

Original Research Article

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Screening of Antimicrobial Potential of *Barleria prionitis* Linn Aerial Parts against Common Respiratory Tract Pathogens

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ABSTRACT

The antimicrobial potential of *Barleria prionitis* Linn. (Aerial parts) were evaluated against common respiratory tract infections causing bacterial and fungal pathogens. The pathogens used in this study were *Streptococcus pneumoniae* (MTCC 655), *Staphylococcus aureus* (MTCC 1144), *Pseudomonas aeruginosa* (MTCC 2474), *Streptococcus pyogenes* (MTCC 442), *Haemophilus influenzae* (MTCC 3826) and two fungal strains *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 921). Antibacterial and antifungal activities were determined by Agar well diffusion method, poison food technique, respectively. Erythromycin and fuconazole were used as positive control to establish the sensitivity of bacterial and fungal strains respectively. Methanol (MeOH) extract was found most active followed by ACE, H₂O and PET respectively. The maximum inhibition zone was found against *S. pneumoniae* (19.40±0.64 mm) followed by *S. aureus* (18.73±0.80 mm), *P. aeruginosa* (14.98±0.28 mm), *S. pyogenes* (14.2±0.47 mm), *H. influenzae* (10.11±0.21 mm), and *Candia albicans* (6.66±32 mm). Minimum inhibitory concentrations (MICs) were observed for MeOH extract between 3.12 to 25 mg/mL against *S. pneumoniae*, *S. pyogenes* and *Candida albicans* respectively. The antimicrobial activity of the crude extracts of plant represents a significant outcome for the treatment of respiratory diseases.

Keywords

Respiratory tract pathogens, antibacterial, antifungal, MIC, *Barleria prionitis*.

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Introduction

Plants are the backbone of all life on the earth and a necessary resource for human wellbeing as raw medicine, food and fuel. Traditional plant derived medicines have been used in most parts of the world and their use in combating microbial disease is becoming the focus of several studies (Bhavnani and Ballow, 2000; Chariandy *et al.*, 1999). Plant derived substances have recently become of great interest owing to their resourceful applications.

It has been estimated that 14- 28 % of higher plant species are used in the medicinal purposes and that 74% of pharmacologically active phytochemicals components were discovered after following up on ethno medicinal use of the plants (Borah *et al.*, 2012). In the last couple of decade, a new development in the research and promotion of plants based drugs has become increasingly towards the herbal medicines (Bisset, 1994; Tyler, 1997).

Respiratory tract infections are the most common ailment including allergies, asthma and chronic obstructive pulmonary disease (COPD). The climatic conditions are very favourable for spread of such diseases commonly transmitted by coughing and sneezing (airborne disease). Some common causative agents are *Escherichia coli*, *Klebsilla pneumoniae* responsible for nosocomial infections (Saonnam *et al.*, 2008), *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Moraxella catarrhalis* for community acquired infections, *Enterobacter cloacae* and *Bacillus subtilis* which cause occupational asthma, respectively (Kayser *et al.*, 1990; Chan-Yenug and Lam, 1986). In 2002, due to the respiratory tract infections there were 3.9 million deaths reported worldwide and 6.91% of all deaths that year (Beaglehole *et al.*, 2004).

Barleria prionitis Linn (Acanthaceae) is distributed throughout Africa, India, Sri Lanka and tropical Asia. The height of the plant is about 1.5 metres. *B. prionitis* is a shrub and flowers are yellow in colour. Flowering occurs during August – October (Kirtikar and Basu, 1999; Kaushik and Dhiman, 2000). In ayurveda it is known by various names like kuranta, kurantaka, kuranda, kurandaka, sahachara, shairiya. In folk medicine it is popularly known as piyaabaaaa, jhinti and ketsariyaa. It is known as ‘Vajradanti because of its antidotal property (Khare, 2007; Banerjee *et al.*, 2012).

The whole plant, root, leaves and bark of the *B. prionitis* reside in a significant place in the indigenous system of medicine in India for controlling the different types of ailments such as inflammations, swellings, boils, glandular etc. (Khare, 2004; Daniel, 2006; Aneja *et al.*, 2010). The decoction of

aerial parts of *B. prionitis* is used in whooping cough, anti-respiratory syncytial virus, antiarthritic, anti-inflammatory and antifertility activities. The antibacterial activity of *B. prionitis* leaf extracts were showed against *S. typhi*, *V. cholerae*, *M. luteus*, *L. sporogens*, *Citrobacter*, *B. subtilis*, *B. cereus*, and *Providencia* (Paul and Saha, 2012). The MeOH extract of root leaves and stems showed potent antioxidant activity. Ethanolic extract of whole plant extract of *B. prionitis* showed considerable antioxidant prosperities (Kapoor *et al.*, 2014; Sharma *et al.*, 2014; Chetan *et al.*, 2010). Preliminary phytochemical screening of hydro-methanol (70%) extract of *B. prionitis* whole plant revealed the occurrence of saponins, glycosides, tannins and flavonoids (Maji *et al.*, 2011). The leaves and flowering tops of *B. prionitis* showed high amount of potassium salts (Khare, 2007).

