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Research article

Comparative quantification of berberine and antimicrobial activity of different extracts from stem and root of *Berberis aristata*

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ABSTRACT

Berberis aristata is used as a traditional medicine in India to accelerate the process of wound healing. Comparative antimicrobial activity of different samples obtained from the stem and root of *Berberis aristata* was evaluated against two Gram-negative bacteria (*Salmonella enterica* and Shigella flexneri) and a lipolytic yeast (*Candida aaseri*). The extraction was done using hot continuous extraction and maceration methods to compare the concentration of berberine and to evaluate the antimicrobial activity by the well diffusion method. The results showed that the hydroalcoholic root extract obtained by the hot continuous extraction process possessed a higher concentration of berberine than other extracts. Moreover, the hydro-alcoholic root extract was also found most effective against microbial strains. The study suggested that berberine might be responsible for the antimicrobial activity of the extract of *Berberis aristata*.

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INTRODUCTION

The genus *Berberis* of the family Berberidaceae is comprised of a number of species including *B. nepalensis*, *B. asiatica*, *B. lycium*, *B. vulgaris* and *B. aristata* (Mazumder et al. 2011). *Berberis aristata* is one of the most useful medicinal plants. Its medicinal properties have been described in the ancient medical treatise *Charaka Samhita* in Sanskrit. *B. aristata* is known as Daruharidra in Ayurveda, which is also known as Kasmoi. *B. aristata* is also mentioned in the ancient scriptures of Ayurveda. Ayurvedic Pharmacopeia of India correlates Daruharidra to *Berberis aristata*.

About 570 species of the Berberidaceae family are widely distributed in the northern Himalayan region. Daruharidra is generally found in India, Sri Lanka, Nepal and Bhutan. In India, this plant grows at 2000-3000 m, especially in the Kumaon and Garhwal regions of Uttarakhand. *B. aristata* is a shrub of 2-3 meters in height, its bark is hard and yellow to brown from outside and deep yellow from inside, removable in longitudinal strips by hand, spines present in leaves, three-branched and 1.5 cm long (Sharma et al., 2011). The root and stem of *B. aristata* contain different chemical constituents like berberine, palmatine, oxycanthine, beramine, aromoline, oxyberberine, karachine, jatrorrhizine, taxilamine, 1-O-methylpakistamine, pseudopalmatine chloride and pseudoberberine chloride. Berberine was found to be a major alkaloid present in all parts of the plant and showed diverse biological activities (Pareek and Suthar, 2010).

Berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium) is a non-basic and quaternary benzylisoquinoline alkaloid with a long history of medicinal use in both Ayurvedic and Chinese medicine. Berberine is widely present in barks, leaves, twigs, rhizomes, roots and stems of several medicinal plants. The *Berberis* genus is well known for being a natural source of berberine (Neag et al., 2018). Extracts containing berberine have demonstrated significant antimicrobial activity against many organisms like bacteria, viruses, fungi, protozoa and helminths (Chander et al., 2017).

Many synthetic antimicrobial agents have been already approved in many countries, yet the usage of plants derived compounds attracts the attention of researchers. Plant-derived compounds have displayed promising results in overcoming antibiotic resistance in bacterial pathogens. The antibacterial action mechanism of berberine includes DNA intercalation, targeting RNA polymerase, gyrase and topoisomerase IV and the inhibition of cell division. Berberine is also able to inhibit the cell function of bacteria through various mechanisms such as damaging the cell structure, protein and DNA synthesis inhibitors that result in bacterial death (Chander et al., 2017).

MATERIAL AND METHODS

Collection and authentication of plant material

The plant material was procured in the month of December 2015 from the Bhimtal region of Uttarakhand (India). The herbarium was prepared and the plant *Berberis aristata* was authenticated from the lab of the National Bureau of Plant Genetic Resources, Regional Station, Niglat, Bhowali, Nainital (Uttarakhand). The voucher (Specimen No. P-01) was deposited there for future reference.

Morphology

The stem and root were collected and observed for their macroscopic parameters like colour, odour, taste, size and shape (Mukharjee, 2003).

Extraction

The dried plant material of stem and root of *B. aristata* was pulverized using a mechanical grinder. Separately prepared powdered material of stem and root was subjected to extraction using the following two methods for comparative study (Houghton and Raman, 2004).

