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Quantitative Estimation of Berberine in Roots of Different provenances of *Berberis aristata* DC by HPLC and Study of their Antifungal Properties

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ABSTRACT

Berberis (Family: Berberidaceae), a genus of stiff shrubs, is distributed in temperate and subtropical parts of Asia, Europe and America. *Berberis aristata* DC is one of the chief sources of the drug (Rasaut) which is useful in the treatment of jaundice and enlargement of spleen. Alkaloids, terpenoids, flavanoids, sterols, anthocyanins, vitamins and carotenoids have been characterized from the different parts of the plant. Berberine is considered to be its active ingredient besides palmatine, jatrorhizine, berbamine, etc. In spite of its use as an ingredient of several formulations, the precise and sensitive analytical method for quantification of berberine is not available. A precise, sensitive and reproducible method using High Performance Liquid Chromatography (HPLC) was developed to quantify berberine alkaloid in the root samples of *B. aristata*. The separation was carried out using C-18 column and mobile phase used was acetonitrile and water (1:1). The detection was performed using UV-VIS detector. The proposed method can be used for detection, monitoring and quantification of berberine in *B.aristata.* Differential antifungal activity against three common forest pathogens was also quantified in varied sources of *B. aristata*.

KEYWORDS: Antifungal, berberine, *Berberis aristata*, high performance liquid chromatography

INTRODUCTION

Berberis aristata DC (Family: Berberidaceae; Hindi: Daruharidra, Rasaut; English: Indian barberry) is one of the economically important and of high medicinal value species of temperate areas. It is an erect, glabrous spinescent shrub, 3–6 m in height with obovate, subacute and entired leaves [1]. It is distributed in India up to an altitude of 1,500–2,400 m. Its stem, roots and fruits are used in many Ayurvedic preparations. The plant got hepatoprotective, antitumour, sedative and wound healing properties. Rasaut is one of the very important and a useful preparation obtained from this plant and is used in curing many human ailments. Berberine is one of the important alkaloidal active principles of this plant [2,3]. Though, *B. aristata* is prescribed in several traditional pharmaceutical preparations, but lack of technological inputs to identify and define molecular landscape of potentially bioactive compounds and their quantification bearing this medicinal plant is missing. In the paper, standardization of berberine alkaloid by High Performance Liquid Chromatography technique that identifies, defines and quantifies active molecular fingerprints has been attempted. This approach may serve as a simple, genuine and appropriate technique to quantify berberine for further therapeutic exploitation.

Botanical fungicides are a potential and effective alternative to synthetic fungicides to save environment from their hazardous effects [4]. Approximately 2,400 plants species have been reported to possess pest-control properties [5]. No report on antifungal properties of *B. aristata* against forest pathogens could be traced in the literature. However, antimicrobial activity of *B. aristata* against human pathogens namely, *Aspergillus* spp., *Candida albicans*, *Bacillus cereus*, *E. coli*, *Staphylcoccus aureus* and *Streptococcus pneumoniae* has been reported [6–8]. In a preliminary screening of the methanol extracts of roots of *B. aristata* collected from seven provenances of Himachal Pradesh and two seasons were screened against three common forest fungi i.e. *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Rhizoctonia solani*.

RESULTS AND DISCUSSION

Calibration curve was constructed by plotting peak area against various concentrations of berberine. It was found to be linear and correlation coefficients were found in the range from 0.9995 to 0.9999. The linearity of equation is expressed as Y = mx + C. Using the proposed method the retention time of berberine was 11.7+ 0.02min.

On the basis of HPLC analysis the percentage of berberine alkaloid in the eleven methanol root extracts of *B. aristata* was determined and given in Table 1. On comparison with standard berberine the root samples collected from Sarhan (Shimla) provenance in winter season have highest berberine content (1.86%). The method allows reliable quantification of berberine and provides a good resolution and separation of berberine from other constituents of *Berberis aristata* DC. The proposed HPLC method is rapid simple and accurate and hence it can be used for quantitative estimation of berberine.