Materials and Methods

Collection and preparation of plant material

The plant material (aerial parts) was collected from the botany garden of the campus, Gurukul Kangri University Haridwar UK (India) during the first week of October, 2013. Collected plant material was washed with tap water and dried under shade at room temperature and crushed into powder using electric grinder.

Extract preparation

Plant extracts were prepared by immersing 100 g of powdered plant material in 300 mL with four different organic solvents *i.e.* petroleum ether (PET), acetone (ACE), methanol (MeOH) and distilled water (H₂O). Powdery material was loaded in Soxhlet apparatus and extracted for 72 h by successive method (Ahmed *et al.*, 1998).

Each extract was filtered through Whatman No. 1 filter paper. Each extract were evaporated with the help of vacuum evaporator at 30°C. The dried extracts obtained from vacuum evaporator were exposed to Ultra Violet rays for 24 h and checked for sterility on nutrient agar plates. Extracts were stored in a refrigerator at 4°C for further analysis (Aneja *et al.*, 2010).

Test Microorganisms

Upper respiratory tract infections causing pathogens were selected for this study. Clinical bacterial strains of gram positive and gram negative bacteria were *Streptococcus pyogenes* (MTCC 442), *Streptococcus pneumoniae* (MTCC 655), *Staphylococcus aureus* (MTCC 1144), *Pseudomonas aeruginosa* (MTCC 2474), *Haemophilus influenzae* (MTCC 3826). Two fungal strains *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 921) were also selected and all pathogens procured from Institute of Microbial Technology (IMTECH), Chandigarh (India).

Antibacterial Activity

Preparation of Stock Cultures

Stock cultures of bacterial and fungal were maintained at 4°C on slopes of nutrient agar medium. Active cultures for experiments were prepared by transferring microbial inoculums from stock cultures to test tubes containing Mueller-Hinton Broth (MHB) for bacteria that were incubated at 37°C for 24 h in BOD incubator. Antibacterial activity was tested by agar well- diffusion method (Gautam *et al.*, 2012). 100 µl of diluted inoculums of 10⁵ CFU mL⁻¹ (IP, 1996) of 24 h old cultures of test organisms were mixed in Mueller Hinton Agar (MHA) medium and shaken. Medium was poured in sterilized Petri plates. Plates were allowed to solidify

for 5 – 10 min. A sterile cork borer of 6 mm diameter was used to punch wells in medium and filled with 45 µL of 200 mg mL⁻¹ final concentration of extracts. DMSO (dimethyl sulphoxide) was used as negative control. All extracts were assayed in triplicate and the mean values were noted. The plates were incubated at 37°C for 24 h in BOD incubator. The antibacterial activity was interpreted from the size of the diameter of inhibition zone measured in millimetre (mm) as experiential from clear zones nearby the wells. This method depends upon the diffusion of the tested material to such an extent that growth of the added microorganisms is prevented completely in a zone around the hole containing a solution of tested material (Prabhat *et al.*, 2005).

Minimum inhibitory concentrations (MICs)

MIC was done using two fold serial dilution method adapted by (Aboaba *et al.*, 2006; Ajaeoba *et al.*, 2003). Sterilized nutrient broth was poured uniformly into all test tubes. Mcfarland's turbidity standard scale number 0.5 was prepared to provide a turbid solution. Saline solution was inoculated with each of the test organism and incubated for 6 h at 37°C to prepare a turbid suspension of the tested microbes. After incubation, dilution of the culture in DMSO was prepared until it matched with the turbidity (1.5 x 10⁶ cfu/mL) of the Mcfarland scale. A solution of MeOH extract was serially diluted with broth to obtain the following concentrations 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.12 mg/mL and 1.56 mg/mL. From the above suspension prepared in DMSO, 0.1 mL was dispensed into the different concentration of the extract in nutrient broth. All test tubes were observed for turbidity after incubating at 37°C for 24 h. The test tube with the concentration at which no detectable growth was observed determined the MIC.