Hot continuous extraction

Powder material of stem and root was subjected to Soxhlet extraction using 70% ethanol and water separately. Before each extraction, the powdered material was dried in a hot air oven below 50°C. Finally, marc (residue) was digested at 50°C for 24 hours to obtain the extract. The extracts were concentrated in a rotary vacuum evaporator (40ºC) and stored at 4ºC until further use in the refrigerator. Weighed the extract and percentage yield were calculated.

Maceration

The powdered material of stem and root was dried at below 50ºC in a hot air oven. Accurately weighed (200 g) powdered material of stem and root was placed in two beakers separately and then added 1.2 L of 70% ethanol in both beakers as a solvent. The same procedure was repeated for stem and root powdered material using water (1.2 L) as a solvent. Beakers containing soaked powdered material were kept at room temperature for 3 days and frequently agitated. After 3 days, the mixture was filtered. The filtrate was concentrated using a rotary vacuum evaporator (40ºC) and stored in a refrigerator at 4ºC until further use. Finally, the dried extract was collected, weighed and calculated the percentage yield.

Preliminary phytochemical screening

The preliminary phytochemical screening of the extracts was performed for different types of chemical constituents using standard methods. The extracts were subjected to different qualitative tests to investigate phytochemicals like alkaloids, carbohydrates, proteins and amino acids, tannins and phenolic compounds, steroids, flavonoids, glycosides and saponins (Khandelwal et al., 2010).

Quantitative analysis of berberine

Preparation of sample solutions

Stock solutions of different extracts were prepared, accurately weighed quantity (10 mg) of each extract was dissolved in 10 ml of methanol in a volumetric flask. Further, sample solutions of various concentrations (2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml) were prepared by diluting the stock solution with methanol in a volumetric flask.

Preparation of standard solution

The standard solution was prepared by accurately weighing the quantity of 10 mg berberine dissolved in 10 ml of methanol in a volumetric flask. The solutions of various concentrations (2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml) were obtained by diluting stoke solution with methanol.

Calibration curve of berberine

A standard calibration plot was generated at 348 nm using known concentrations of berberine. The absorbance of each concentration was measured, using methanol as a blank. The linear correlation between these concentrations (x-axis) and absorbance (y-axis) was statistically presented. The linear equation ($y = mx + c$) was determined by calculating slope (m), intercept (c), and correlation coefficient (r^2).

Estimation of berberine

Appropriate aliquots of extract solutions were taken and absorbance was measured at 348 nm. The concentration of berberine in the sample solutions of various extracts of *Berberis aristata was* calculated from the regression equation in the calibration plot and expressed as a percentage by weight (Joshi and Kanaki, 2013).

Microbiological screening

The antimicrobial activities of various extracts of *B. aristata* were evaluated by following the method described by Jyostna and Malathi (2009).

Antimicrobial assay

To screen the antibacterial and antifungal activity of *B. aristata*, different concentrations of plant extracts of *B. aristata* were tested against two pathogenic gram-negative bacterial strains (*Salmonella enterica* and *Shigella flexneri*) and one pathogenic fungal strain (*Candida aaseri*) by agar well diffusion-based method.

Petri plates containing 25 ml of nutrient agar media were inoculated with broth culture of respective suspension of bacteria and fungi by swabbing (sterile cotton) the medium. Then, well holes of diameter 6-8 mm were aseptically punched 20 mm apart in each of these

nutrient agar Petri plates using a sterile cork tip. Twenty µl of different concentrations of test samples were introduced by sterile syringe into the wells. The agar Petri plates were incubated under the suitable aerobic condition at 36°C±1°C for 24 hrs in case of bacterial pathogens and 18-48 hrs for fungal pathogens at 25°C±1°C. The standards consist of a disc of chloramphenicol and gentamycin for antibacterial activity and terbinafine for antifungal activity under similar conditions as test samples.

DMSO served as a control for both antibacterial and antifungal activities. To determine the sensitivity of microorganism species of different concentrations of test samples, the diameter of the zone of inhibition (mm) around the wells was measured and the activity index was calculated.

