also tried[11]. The developed plate was dried in air, visualized in UV at wavelengths 366 nm and photographed. Solvent system: n-butanol: ethyl acetate: formic acid: water (3: 5: 0.5: 1).

Physico-chemical parameters of *B. aristata* stem bark

Physicochemical parameters were determined for *B. aristata* aerial parts according to methods described in WHO guidelines[12].

Moisture Content

The powdered material (10 g) was placed in a moisture dish and dried to a constant weight in an oven at 100-105^{0}C. The loss of weight (in mg/g) of air dried was calculated as follows:

$$\% \text{ Moisture content} = \frac{\text{weight loss}}{\text{weight of sample}} \times 100$$

Total Ash Content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600 °C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a dessicator. The content of total ash (in mg/g) of air-dried material was calculated as follows:

$$\% \text{ Total ash} = \frac{\text{weight ash}}{\text{weight of sample}} \times 100$$

Acid Insoluble Ash Content

HCl (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a dessicator and weighed. The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

$$\% \text{ Acid insoluble ash} = \frac{\text{weight ash}}{\text{weight of sample}} \times 100$$

Determination of water soluble ash content

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water soluble ash content was calculated using the following equation.

Methanol Soluble Extractive Matter

Accurately weighed powdered material (4 g) was placed in a glass stoppered conical flask. Methanol (100 ml) was added to the flask and it was weighed to obtain the total weight, including the flask. Then, the flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h, and then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of methanol. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25 ml of the filtrate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h and cooled in a dessicator and weighed. The content of extractable matter (in mg/g) air-dried material was calculated as follows:

$$\% \text{ Water soluble ash} = \frac{\text{total ash content-water insoluble residue in total ash}}{\text{weight of sample}} \times 100$$

Water Extractable Matter

The same procedure as described for the methanol extractable matter was followed for the determination of water extractable matter using distilled water instead of methanol.

Chloroform, DCM, Pet. Ether and Acetone Soluble Extractive Matter

$$\% \text{ Methanol soluble extractive matter} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 4 \times 100$$

The same procedure as described for the methanol extractable matter was followed for the determination of chloroform extractable matter using distilled chloroform instead of methanol.

RESULTS

The results of standardization parameters are:

Phytochemical Screening

The results of preliminary phytochemical investigation of methanolic extract of *B. aristata* showed the presence of alkaloids, glycoside, phenolic compounds, carbohydrate, flavonoids and sterols.

Behavior of Powder Drugs with Different Chemical Reagents

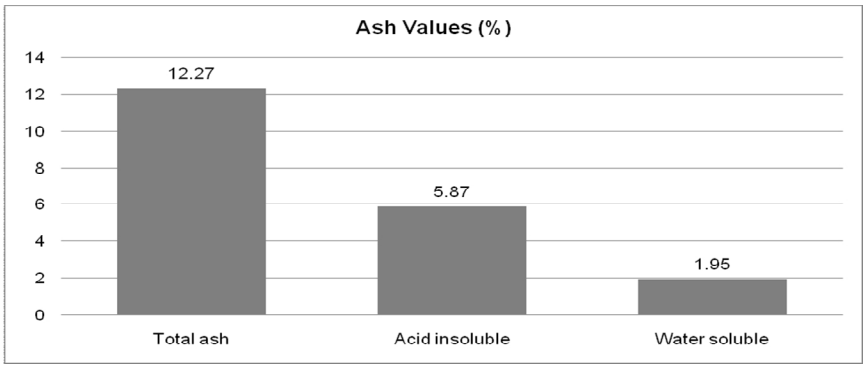
The observations are recorded in **Table –I**

Table I Effect of different chemical reagents on *B. aristata*

S. No.	Chemical reagents	*B. aristata*
1	Iodine (I_2)	Light Yellow
2	Glacial acetic acid	Yellow
3	$FeCl_3$ solution (5 %)	Light Gray
4	Lead acetate (10%)	Yellow
5	Picric acid	Light Yellow
6	KOH (1 %)	Light Yellow
7	NaOH (5 %)	Orange
8	HNO_3 (50%)	Red

