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Screening of *Berberis aristata* DC. for Antimicrobial Potential against the Pathogens Causing Ear Infection

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Abstract: In the present study, the antimicrobial and minimum inhibitory concentration of *B. aristata* leaves and roots extracts were tested by agar well diffusion method against the six ear pathogens namely, *Staphylococcus aureus, Proteus mirabilis, Escherchia coli, Pseudomonas aeruginosa, Acinetobacter* sp. and *Candida albicans*. Root extracts displayed antimicrobial activity against all the tested ear pathogens, while leaf extracts showed activity against five tested ear pathogens. The highest antimicrobial activity of *B. aristata* root extract was found against *S.aureus* (28.3 mm) in acetonic extract with an MIC of 3.12 mg mL^{-1} and that of leaf also against *S.aureus* (27.6 mm) in hot aqueous extract with an MIC of 3.12 mg mL^{-1}. Of the three organic extracts of *B. aristata* screened, the acetonic extract showed the highest antimicrobial activity. This study suggests that the organic extracts of *B. aristata* showed broad spectrum antimicrobial activity and may be useful in treating ear infections.

Key words: *Berberis aristata,* ear infection, antimicrobial activity, minimum inhibitory concentration

INTRODUCTION

Ear infection is amongst the most common diseases encountered in medical practice today throughout the globe, affecting people of all ages from neonate to geriatric groups (El-Mahmood *et al*., 2010). Ear infection is mainly caused by bacterial (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Proteus mirabilis*) and fungal pathogens (*Aspergillus niger*, *A. fumigatus, A. flavus*, *Candida albicans*) (Roland and Stroman, 2002; Rosenfeld *et al*., 2006; Dhingra, 2006; Aneja *et al*., 2010). Increasing resistance amongst microorganisms against available antimicrobial agents is of major concern among scientists and clinicians worldwide. To overcome the non-efficacy of the current antimicrobial drugs and to obtain more efficacious drugs, the impetus is to search for new antimicrobial drugs from medicinal plants (Cordell, 2000; Khalafi-Nezhad *et al*., 2005; Nebedum *et al*., 2009; Jana and Shekhawat, 2010).

Plant extracts have been used for centuries as a popular method for treating several health disorders. Indian plants constitute a rich untapped pool of medicinal plants which are commonly used for the treatment of various ailments, as these are considered to have advantages over the conventionally used drugs that are expensive and known to have harmful side effects (Khare, 2004; Tijjani *et al*., 2009; Arya *et al*., 2010; Vaghasiya *et al*., 2011). Therefore, fewer side effects, better compatibility and only available treatment for some diseases makes the medicinal plants an ideal remedy for treatment of the diseases (Karim *et al*., 2011). *Berberis aristata* DC. (family *Berberidaceae*), commonly called Indian barberry and Daru haldi, a taxon of *Berberis* comprising of about 450-500 species. It is an erect, glabrous, spinescent, deciduous shrub, natively belongs to mountainous parts of North India and Nepal, growing upto a height of 3-6 m. Leaves of *B. aristata* are obovate to elliptic producing yellow flowers arranged in corymbose drooping racemes. Fruits are long, ovoid, bluish black or bright red in colour and covered with a thick pale or bluish white bloom and are born in pendulous clusters (Khory and Kartak, 1985; Kirtikar and Basu, 1995; Rashmi *et al*., 2008; Das *et al*., 2009).

Traditionally, the leaves and roots of *B. aristata* plant have been used in indigenous system of medicine for curing different disorders. Decoction of leaves, commonly called Rasaut, is used to treat skin diseases, menorrhagia, diarrhoea, cholera, jaundice, urinary tract infections, eye and ear infections. The decoction of root is used as a wash for infected wounds and ulcers and helps in healing and promotes cicatrisation. The dried extract of the roots are applied externally to the eyelids to cure ophthalmia and other eye diseases (Jain and Singh, 1994; Malik *et al*., 2011). The other uses of this plant are as a laxative useful in rheumatism, antihepatotoxic,

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anti-inflammatory and a useful tonic in curing ulcers and fevers (Gilani and Janbaz, 1992; Gupta *et al*., 2008).