Interaction between extract and fungus (E × F) are differential in nature (Table 2; Figure 1). For example, practically all the sources of *B. aristata* suppressed less than 50 per cent growth of *C. gloeosporioides*, excepting S-3 source (75.2%) at 0.50 per cent concentration. The effectivity of *B. aristata* was more for *F. oxysporum,* where six sources showed more than 50 per cent inhibition (S-3: 85.2%, S-4: 72.3%, S-7: 86.1%, S-5: 71.9%, S-11: 81.9%, S-6: 60.5%). While in case of *R. solani*, barring one source (S-11; 46.2%), all other sources could reduce the fungal growth more than 50 per cent. In case of *F. oxysporum*, the maximum inhibition of 85.2 per cent was recorded with S-3, while it was lowest of 60.5 per cent with S-6. The former sources suppressed the fungal growth at par with S-7 (w: 86.1%). In a similar manner, two more sources had at par inhibition i.e. 72.3 and 71.9 per cent for S-4 and S-5, respectively. Cent per cent inhibition of *R. solani* was recorded with S-1 and S-8 sources. Likewise, S-2 (73.3 %) and S-7 (73.3%) registered at par inhibition of *R. solani*. Best performance in terms of fungal growth inhibition may be assigned to S-3 source of *B. aristata* as it inhibited all the three fungi to highest extent excluding *R. solani* (71.9 %).

The methanol extract of *B. aristata* from different sources showed varied activity against 3 forest fungi. For example, only 1 source of S-3 (Chaupal, Shimla) showed IC_{50} against the aerial pathogen, *C. gloeosporioides*. While, 6 and 10 sources of *B. aristata* exhibited IC_{50} against two common forest fungi, *F. oxysporum* and *R. solani*, respectively. Only 1 source, S-3 (Chaupal, Shimla) had IC_{50} against all the three test fungi showing its universal and high antifungal nature. Berberine, an isoquninoline alkaloid is composed of a planar aromatic cationic center that is thought to be the primary pharmacophore responsible for both antibacterial activity and recognition by efflux proteins in microbial cells, rendering them ineffective against efflux-resistant pathogens [9]. The variation in

Table 1: Percentage of berberine alkaloid in the roots of *B. aristata*

S. no.	Place (District, code)	Berberine (%)
1.	Sarhan (Shimla, S1)	1.36
2.	Narkanda (Shimla, S2)	1.29
3.	Choupal (Shimla, S3)	0.82
4.	Kharapathar (Shimla, S4)	1.58
5.	Sojha (Kullu, S5)	1.20
6.	Sangli (Kullu, S6)	1.30
7.	Sarhan (Shimla, S7) Winter collection	1.86
8.	Narkanda (Shimla, S8) Winter collection	1.45
9.	Choupal (Shimla, S9) Winter collection	1.26
10.	Kharapathar (Shimla, S10) Winter collection	1.31
11.	Jinjheli (S11)	1.45

Table 2: Effect of methanol extract of different sources of *B. aristata* at 0.50 per cent concentration on the radial growth of test fungi

	Fungus / Growth Inhibition (%)			Mean
Extract	*C. gloeosporioides*	*F. oxysporum*	*R. solani*	
S-1	30.0	23.3	100.0	**51.1**
S-2	10.5	20.5	73.3	**34.7**
S-3	75.2	85.2	71.9	**77.4**
S-4	10.0	72.3	70.5	**50.9**
S-5	16.6	71.9	69.0	**52.5**
S-6	5.2	60.5	56.2	**46.6**
S-7	42.3	86.1	73.3	**67.3**
S-8	22.8	24.2	100.0	**49.0**
S-9	5.2	16.6	50.5	**24.1**
S-10	17.6	28.0	54.7	**33.5**
S-11	16.2	81.9	46.2	**48.1**
Mean	**22.9**	**51.9**	**69.6**	
	Extract (E)	***Fungus (F)***	***Interaction (E × F)***	
SEM	**0.3**	**0.1**	**0.5**	
CD (5%)	**0.8**	**0.4**	**1.3**	