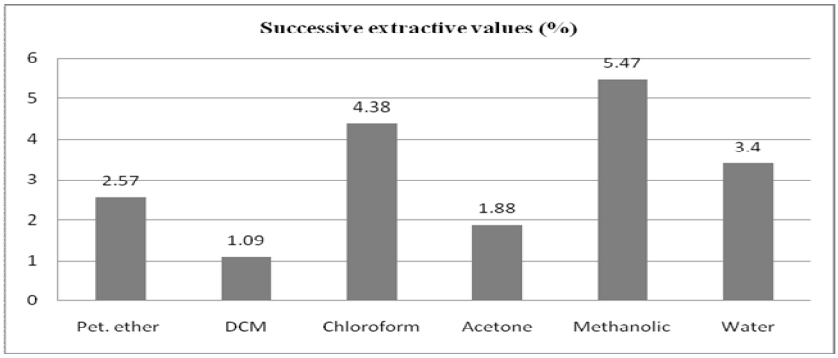
Ash Values

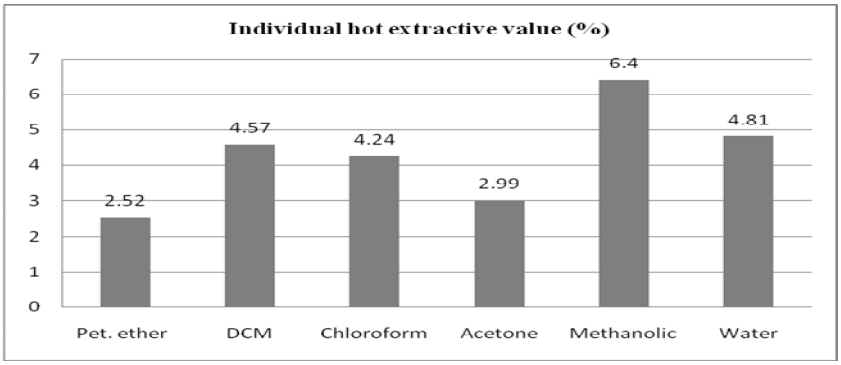
The results of ash values for *B. aristata* shown below

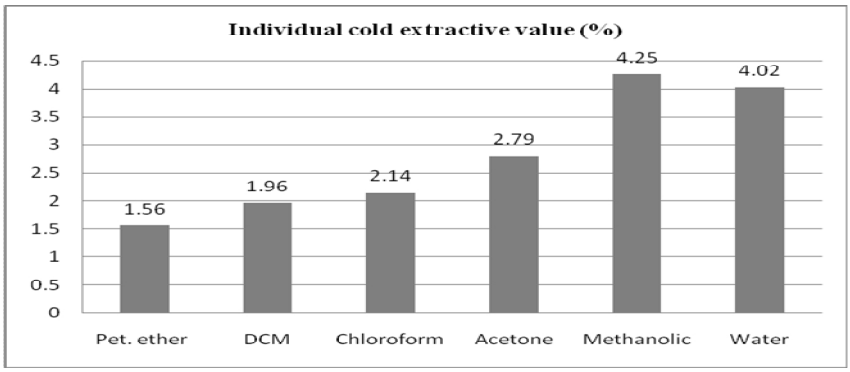


Extractive Value

The successive and individual extractive values of *B. aristata* with different solvents have been determined and results are







Foreign Matter

The results for foreign matter are given in **Table II** for *B. aristata.*

Table II: Foreign matters in *B. aristata* stem bark (200g).

Drugs	Wt. of Drug (g) (A)	Wt. of Drug After removal of F. M. (g) (B)	Wt. of foreign matter (g) (A-B)	% F. M.
B. aristata	201	196	5	2.5
	199	195	4	2.0
	200	196	4	2.0
Mean				**2.17**

Moisture Content

The moisture content of stem bark of *B. aristata* was found to be 6.50%.