Among the various phytocompounds of antimicrobial significance present in medicinal plants, Berberine is the chief alkaloid present in *B.aristata*. Palmatine, Oxyacanthine, Beramine, Aromoline, Oxyberberine, Karachine, Jatrorrhizine, Taxilamine, 1-O-methylpakistamine, Pseudopalmatine chloride and Pseodoberberine chloride are the other alkaloids present in this plant (Chakravati *et al*., 1950; Blasko and Sharma, 1982; Sharma *et al*., 2002; Jha, 2004).

Hence, the present study was carried out to find out the antibacterial and antifungal potential of this plant against the locally isolated microorganisms from the patients having ear infection and its comparison with locally available ear drops.

MATERIALS AND METHODS

Plant collection: The leaves and roots of *Berberis aristata* were collected from the local areas of Mandi (Himachal Pradesh) in March, 2010. The taxonomic identity of this plant was confirmed by Dr. B.D. Vashishta of Botany Department, Kurukshetra University, Kurukshetra.

Extraction of plant material: The extract of roots or leaves of *B.aristata* were prepared following the methodology adopted earlier by Aneja and Sharma (2010).

Test microorganisms: Five bacteria namely *Staphylococcus aureus* (HM626197)*(Gram-positive), *Acinetobacter* sp. (HM626198), *Proteus mirabilis* (HM626199), *Escherchia coli* (HM626200), *Pseudomonas aeruginosa* (HM626201) (Gram-negative) and one yeast, *Candida albicans* used in this study, were isolated from the patients having ear infection from the local ENT clinics of Kurukshetra (Aneja *et al*., 2010). Bacterial strains were identified on the basis of staining, biochemical and molecular characteristics (16S rRNA sequencing) (Lawongsa *et al*., 2008) and yeast on the basis of staining, morphological and cultural characteristics (Aneja, 2003; Cappuccino and Sherman, 2008). The bacterial isolates were subcultured on Nutrient agar and yeast on Malt yeast agar and incubated aerobically at 37°C. The media were procured from Hi Media Laboratory Pvt. Ltd., Bombay, India (*Nucleotide sequence of all the five bacteria have been submitted to GenBank database which have provided GenBank accession number, HM626197-HM626201).

Ear drops: Three commonly prescribed ear drops by otolaryngologist, two allopathic Ciplox (antibacterial), Candid (antifungal) and a herbal ear drop Bilwa Tel (antimicrobial), used in this study, were procured from the local market of Kurukshetra.

Screening for antimicrobial activity: The acetone, methanol, ethanol, hot and cold aqueous *B. aristata* leaves and roots extracts were used for evaluation of the antimicrobial activity by the agar well diffusion method and Minimum Inhibitory Concentration (MIC) for each test organism was determined by following the modified agar well diffusion method (Adeniyi and Ayepola, 2008; Aneja and Sharma, 2010).

Statistical analysis: The experimental results were repeated thrice in triplicates each time and expressed as Mean±SD (Standard deviation) and results were statistically evaluated using Dennett's t-test. The p-value less than 0.01 were considered significant.

RESULTS

The antibacterial activity of *B. aristata* leaves and root extracts on the agar plates varied in different solvents. Positive controls produced significantly sized inhibition zones against the tested bacteria (ranging between 11.6 and 56.3 = mm) and the yeast (with zone of inhibition of 21.3 mm) and the negative control produced no observable inhibitory effect against any of the test organism. Among the three organic solvent extracts of root and leaves, acetonic extract has been found most effective showing maximum zone of inhibition as compared to the ethanolic and methanolic extracts and lowest MIC against the tested bacterial pathogens. Aqueous leaves extracts (both hot and cold), possessed antibacterial activity against three pathogens and root extract against one bacterial pathogen only. Root and leaves aqueous extract did not produce any inhibition zone against the tested fungal pathogens (Table 1, 2).

Among the tested root extracts, all the five solvent extracts (organic and aqueous) showed antibacterial activity against *S. aureus* with highest zone of inhibition in acetonic extract (28.3 mm) followed by methanolic extract (26.3 mm), ethanolic extract (24 mm), hot (17.6 mm) and cold aqueous extracts (15.3 mm). *S. aureus* was found to be the most sensitive pathogen having an MIC of 3.12 mg mL^{-1} in acetonic root extract followed by the methanolic and ethanolic extracts, 6.25 mg mL^{-1}. Organic root extracts showed moderate activity against

Table 1: Antimicrobial activity of *B. aristata* leaves and root extract, allopathic and ayurvedic drugs on ear pathogens determined by agar well diffusion method