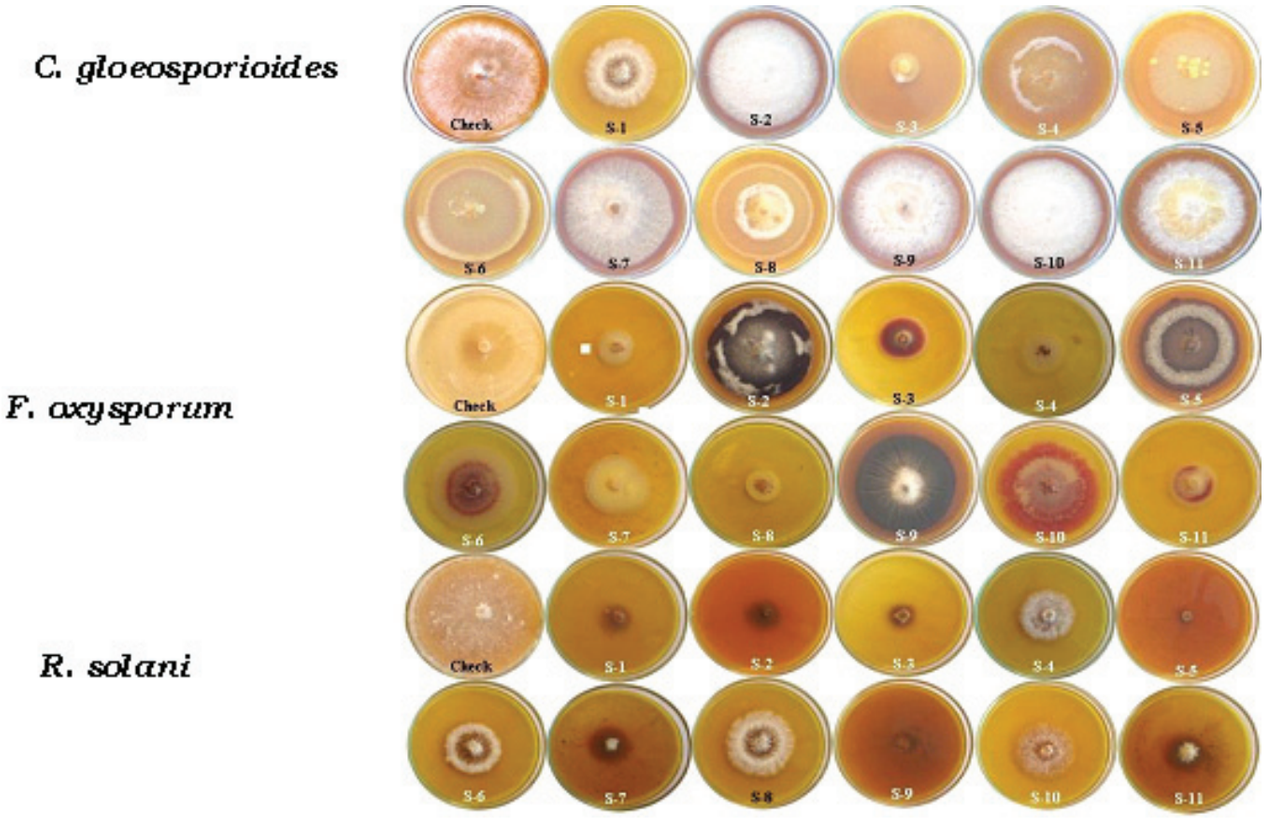

Figure 1: Effect of methanol extract of different sources of *B. aristata* root on the mycelial growth of test fungi.

azadirachtin content (0.194 to 0.670% by weight) of neem (*Azadirachta indica*) seed kernel of different agroclimatic zones of Rajasthan, India [10], variation in soluble sugars and proteins and phenols of jack pine (*Pinus banksiana*) seeds from various provenances across the natural range and clinal variation in soluble sugar in *Pinus sylvestris* [11] are few examples of different quantities of biochemicals in trees from varied agroclimatic zones/provenances. These quantitative differences in chemicals (also of berberine) among provenances may be responsible for differential antifungal activities against single or array of fungal species.