RESULTS

Morphology

Stem

The stems are often much cylindrical in shape have a rough surface, bitter in taste and phenolic odour. The branched stems contain thin bark, short fractures on the surface and dark yellow colour from the inner side of the wood. Stem branches are soft, pale yellowish-brown in colour having variable thickness and length of about 18-22 mm and bark about 6-8 mm in size, fibrous with fine ridges and rough (Fig. 1).

Fig. 1. Root and stem of *Berberis aristata*

Root

The hard cylindrical roots are prominently fissured on the surface longitudinally and transversely. The outer surface is corky, 5 cm or more in diameter having a greyish brown colour. Lemon yellow coloured wood is distinctly radiated. The root bark is internally smooth and striated, externally friable and corky, 4-6 mm thickness having yellowish-brown colour (Fig. 1). Roots have very hard fractures with characteristic odour and bitter taste.

Extractive yields

A comparative study was carried out by extraction of stems and roots of *B. aristata*. The yields obtained from various extracts are shown in Table 1. The study showed the highest yield of extracts when using the hot continuous extraction method. It was also found that stem and root gave higher extractive yield in the aqueous extract as compared to hydroalcoholic extract. On the other hand, the maximum extractive yield was obtained from roots (34.9%) as compared to stems of *B. aristata*.

Table 1. Yields of different extracts of *B. aristata* (n=3)

Plant Part	Extracts	Yields (% w/w)	
		Hot extraction	Maceration
Stem	Hydroalcohol	16.164 ± 0.12	14.4 ± 0.13
	Water	21.66 ± 0.21	10.6 ± 0.22
Root	Hydroalcohol	20.74 ± 0.11	18.55 ± 0.12
	Water	34.9 ± 0.14	24.62 ± 0.13

Phytoconstituents

Preliminary phytochemical screening of the extracts showed the presence of various phytochemicals like alkaloids, glycosides, flavonoids, tannins and phenolic compounds in the plant extracts. The classes of constituents present in the stem and root of *B. aristata* are given in Table 2 and Table 3, respectively. Preliminary phytochemical screening also suggests that there is a similarity in the phytochemical profile of stem and root.

Table 2. Phytochemicals present in the stem of *B. aristata*

Phytoconstituents	Test	Hot Continuous Extraction		Maceration	
		Hydro-alcoholic extract	Water extract	Hydroalcoholic extract	Water extract
Alkaloids	Mayer's	+	+	+	+
	Wagner's	+	+	+	+
	Hager's	+	+	+	+
Carbohydrates	Molise	-	-	-	-
	Benedict	-	-	-	-
	Fehling	-	-	-	-
Proteins and amino acids	Biuret	+	+	+	+
	Milan's	+	+	+	+
Tannins and phenolic compounds	Basic test	+	-	+	+
	Nitric acid	-	+	+	+
	Bromine water	+	+	+	-
Steroids	Salkowski	-	-	-	-
Flavonoids	Zink dust	+	+	+	+

	Alkaline	+	+	+	+
Glycosides	Legal's	+	+	+	+
	Keller Killiani	-	-	-	-
	Bontrager's	-	-	-	+
Saponins	Foam	-	-	-	-

Table 3. Phytochemicals present in the root of *B. aristata*

Phytoconstituents	Test	Hot Continuous Extraction		Maceration	
		Hydro-alcoholic extract	Water extract	Hydroalcoholic extract	Water extract
Alkaloids	Mayer's	+	+	+	+
	Wagner's	+	+	+	+
	Hager's	+	+	+	+
Carbohydrates	Molise	-	-	-	-
	Benedict	-	-	-	-
	Fehling	-	-	-	-
Proteins and amino acids	Biuret	+	+	+	+
	Milan's	+	+	+	+
Tannins and phenolic compounds	Basic test	+	+	+	+
	Nitric acid	+	-	+	-
	Bromine water	+	+	+	+
Steroids	Salkowski	-	-	-	-
Flavonoids	Zink dust	+	+	+	+
	Alkaline	+	+	+	+
Glycosides	Legal's	+	+	-	+
	Keller Killiani	+	-	+	-
	Bontrager's	-	+	+	-
Saponins	Foam	+	-	-	+

Quantitative analysis of berberine by UV spectrometry

Calibration curve

The calibration curve was obtained by plotting various concentrations of pure berberine alkaloid against absorbance. It was observed that the Beer-Lambert law is followed within the concentration range of 2-10 µg/ml at 348 nm. The curve was found to be linear with a regression value of 0.983 and a slope of 0.043 as shown in Fig. 2. The linearity of calibration plot is expressed with the regression equation was $y = 0.0434x + 0.0244$ and $R^2 = 0.9835$.