	Diameter of growth of inhibition zones (mm)											
	Sa		Pm		Pa		Ec		Asp		Ca	
Solvent extracts (mg mL^{-1})	Le	Re	Le	Re	Le	Re	Le	Re	Le	Re	Le	Re
Methanol	20.6±0.57[a]	26.3±0.57	17.3±0.57	20.6±0.57	17.6±0.57	15.6±0.57	-	17±0	19.6±0.57	18.3±0.57	16.3±0.57	14.6±0.57
Ethanol	18.4±0.57	24.0±0	17.6±0.57	18.3±0.57	15.3±0.57	15.3±0.57	-	16.3±0.57	18.3±0.57	17.6±0.57	15±0	14.6±0.57
Acetone	24.6±0.57	28.3±0.57	19.3±0.57	22.6±0.57	18±0	17.6±0.57	-	18.6±0.57	22.3±0.57	21±0	18.3±0.57	15.6±0.57
Hot aqueous	27.6±0.57	17.6±0.57	-	-	17.3±0.57	-	-	-	20.6±0.57	-	-	-
Cold aqueous	25.3±0.57	15.3±0.57	-	-	15.3±0.57	-	-	-	16.3±0.57	-	-	-
DMSO	0		0		0		0		0		0	
Ciplox ear drop	56.3±0.57		32.6±0.57		34±0		46.3±0.57		36±0		Nt	
Bilva tel ear drop	13.6±0.57		-		-		11.6±0.57	-	-			
Candid ear drop	Nt		Nt		Nt		Nt		Nt		21.3±0.57	

Sa: *Staphylococcus aureus*, Pm: *Proteus mirabilis*, Pa: *Pseudomonas aeruginosa*, Ec: *Escherchia coli*, Asp.: *Acinetobacter* Sp., Ca: *Candida albicans;* Re: Root extract, Le: Leaves extract, -: No activity, nt: Not tested, [a]Values, including diameter of the well (8 mm), are means of three replicates±Standard deviation. The data were analyzed by one way ANOVA followed by Dunnett's t-test

Table 2: Minimum inhibitory concentration of *B. aristata* leaves and root extract on ear pathogens determined by modified agar well diffusion method

	MIC (mg mL^{-1})											
	Sa		Pm		Pa		Ec		Asp		Ca	
Solvent extracts	Le	Re	Le	Re	Le	Re	Le	Re	Le	Re	Le	Re
Methanol	12.5	6.25	25	12.5	25	50	Nt	25	25	25	50	50
Ethanol	25	6.25	25	25	50	50	Nt	50	25	25	50	50
Acetone	6.25	3.12	25	12.5	25	25	Nt	25	12.5	12.5	25	50
Hot aqueous	3.12	25	Nt	Nt	25	Nt	Nt	Nt	12.5	Nt	Nt	Nt
Cold aqueous	6.25	50	Nt	Nt	50	Nt	Nt	Nt	50	Nt	Nt	Nt

Sa: *Staphylococcus aureus*, Pm: *Proteus mirabilis*, Pa: *Pseudomonas aeruginosa*, Ec: *Escherchia coli*, Asp.: *Acinetobacter* Sp., Ca: *Candida albicans*; Re: Root extract, Le: Leaves extract; Nt: Not tested

P. mirabilis and *Acinetobacter* sp. with zones of inhibition ranging between 22.6 and 17.6 mm and MIC ranging between 12.5 and 25 mg mL^{-1}. The inhibition zones produced by organic solvents against *P. aeruginosa* and *E. coli* was almost equal and ranged between 18.6 mm and 15.3 mm and these two bacteria were found to be less sensitive having an MIC ranging between 25 and 50 mg mL^{-1}. However, *C. albicans* was found to be least sensitive having an MIC of 50 mg mL^{-1} (Table 1, 2).