Antifungal activity

The antifungal potential of aerial parts extracts were determined by poisoned food (Grover and Moore, 1962; Nene and Thapliyal, 2002; Pal and Kumar, 2013; Pal *et al.*, 2013). An aliquot of 250 mg/ml concentration of different extracts were poured into Petri dishes and followed by adding 19 mL of melted Sabouraud dextrose agar (SDA) medium. 6 mm mycelial disc was punched with a sterile cork borer from the borders of two to three days old culture of *A. niger* and placed the disc in the centre of agar plate. Percentage inhibition zone of mycelial growth was determined by measuring the relative growth of the fungus in the treatment and control by applying the following formula.

$$I = (C - T) / C \times 100$$

Where I is the percentage inhibition, C mean growth rate of the control and T for the treatment. Extract is not used in the control and fuconazole was used as the reference drug for comparison. All plates were incubated for 48 – 72 h at 25⁰C in BOD incubator. All samples were assayed in triplicate and mean values were observed.

Result and Discussion

Antimicrobial activities of MeOH, ACE, H₂O and PET aerial parts extracts of *B. prionitis* are presented in Table no1. MeOH extract showed the highest antibacterial activity among all solvents followed by ACE, H₂O, and PET. Maximum inhibition zone was found against *S. pneumoniae* (19.40±0.64 mm) followed by *S. aureus* (18.73±0.80 mm), *P. aeruginosa* (14.98±0.28 mm), *S. pyogens* (14.2±0.47 mm) *H. influenzae* (13.24±0.80 mm) and *C. albicans* (8.89±0.58 mm). Different solvent extracts of barks, leaves and stems of *B.*

prionitis showed the good antibacterial potential against *S. aureus*, *P. aeruginosa*, *B. cereus*, and *S. mutans*. It was also reported that among the extracts, MeOH bark extract showed potential antibacterial activity against all the pathogens. Crude MeOH extract revealed good antibacterial activity against MDR *E. coli* with 12 mm of inhibition zone (Aneja *et al.*, 2010; Khobragade and Bhande, 2012). Chetan *et al.*, 2010 were reported the antibacterial activity of ethanolic leaf extract of *B. prionitis* against *S. aureus*, *B. subtilis*, *P. vulgaris*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*. Antibacterial activity of four extracts *i.e.* H₂O, PET, CHCl₃ and ACE were reported against *L. rhamnosus* (MTCC1408), *S. mutans* (MTCC 890), *S. aureus* MTCC 3408), *A. viscosus* (MTCC 7345), *S. epidermidis* (MTCC 3639), *E. coli* (MTCC 732) and *B. subtilis* (MTCC 3160). Pronounced inhibition of the four extracts was observed for bacterial species, *L. rhamnosus* CHCl₃ extract was found to be more effective against the entire test microorganism (Diwan *et al.*, 2012). *In vitro* propagated shoot tips and leaves of *B. prionitis* with ethanol, ether and chloroform extracts showed the antibacterial activity (Shukla *et al.*, 2011). Antibacterial activities of *B. prionitis* bark and leaf MeOH extracts against *B. cereus* (22.66 mm) followed by PET leaf extract against *E. coli* (21.66 mm). Minimum inhibition was showed by PET leaf extract against *A. faecalis* (4.66 mm) followed by MeOH bark extract against *A. faecalis* (5.33 mm) (Kumar *et al.*, 2013).

The antifungal potential of *B. prionitis* Linn. of all extracts were measured in % inhibition that is presented in table 2. Maximum % of inhibition was observed by the standard drug (74.31%), fuconazole followed by H₂O (35.11%), ACE (35.02%), MeOH (24.51%), and PET (20.18%), respectively.

Table.1 Diameter of inhibition zone all extracts of *B. prionitis*

Pathogen	Inhibition zone (mm in diameter)				Positive control Erythromycin
	PET	ACE	MeOH	H ₂ O	
<i>S. pneumoniae</i>	13.33±0.57	14.22±0.36	19.40±0.64	17.34±0.70	27.56±0.95
<i>S. aureus</i>	12.88±0.22	18.73±0.80	15±0.45	14.22±0.62	29.42±0.73
<i>H. influenzae</i>	10.11±0.21	11.74±0.22	13.24±0.80	10.39±0.21	21.68±0.43
<i>P. aeruginosa</i>	12.63±0.91	11.54±0.62	14.98±0.28	12.71±0.41	16.91±0.49
<i>S. pyogenes</i>	10.93±0.69	13.80±0.25	14.2±0.47	14.04±0.33	25.01±0.57
<i>C. albicans</i>	6.66±32	7.34±0.51	7.55±46	8.89±0.58	22.55±0.56