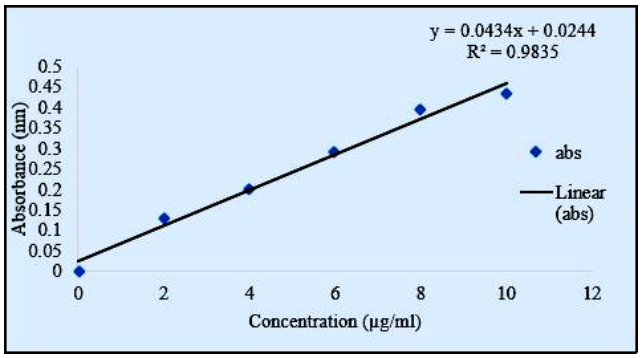

Fig. 2. Calibration curve of berberine

Quantification of berberine

The comparative study on quantitative estimation of berberine in stem and root extracts of *B. aristata* shows that roots contain a higher amount of berberine as compared to stems. It was found that the hydro-alcoholic extract of the root by hot continuous extraction gave a higher concentration of berberine (19.71±3.77%) as compared to all other extracts (Table 4).

Table 4. Percentage yield of berberine in the stem and root extracts of *B. aristata* (n=3)

Plant Part	Extracts	Yields of berberine (% w/w)	
		Hot Extraction	Maceration
Stem	Hydroalcohol	15.25 ± 4.60	9.60 ± 2.81
	Water	9.28 ± 2.77	9.15 ± 2.90
Root	Hydroalcohol	19.71 ± 3.77	13.43 ± 3.73
	Water	10.01 ± 1.61	14.81 ± 3.77

Antimicrobial activity

The antibacterial and antifungal activities of different extracts of stems and roots of *B. aristata* were evaluated against bacterial and fungal strains. The comparative diameter of the inhibitory zone (mm) with the activity of standard was calculated. Results (Table 5 and Table 6) are expressed in terms of mean ± SD (n=3). The present investigation showed that all the extracts showed an inhibitory effect against all the microbial strains. The present study revealed that hydro-alcoholic extract of the root by hot continuous extraction produced a high degree of inhibition zone against all the bacterial and fungal strains at different concentrations. Extracts of stem showed antimicrobial activity against all microbial strain, hydro-alcoholic extract (by hot continuous extraction) at a concentration of 2000 (µ/ml) possess maximum inhibitory

zone towards *C. aaseri* (17.66±1.52 mm) followed by *S. enterica* (17.33±1.15 mm) and *S. flexneri* (17±2 mm) in all the stem extracts. Similarly, in root extracts, hydro-alcoholic extract (by hot continuous extraction) showed a maximum zone of inhibition at a concentration of 2000 (µ/ml) for tested strain *C. aaseri* (18.33±1.15mm) followed by *S. enterica* (18±1 mm) and *S. flexneri* (17.33±1.15 mm).

Table 5. Antimicrobial activity of stem extracts of *B. aristata* (n=3)

Extraction methods	Extracts	Concentrations (µg/ml)	Zone of inhibition (mm)		
			S. enterica	***S. flexneri***	***C. aaseri***
Hot extraction	Hydroalcohol	2000	17.33±1.15	17±2	17.66±1.52
		1000	13.33±1.15	13.33±1.52	15.66±1.52
		500	10±1	10.66±0.57	11.66±1.52
		250	07.66±1.52	07±1	09.33±0.57
	Water	2000	14±2	12.33±2.08	12.33±2.51
		1000	10.66±2.08	11±1	11.33±1.52
		500	06.66±1.52	07.33±1.52	08.33±2.08
		250	04.66±1.15	06.33±1.52	05±1
Maceration	Hydroalcohol	2000	12.66±2.08	13±1	14.33±2.08
		1000	10.66±2.51	08±2.64	10.33±1.52
		500	07±2	07.66±1.52	07±2.64
		250	06.33±1.52	05.66±1.52	06±1.73
	Water	2000	14.33±1.52	13.66±1.52	14±2
		1000	08.33±2.08	09.66±1.52	09±1
		500	06.66±1.52	08.33±2.08	08.66±2.51
		250	04.33±0.57	04.33±1.52	06±1
Chloramphenicol (30 µg/ml)			19	20	-
Gentamycin (10 µg/ml)			21	19	-
Terbinafine (1 µg/ml)			-	-	23