When antifungal activity was analyzed in relation to seasons (summer vs. winter), no consistent trend was observed. In case of S-1 (Sarhan, Shimla) the growth of *R. solani* was suppressed 100.0 per cent by summer collection but 73.3 per cent by winter collection. Exactly opposite trend was showed by S-2 (Narkanda, Shimla) for *R. solani*. This fact regarding random seasonal efficacy of *B. aristata* in relation to antifungal activity can also be substantiated for *F. oxysporum* in relation to source S-7 vs. S-1(Sarhan, Shimla) and S-3 vs. S-9 (Chaupal, Shimla). However, in Senegal, the difference in aza contents could be attributed to seasonal factors. In that country, neem seeds are harvested in some regions twice a year, namely in June/July and during October to December. The dry conditions during the months of October- December are conducive to high aza yield from the neem seeds [12].

These trends underline two important facts- one the geographical distribution of a plant not only influences its phenotypes but also its chemical composition to appreciable extent and two, the impact of season on the efficacy of secondary metabolites does not follow a set pattern especially in the present case. Therefore, plant collection over a wide space and period seem critical for testing and understanding the extent of antimicrobial activity of any plant.

EXPERIMENTAL

3.1 Collection of Plant material

Eleven root samples were collected from seven provenances and three districts of Himachal Pradesh during summer and winter season of 2006 (Shimla, Kullu and Kinnaur; Table 3. The root samples were collected on

Table 3: Yield of methanol extracts of roots of *B. aristata*

S. no.	Place (District, code)	Yield (%)*
1.	Sarhan (Shimla, S1)	8.46
2.	Narkanda (Shimla, S2)	9.60
3.	Choupal (Shimla, S3)	5.99
4.	Kharapathar (Shimla, S4)	8.94
5.	Sojha (Kullu, S5)	8.73
6.	Sangli (Kullu, S6)	9.72
7.	Sarhan (Shimla, S7) Winter collection	10.63
8.	Narkanda (Shimla, S8) Winter Collection	9.83
9.	Choupal (Shimla, S9) Winter Collection	9.29
10.	Kharapathar (Shimla, S10) Winter Collection	10.55
11.	Jinjheli (S11)	11.75

*Moisture free basis

complete maturation of plant (at the time of flowering and fruiting). The roots were air dried, cut into small pieces and coarsely powdered.

3.2 Extraction of Root samples

Powdered root material was extracted with methanol by using soxhlet apparatus and extract was concentrated to small volume on water bath at 100°C. The solvent was completely removed on a flash evaporator and yield of the extract was determined on the moisture free basis of root weight. Yield of methanol extracts of roots are given in Table1. Maximum yield was found in root samples collected from Jinjheli (11.75%).

3.3 HPLC analysis

The methanol extracts of roots of seven provenances were subjected to HPLC analysis and a method was developed and standardized to obtain the quantitative yield of berberine in the extracts. The conditions of HPLC analysis of root methanol extract are as follows:

3.4 Instrumentation

A Waters High Performance Liquid Chromatography system consisting of Chromolion Dionex (1996-2001) software with UV detector using C-18 column (3.9 × 300 mm column) in isocratic mode was used.

3.5 Standard solutions

The stock solution of berberine standard was prepared at concentration of 1mg/ml in mobile phase. The quantification of berberine was performed using five standard solutions in a 0.1-0.5mg/ml concentration range prepared by diluting the stock solution.

3.6 Procedure

The mobile phase was prepared by mixing sodium acetate buffer (0.01M) and acetonitrile and water (HPLC grade) in 1:1 ratio and filtered through 0.45 micron filter and degassed by ultrasonication for 30 min. Linearity of the method was investigated by serially diluting the working standard solution of berberine with mobile phase to give a concentration range of 100 to 500µg/ml and injecting 20µL with injector. The flow rate was maintained at 0.8ml/min. Temperature of the column was kept at ambient and scanning wavelength was monitored at 228nm.

3.7 Antifungal activity of extracts

The extracts of *B. aristata* were tested for their toxicity against three fungal pathogens (*C. gloeosporioides*, *F. oxysporum* & *R. solani*) by the Poisoned Food Technique [13]. A culture of test fungi was grown on potato dextrose agar (PDA) medium for about seven days. Methanol extract was dissolved in sterilized distilled water to 0.50 per cent concentration. Small disc (0.7 cm dia.) of the fungus culture was used. Suitable check was maintained where the culture discs were grown under same conditions on PDA without extract. Inoculated Petri plates were incubated at $25^0 \pm 1^0$C.

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