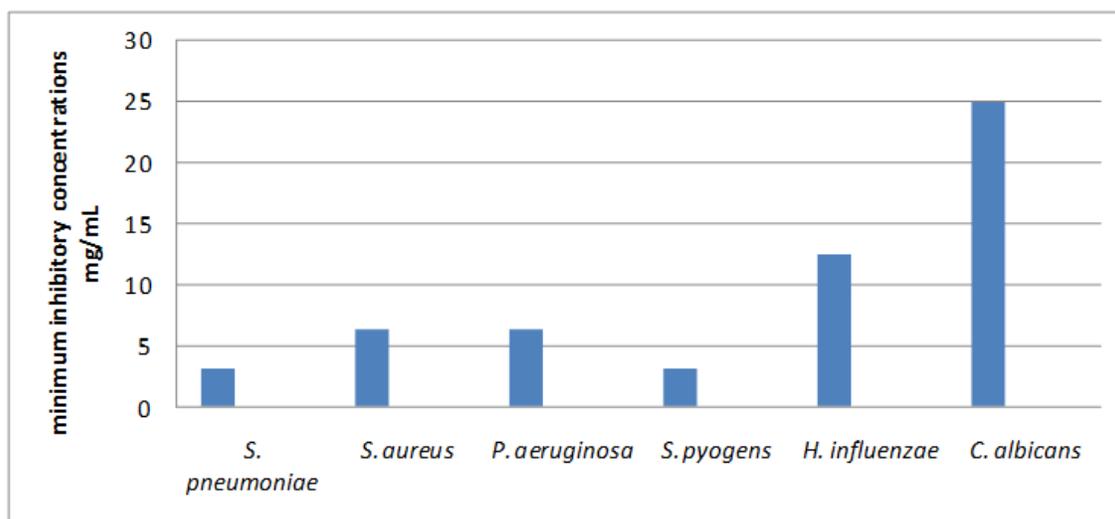
cork borer diameter (6 mm), all values were mean of three replicates with standard error , PET = Petroleum ether, ACE= Acetone, MeOH = Methanol, H₂O= Aqueous

Table.2 Effect of aerial parts extract and fuconazole on the mycelial growth rate of *Aspergillus niger*

Fungicide/extract	Mycelial growth (mm)	Control	% inhibition
PET	30.44±0.58	38.14±0.28	20.18
ACE	23.3±0.28	35.86±.54	35.02
MeOH	28.6±0.76	37.89±0.45	24.51
H ₂ O	21.56±0.54	33.23±0.76	35.11
Fuconazole	9.44±56	36.76±34	74.31

cork borer diameter 6 mm, all values were mean ± standard error, all values of three replicates

Fig.1 Minimum inhibitory concentrations (MICs) of MeOH extract of *B. prionitis* Linn. The minimum inhibition is observed at 3.12 mg/mL for *S. pneumoniae*, *S. pyogens*, 6.25mg/mL for *S. aureus*, *P. aeruginosa*, 12.5mg/mL for *H. influenzae* and 25 mg/mL against *C. albicans*.



The antifungal activity of ACE, EtOH and MeOH bark extracts of *B. prionitis* against *S. cerevisiae*, *C. albicans* and MeOH extract was found more active against all the fungal strains (Shukla *et al.*, 2011; Aneja *et al.*, 2010). Antifungal activity of *B. prionitis* were reported against *C. neoformans*, *C. albicans*, *C. vaginitis*, *B. dermatidis* using chloroform, acetone and ethanol extract of stem, leaves and roots (Panchal and Singh, 2015).

PET, dichloromethane and ethanol stem and root extracts of *B. prionitis* showed fungistatic and fungicidal properties against *C. albicans* (Banerjee *et al.*, 2012).

MICs of MeOH extract of *B. prionitis* Linn. are presented in fig. 1. Minimum inhibitory concentrations (MIC) of MeOH extract of *B. prionitis* Linn. was observed at 3.12 mg/mL for *S. pneumoniae* and *S. pyogenes*, 6.25 mg/mL for *S. aureus* and *P. aeruginosa*, 12.5mg/mL for *H. influenzae* and 25 mg/mL against *C. albicans*.

In conclusion, present study concluded that the aerial parts of *B. prionitis* Linn. have the potent antimicrobial activity against some respiratory bacterial and fungal strains. The MeOH extract was found most potent in comparison to other solvents. The antimicrobial activity of crude extracts represents a considerable outcome for the treatment of respiratory diseases.

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