

COMPARATIVE STUDY ON THE ANTIMICROBIAL ACTIVITY OF *BERBERIS ARISTATA* FROM DIFFERENT REGIONS AND BERBERINE IN VITRO

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ABSTRACT

Berberis aristata belongs to genus *Berberis* of family *Berberidaceae*. Most of the species belonging to this genus are very popular indigenous drugs found in India [1]. The present work is mainly focused on screening the antimicrobial activity of aqueous and ethanolic extracts of *Berberis aristata* stems from three different regions viz. Uttaranchal (BR-1), Bihar (BR-2) and Nepal (BR-3) and berberine, an active principle of *Berberis aristata*, against five bacterial strains including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis* and one fungal strain *Aspergillus niger* by agar diffusion method by measuring the zone of inhibition by turbidity method and a comparative study was done for the antimicrobial activity of the plants from these regions. HPTLC fingerprinting of *Berberis aristata* extracts showed that berberine was present in all the three *Berberis aristata* samples, it was concluded that the observed antimicrobial activity was due to berberine present in the samples used in this investigation.

Key Words : *Berberis aristata*, antimicrobial activity, alcoholic extract

INTRODUCTION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical uses[2-7]. Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects [8].

Berberis extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminths, and Chlamydia. Currently, the predominant clinical uses of berberine include

bacterial diarrhea, intestinal parasite infections, and ocular trachoma infections. The most active ingredient of the plant *Berberis aristata* is berberine, a quaternary isoquinoline alkaloid and the content of berberine is used for monitoring the quality of the plant. It is mostly found in the roots, rhizomes and stem bark. Present research work mainly deals with comparative study on antimicrobial activity of *Berberis aristata* stems collected from different regions of India and berberine, an active principle of *Berberis aristata*.

MATERIALS AND METHODS

Dried stem pieces of *Berberis aristata* DC were received from the Taxonomist, Dabur Research and

Development Centre, Sahibabad (Ghaziabad). As per the information given by Supplier, the raw materials have been collected from the different places such as, BR-1 from Uttaranchal, BR-2 from Bihar and BR-3 from Nepal.

The plant material was identified by Dr. G.P. Kimothi, Taxonomist, Dabur Research and development Centre, Sahibabad (Ghaziabad).

Berberine marker was obtained from Natural Remedies, Bangalore.

Extraction

Fifty grams (50g) of dried plant material was extracted with 200ml of solvent. The coarsely powdered dried stem pieces were completely submerged and then covered with aluminum foil. Extraction was allowed to proceed for 48h. The extract was decanted and the solvent removed using rotary evaporator (buchi).

Antimicrobial Screening [9]

The extracts were tested for their effect on some human pathogenic microorganisms.

Antimicrobial activity was tested by Agar diffusion method employing 24hr cultures of *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 9372) and *Aspergillus niger* (MTCC 1344).

Bacteria were seeded into sterile nutrient agar medium and fungi on Sabouraud dextrose agar medium by uniformly mixing 1ml of inoculum with 20 ml of sterile melted nutrient agar and Sabouraud dextrose agar cooled to 49-50 °C into sterile petridish. The strains were inoculated into conical flask containing sterile nutrient broth and incubated at 37°C ± 1°C (bacteria) for 24 h and 25°C ± 1°C (fungus) for 7 days. From these various serial dilutions were made and suitable dilutions selected. Then the sterilized nutrient agar media was poured into stock petridishes (containing 0.1 ml microbial suspension) and allowed to solidify. Cups

were made by punching into agar surface with a sterile cork borer (6 mm i.d). Specific numbers of cups were made in each plate and into these 0.1 ml of various concentration of test compound were added. One was filled with pure solvent DMF (N, N-dimethyl formamide) and one with standard antibiotic. The plates were allowed to stand for 1 h for diffusion of solution and then incubated for 24 h at 37°C for bacteria and for 7 days at 25°C for fungus. The zone of inhibition formed around the cups after incubation were measured.

RESULTS AND DISCUSSION

Of all the tested concentrations, ethanolic extracts [Table I] of all the raw materials showed significant activity against *S.aureus*, *B.subtilis* and *A.niger* whereas only the raw material from Nepal showed significant activity against *S.epidermidis* and other two i.e. from Uttaranchal and Bihar failed to show any activity against *S.epidermidis*. Significant antimicrobial activity of the aqueous extracts [Table II] from Uttaranchal and Nepal was observed for *S.aureus* but aqueous extract from Bihar failed to show any activity against *S.aureus*. Aqueous extract from Uttaranchal showed activity against *S.epidermidis* whereas aqueous extract from other two regions failed to show any activity against *S.epidermidis*. Aqueous and ethanolic extracts of all the three raw materials showed significant activity against *A.niger*. None of the extracts showed any activity against *P.aeruginosa* and *E.coli* in any of the tested concentrations. Also, no antimicrobial activity of berberine [Table III] was observed against *P. aeruginosa* and *E.coli* in any of the tested concentrations but it showed significant activity against all other pathogens tested. Given that TLC of *Berberis aristata* extracts showed that berberine was present in all the three *B.aristata* extracts, it was concluded that the observed antimicrobial activity was due to berberine present in the extracts used in this investigation.