All the five solvent extracts (organic and aqueous) of leaves showed antibacterial activity against *S. aureus* with highest zone of inhibition in hot aqueous extract (27.6 mm) followed by cold aqueous extract (25.3 mm), acetonic extract(24.6 mm), methanolic extract (20.6 mm) and ethanolic extract (18.4 mm). *S. aureus* was again found to be the most sensitive pathogen, thus having an MIC of 3.12 mg mL^{-1} in hot aqueous extract followed by the cold aqueous and acetonic extract (6.25 mg mL^{-1}), methanolic extract (12.5 mg mL^{-1}) and ethanolic extract (25 mg mL^{-1}). The inhibition zones produced by the five solvents against *Acinetobacter* sp. ranged between 22.3 mm and 16.3 mm. *Acinetobacter* sp. was comparatively less sensitive, thus having an MIC ranging between 12.5 and 50 mg mL^{-1}. The zone of inhibition produced by the organic solvent leaves extracts against *P. mirabilis*, *P. aeruginosa* and *C. albicans* were almost equal and ranged between 19.3 and 15 mm. *P. mirabilis* showed an MIC of 25 mg mL^{-1} against all the tested organic extracts whereas *P. aeruginosa* showed an MIC of 25 mg mL^{-1} (acetone and methanol) and 50 mg mL^{-1} (ethanol) while *C. albicans* showed an MIC of 25 mg mL^{-1} (acetone) and 50 mg mL^{-1} (methanol and ethanol), respectively (Table 1, 2).

Bilwa tel ear drop showed the inhibition of only two bacterial pathogens, *S. aureus*, *E.coli* with zone of inhibition ranging between 13 mm and 11 mm but allopathic antibacterial ear drop ciplox, containing ciprofloxacin and antifungal ear drop, candid, containing clotrimazole, produced zone of inhibition ranging between 56 mm and 21 mm , showed higher inhibitory activity against the ear pathogens than the tested root and leaf extracts of *B. aristata*. All the obtained results are statistically significant as they showed ($p<0.01$) as compared with control (Table 1).

DISCUSSION

Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drugs, many of which have adverse side

effects (Nair and Chanda, 2007; Agbafor *et al*., 2011). Therefore, in the present investigation different organic (ethanol, methanol, acetone) and aqueous (hot and cold) extracts of *B. aristata* were evaluated for their antibacterial and antifungal potential for the first time against the pathogens causing ear infection.

Ethnobotanically, *B. aristata* has been used against a wide range of ailments (Shahid *et al*., 2009).Variable activities of different plant parts (root, stem, fruit) of this plant have been reported against different bacterial and fungal human pathogens in different solvents (Singh *et al*., 2007; Malik *et al*., 2010).

A majority of the described antimicrobial effects of *B. aristata* extracts have been attributed to their secondary metabolites, notably alkaloids compounds (Singh *et al*., 2010). In this study, the organic root extracts of *B. aristata* were found to be the most active in inhibiting the growth of all the six tested microbial ear pathogens. However, hot aqueous extract of *B. aristata* leaves was found to be best in inhibiting the growth of Gram Positive bacteria whereas organic leaves extracts was found to be most effective against Gram negative bacteria and tested yeast.

The antimicrobial activity of *B. aristata* extracts against the tested ear pathogens may be due to the presence of secondary metabolites mainly, Berberine, an isoquinoline alkaloid, with a bright yellow colour. Berberine was also found to inhibit the intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins without causing histological damage to the intestinal mucosa. Berberine is also active against other intestinal infections that cause acute diarrhea such as *Shigella dysenteriae, Salmonella Paratyphi* and various *Klebsiella* species. Berberine sulphate has been shown to block the adherence of *Streptococcus Pyrogenes* and *E. coli* to host cells, possibly explaining its mechanism of action against numerous pathogens (Sack and Froehlich, 1982; Sun *et al*. 1988; Naika and Krishna, 2008).

Of the six organic extracts of two parts of this plants screened, the acetonic extract has been found to be most active and have a better antimicrobial activity than the corresponding ethanolic and methanolic extracts (Table 1).These results confirm the finding made by Eloff, (1998), Cowan (1999) and Nair *et al*. (2005), who had rated acetone as the best solvent. Interestingly, *B. aristata* extracts were found to be more potent against the tested ear pathogens compared to the standard herbal ear drop (Bilwa tel) showing a great potential to be developed as an herbal ear drop to control the microbial ear infections.

CONCLUSION

In conclusion, this study has shown that the acetonic extracts of *B. aristata* showed promising antimicrobial activity against all the tested bacterial and fungal ear pathogens. This result lend credence to the folkloric use of *B. aristata* plant in treating ear infections and shows that *B. aristata* could be exploited for new potent antimicrobial agents. However, further experiments including phytochemical analysis are needed to identify the active constituents responsible for the observed antimicrobial activity and *in vivo* studies on this plant to determine its toxicity and their pharmacokinetics properties, for therapeutic utility in treating the ear infections.

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