Swelling Index

No swelling was found in both *B. aristata.*

Foaming Index

The foaming index was less than 100 in both *B. aristata.*

HPTLC/TLC Profile of Methanolic Extract of Stem Bark of *B. aristata*

Table III: Finger print profile of *A. absinthium*

Plant	Solvent system	Wavelength (nm)	No. of spots (R_f values)
B. aristata	n-butanol: ethyl acetate: formic acid: water (3: 5: 0.5: 1)	366	4 (0.47, 0.52, 0.74, 0.86)

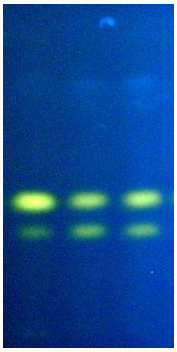


Fig I: HPTLC Chromatogram of methanolic extract of *B. aristata* at 366nm

DISCUSSION

Standardization of crude drug is an integral part of establishing its correct identity. The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. Phytochemical screening revealed the presence of alkaloids, polyphenolic compounds, flavonoids and steroid glycosides in both HME. The physicochemical analysis of plant drugs is an important for detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity and quality of drugs. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition[14]. The total ash usually consists of carbonates, phosphates, silicates and silica, which include both physiologic ash and nonphysiologic ash. A high ash value is indicative of contamination, substitution, adulteration, or carelessness in preparing the crude drug for marketing[13]. Acid insoluble ash indicates contamination with silica, for example, earth and sand. Comparison of this with the total ash value of the same sample will differentiate between contaminating materials and variations of the natural ash of the drug. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Insufficient drying leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles[13]. HPTLC fingerprint profile will serve as a reference for quick quality control approval of the drug. All these parameters, which are being reported, could be useful in identification of distinctiveness features of the drug and also valuable in manufacturing as raw material or in prescription medicine. In conclusion, the results obtained from phytochemical screening studies and physico-chemical parameters can be used to standardize stem bark of *B. aristata.*

REFERENCES

1. Anonymous. "Reviews on Indian medicinal plants.'' 2004; 4: pp. 150-157.
2. Malhotra CL, Balodi, B. Medicinal plants of alpine region of Gori Vally, Kumaon. J Sci Res Plant Med. 1984; 5(1): 1-8.
3. Srivastava TN, Rajasekharan S, Badola DP, Shah DC. An index of an available medicinal plant, used in Indian system of medicine from J & K state. Ancient Sci Life. 1986; 6: 49-63.
4. Jha PK, Choudhary RS, Choudhary SK. Studies of medicinal plants of Palamau (Bihar)-(IInd part). Biojournal. 1997; 9: 21-38.
5. Uniyal MR, Issar RK. Commercially and traditionally important medicinal plants in Mandakini vally of Uttarakhand Himalayas. J Res Indian Med. 1969; 4(1): 83-96.
6. Chauhan V, Chauhan NS. Ethno-botany of the Giri area of Sirmour district of Himachal Pradesh. Bull Med Ethnobot Res. 1988; 9: 106-122.
7. Sharma PK. Herbal remedies for treating rheumatic pains in J & K. Indian J. For. 1991; 14: 206-210.
8. Singh PB. Medicinal plants of Ayurvedic importance from Mandi district of Himachal Pradesh. Bull Med Ethnobot Res. 1993; 14: 126-136.
9. Chakravarti KK, Dhar DC, Siddiqui S. Alkaloidal constituents of the bark of *B. aristata* (Rassaut). J Sci. Ind. Res. 1950; 9B:161-164.
10. Farnsworth NR. Biological and Phytochemical screening of plants. J Pharm Sci. 1996; 55: 225-276.
11. Wagner H, Bladt S, Zgainsk EM. Plant drug Analysis: A thin layer chromatography Atlas, Springer, Verlag, Berlin. 1984.
12. WHO. Quality control methods for medicinal plant materials. Geneva: Word Health Organization. 1998.
13. Mukherjee PK. Quality Control of Herbal Drugs. New Delhi, India: Business Horizons. 2002.
14. Singh MP, Sharma CS. Pharmacognostical evaluation of *Terminalia chebula* fruits on different market samples. Int J ChemTech Res. 2010; 2: 57-61.

Source of support: Nil, Conflict of interest: None Declared