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, Escherichia 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 acetone 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 acetone 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 amongst 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; Jura 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; Tijani 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 up to 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, mencerhagia, 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 cicatrization. The dried extract of the roots are applied externally to the eyelids to cure opthalmia and other eye diseases (Jain and Sigh, 1994; Malik et al., 2011). The other uses of this plant are as a laxative useful in rheumatism, antihypertensive,
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. arisata*. Palmitate, Oxycanthine, Beramine, Aromoline, Oxysterberine, Karachine, Jatrohorzine, Taxilamine, 1-O-methylpakistanine, Pseudopalmatine chloride and Pseudoberberine chloride are the other alkaloids present in this plant (Chakravati et al., 1950; Blaska 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 arisata* 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. Vashihshta of Botany Department, Kurukshetr University, Kurukshetra.

**Extraction of plant material:** The extract of roots or leaves of *B. arisata* 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), Escherichia 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. arisata* 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 Ayeepola, 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. arisata* 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 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

<table>
<thead>
<tr>
<th>Solvent extracts</th>
<th>Sa</th>
<th>Pm</th>
<th>Pa</th>
<th>Ec</th>
<th>Asp</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Le</td>
<td>Re</td>
<td>Le</td>
<td>Re</td>
<td>Le</td>
<td>Re</td>
</tr>
<tr>
<td>Methanol</td>
<td>20.6±0.57</td>
<td>26.3±0.57</td>
<td>17.3±0.57</td>
<td>20.6±0.57</td>
<td>17.6±0.57</td>
<td>15.6±0.57</td>
</tr>
<tr>
<td>Ethanol</td>
<td>18.4±0.57</td>
<td>24±0.57</td>
<td>17.6±0.57</td>
<td>18.3±0.57</td>
<td>15.3±0.57</td>
<td>15.3±0.57</td>
</tr>
<tr>
<td>Acetone</td>
<td>24.6±0.57</td>
<td>28.3±0.57</td>
<td>19.3±0.57</td>
<td>22.6±0.57</td>
<td>18±0</td>
<td>17.6±0.57</td>
</tr>
<tr>
<td>Hot aqueous</td>
<td>27.6±0.57</td>
<td>17.6±0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cold aqueous</td>
<td>25.3±0.57</td>
<td>15.3±0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin ear drop</td>
<td>56.3±0.57</td>
<td>32.6±0.57</td>
<td>34±0</td>
<td>46.3±0.57</td>
<td>36±0</td>
<td>-</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>

Sa: Staphylococcus aureus, Pm: Proteus mirabilis, Pa: Pseudomonas aeruginosa, Ec: Escherichia coli, Asp: Acinetobacter sp., Ca: Candida albicans; Re: Root extract, Le: Leaves extract, *: No activity, nt: Not tested. *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

<table>
<thead>
<tr>
<th>MIC (mg mL⁻¹)</th>
<th>Sa</th>
<th>Pm</th>
<th>Pa</th>
<th>Ec</th>
<th>Asp</th>
<th>Ca</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Le</td>
<td>Re</td>
<td>Le</td>
<td>Re</td>
<td>Le</td>
<td>Re</td>
</tr>
<tr>
<td>Methanol</td>
<td>12.5</td>
<td>6.25</td>
<td>25</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Cold aqueous</td>
<td>6.25</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>50</td>
</tr>
</tbody>
</table>

Sa: Staphylococcus aureus, Pm: Proteus mirabilis, Pa: Pseudomonas aeruginosa, Ec: Escherichia coli, Asp: Acinetobacter sp., Ca: Candida albicans; Re: Root extract, Le: Leaves extract, nt: Not tested. All the obtained results are statistically significant as they showed (p<0.01) as compared with control (Table 1).

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 less 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 ciprofloxacin, 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 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 Pyogenes 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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