Table 1: Zone of inhibition of aqueous extracts of *Berberis aristata*

Extract	Conc. (µg/ml)	Zone of inhibition (mm)					
		<i>S. aureus</i>	<i>S.epidermidis</i>	<i>B.subtilis</i>	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>A.niger</i>
Raw 1 Aqueous	2000	15.94	16.12	-	-	-	10
	1000	16.08	13.04	9.16	-	-	7
	500	12.96	11.94	-	-	-	6
	250	10.65	9.53	-	-	-	2
Raw 2 Aqueous	2000	-	-	-	-	-	15
	1000	-	-	-	-	-	14.8
	500	-	-	-	-	-	13.0
	250	-	-	-	-	-	10
Raw 3 Aqueous	2000	17.11	-	-	-	-	20
	1000	15.86	-	-	-	-	16
	500	14.16	-	-	-	-	12
	250	13.50	-	-	-	-	-

S. aureus – *Staphylococcus aureus*, *S. epidermidis* – *Staphylococcus epidermidis*, *B. subtilis* – *Bacillus subtilis*, *P. aeruginosa* – *Pseudomonas aeruginosa*, *E. coli* – *Escherichia coli*, *A. niger* – *Aspergillus niger*

Table2: Zone inhibition of ethanolic extracts of *Berberis aristata*

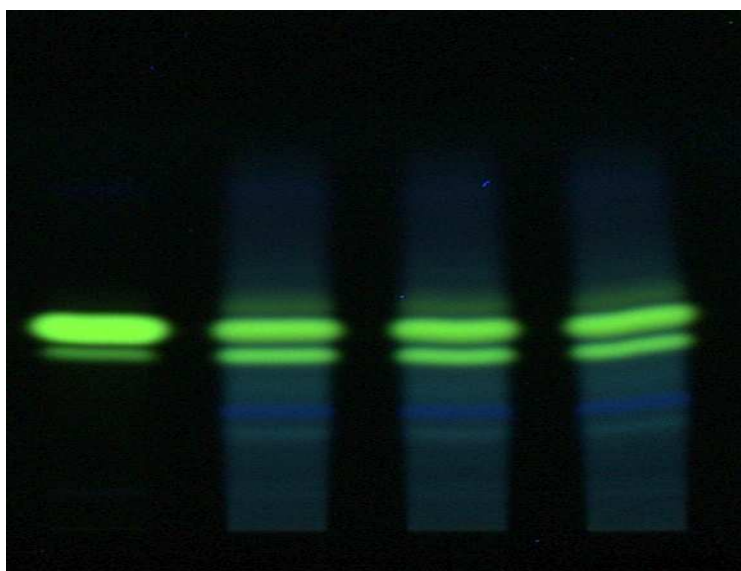
Extract	Conc. (µg/ml)	Zone of inhibition (mm)					
		<i>S. aureus</i>	<i>S.epidermidis</i>	<i>B.subtilis</i>	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>A.niger</i>
Raw 1 Aqueous	2000	15.79	-	8.05	-	-	15
	1000	17.82	-	8.31	-	-	11.84
	500	15.15	-	-	-	-	8.25
	250	10.79	-	-	-	-	6.24
Raw 2 Aqueous	2000	22.76	-	14.39	-	-	10.24
	1000	23.91	-	16.08	-	-	8.25
	500	21.60	-	12.40	-	-	6.5
	250	18.36	-	8.79	-	-	-
Raw 3 Aqueous	2000	18.30	24.59	11.95	-	-	13.35
	1000	19.63	23.88	13.14	-	-	11.1
	500	17.77	20.37	9.73	-	-	8.60
	250	14.41	18.70	8.21	-	-	8.0

S. aureus – *Staphylococcus aureus*, *S. epidermidis* – *Staphylococcus epidermidis*, *B. subtilis* – *Bacillus subtilis*, *P. aeruginosa* – *Pseudomonas aeruginosa*, *E. coli* – *Escherichia coli*, *A. niger* – *Aspergillus niger*

Table 3: Zone of inhibition of Berberine marker

Extract	Conc. (µg/ml)	Zone of inhibition (mm)					
		<i>S. aureus</i>	<i>S.epidermidis</i>	<i>B.subtilis</i>	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>A.niger</i>
Berberine marker	2000	21.97	10.87	12.02	-	-	26
	1000	23.30	10.80	15.06	-	-	18
	500	20.89	8.90	14.31	-	-	13
	250	20.43	5.12	12.74	-	-	12

S. aureus – *Staphylococcus aureus*, *S. epidermidis* – *Staphylococcus epidermidis*, *B. subtilis* – *Bacillus subtilis*, *P. aeruginosa* – *Pseudomonas aeruginosa*, *E. coli* – *Escherichia coli*, *A. niger* – *Aspergillus niger*



Berberine Sample 1 sample 2 sample 3
Fig: HPTLC Fingerprint Print of *Berberis aristata*

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