Table 6. Antimicrobial activity of root extracts of *B. aristata* (n=3)

Extraction methods	Extracts	Concentrations (µg/ml)	Zone of inhibition (mm)		
			S. enterica	***S. flexneri***	***C. aaseri***
Hot extraction	Hydroalcohol	2000	18±1	17.33±1.15	18.33±1.15
		1000	14.33±0.57	12.66±1.52	15.66±0.57
		500	11.33±11.15	08.66±0.57	10.66±1.52
		250	06.66±1.15	04.33±0.57	07.33±1.15
	Water	2000	13.66±1.52	14.33±1.52	15.33±3.05
		1000	11±2	11.66±2.08	11.33±1.52
		500	07.66±1.52	10±1	08±1
		250	05.66±2.08	04.33±0.57	04±1
Maceration	Hydroalcohol	2000	16.33±1.52	14.66±1.52	15.66±2.08
		1000	11.66±1.52	12.66±2.08	12.33±1.52
		500	07.66±1.52	08.33±1.52	08.66±1.52
		250	05±1	05.33±0.57	05.66±1.52
	Water	2000	16.33±1.52	14.66±1.52	16.66±0.57
		1000	13.33±1.52	13±1	14±1.52
		500	08±1	09.66±1.52	08.66±0.57
		250	05.66±1.52	07±1	05.33±0.57
Chloramphenicol (30 µg/ml)			19	20	-
Gentamycin (10 µg/ml)			21	19	-
Terbinafine (1 µg/ml)			-	-	23

DISCUSSION

Earlier literature reported various mechanisms supporting the antimicrobial activity of berberine. He et al. (2010) reported inhibition of DNA synthesis by berberine in their study. Berberine is also found to inhibit protein synthesis and RNA transcription, destruction of bacterial cell surface and inhibition of enzyme activities (Jin et al., 2010). Sun et al. (1988) reported the bacteriostatic property of berberine against *Streptococci* by inhibiting the streptococci adherence to the host cell. Berberine also influences by decreasing the intracellular invasion and adhesion ability of methicillin-resistant *S. aureus* (Yu et al., 2005). Berberine can be shown functional effects in the treatment of bacterial infections, berberine could be attached to amyloid proteins in bacterial biofilm, thus resulting in an interruption in the stability of bacteria and increasing the antibacterial activity of antibiotics.

The results obtained from our comparative study show that berberine content is obtained in a higher concentration in hydro-alcoholic extract (by hot continuous extraction) of the root of *B. arista* and also indicates that all microbial strains (*S. enterica*, *S. flexneri* and *C. aaseri*) showed higher susceptibility to this extract. Berberine possesses multifunctional antimicrobial activity and has the potential role to be used as an antibacterial and antifungal agent.

CONCLUSION

Preliminary phytochemical screening data showed the presence of various phytoconstituents in various extracts of stems and roots of *B. arista* which are also helpful for further characterization and identification of phytochemicals and their isolation. A comparative study on extractive yields of various extracts also seems to suggest the hot continuous extraction method (water as solvent) should be used for better extractive yield from roots. In quantification of berberine, hydroalcoholic extract (by hot continuous extraction) of a root possesses a higher concentration of berberine. Moreover, a comparative study on antimicrobial activity reflects that hydroalcoholic extract (by hot continuous extraction) from the root is more potent against both bacterial and fungal strains. Further studies are suggested to isolate berberine from such potent extracts and *in vivo* bioassay should be performed for microbiological screening which will be helpful in new pharmaceutical formulation development.

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CONFLICTS OF INTEREST

The author(s) declare(s) no conflicts of interest.

DECLARATION

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