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Research Article

Antibacterial Potential and Phytochemical Analysis of *Barleria lupulina* Lindl. (Aerial Parts) Extracts Against Respiratory Tract Pathogens

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ABSTRACT

The antibacterial and phytochemical investigation of *Barleria lupulina* Lindl. aerial parts extracts were examined against common respiratory tract pathogens i.e., *Streptococcus pneumoniae* (MTCC 655), *Staphylococcus aureus* (MTCC 1144), *Pseudomonas aeruginosa* (MTCC 2474), *Streptococcus pyogens* (MTCC 442), *Haemophillus influenzae* (MTCC 3826). The plant material was extracted with solvents *i.e*., petroleum ether (PET), acetone (ACE), methanol (MeOH) and aqueous (H_2O) with increasing polarity by Soxhlet apparatus and removed the solvent using vacuum evaporator at 30˚C. Antibacterial activity and minimum inhibitory concentration (MICs) were examined by Agar well diffusion two fold serial dilution method respectively. The maximum inhibition zone was found against *S. aureus* (17.34±0.78 mm) of methanol extract and minimum against *S. pyogens* (9.44±0.32 mm). MICs were observed for MeOH extract between 3.12 to 12.5 mg/mL against *S. pneumoniae* and *S. pyogens* respectively. Phytochemical examination of plant extracts showed the occurrence of alkaloids, saponins, steroids, flavonoids, glycosides, tannins, resins and phenolic compounds. The antimicrobial activity of the crude extracts of plant represents a significant outcome for the treatment of respiratory tract diseases.

Keywords: *Barleria lupulina* Lindl., Respiratory tract pathogens, antibacterial, phytochemical analysis, Minimum inhibitory concentration.

INTRODUCTION

Plants derived medicines have been widely used in most parts of the world. The use of the traditional plants for warfare infectious diseases is becoming the focus of various studies[1-3]. Plants have unlimited capacity to produce secondary metabolites like tannins, saponins, terpenoids, alkaloids, flavo- glycosides and phenols which having antimicrobial properties[4,5]. Phytochemical substances have recently become of great awareness owing to their ingenious applications. It has been estimated that 14- 28 % of higher plants are used for therapeutic purposes and that 74 % of pharmacologically active phytochemicals were revealed after following up on ethno medicinal use of the plants[6]. In the last couple of decades, it is evident that there is a new development in the research and promotion of plants based drugs. The interest of the peoples has become more and more towards the herbal medicines[7-9].

Respiratory tract infections are the most common ailment including allergies, asthma and chronic obstructive pulmonary disease (COPD). The proportion of non-communicable diseases deaths in 2008 due to respiratory diseases were 3.9%, with 4.2 million deaths occurred due to asthma and COPD worldwide[10]. The climatic conditions are very complimentary for spread of such diseases commonly transmitted by coughing and sneezing (airborne disease). Some widespread causal agents are *E. coli*, *K. pneumoniae* responsible for nosocomial infections[11], *H. influenzae*, *S. pneumoniae*, *S. pyogenes* and *Moraxella catarrhalis* for community acquired infections, *Enterobacter cloacae* and *Bacillus subtilis* which cause occupational asthma, respectively[12-14].

Barleria lupulina Lindl. is belongs to the family Acanthaceae. It is a small shrub and commonly known as Bishalyakarani or Vishalyakarani in Bengali, Sornomukhi and Hophead, Philippine violet in English. *B. lupulina* is a cultivated medicinal plants and introduced from Mauritius, now somewhat naturalized besides its cultivation in the garden as ornamental shrub[15]. Its greatest representation is in Africa and Asia[16].

It is used for its medicinal importance as the leaf juice is given to stop bleeding when cut and the paste of leaf is used as poultice to relief pain. It is also used as an anti-inflammatory against insect bites, snake bites, dog bite, herpes simplex virus, rheumatism[17,18]. Compounds found in the leaves of *B. lupulina* include barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester, ipolamiidoside and iridoid glucosides[19]. Virucidal activity against HSV type 2 strain G, *in vitro* anti-HSV activity, antimicrobial activity against acne-inducing bacteria, antidiabetic potential, antiulcer activity[20,19,21,22]. Leaves, stems and roots of *B. lupulina* and flower of *B. prionitis* possess potential antibacterial and anti-inflammatory activities[23,24,20]., ameliorate secondary complications of diabetes including cataract[25,26], antiarthritic[27],

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antiinflammatory[28], antimicrobial[26], anticlastogenic, antitumor, and anticancer activities[29].

MATERIALS AND METHODS

Collection and preparation of plant material

Plant material was collected from the garden of Gurukul Kangri University, Haridwar India, during September, 2013. Collected plant material was washed using distilled water and dried at room temperature. Well dried material was crushed into the powder form using electric grinder.

Extract preparation

Plant extracts were prepared by immersing 100 g of powdered plant material in 300 mL (1:3) with four different organic solvents *i.e.* PET, ACE, MeOH and H_2O. Loaded the powdery material in Soxhlet apparatus and extracted for 72 h by successive method[30]. Each preparation was filtered through a sterilized Whatman No. 1 filter paper. Extracts were evaporated with the help of vacuum evaporator at 30˚C. The dried extracts obtained from vacuum evaporator were exposed to Ultraviolet rays for 24 h to checked sterility on nutrient agar plates and stored in a refrigerator at 4˚C for further use[31].

Test Pathogens

Upper respiratory tract infections pathogens were selected for this exploration. Clinical bacterial strains of Gram positive (*S. pyogenes* MTCC 442, *S. pneumoniae* MTCC 655, *S. aureus* MTCC 1144) and Gram negative *P. aeroginosa* MTCC 2474, *H. influenzae* MTCC 3826 were selected and all pathogens were procured from Institute of Microbial Technology (IMTECH), Chandigarh (India).

Antibacterial Activity

Antibacterial activity of seeds was determined by agar well- diffusion method[30]. Stock cultures were maintained at 4˚C on slopes of nutrient agar medium (NAM). Active cultures for experiments were prepared by transferring microbial inoculum from stock cultures to test tubes containing Mueller-Hinton Broth (MHB) for bacteria that were incubated at 37˚C for 24 h. 100 µL of diluted inoculums of 10^5 CFU mL^{-1} [32] of 24 hours aged culture of test organisms were poured and mixed in Mueller Hinton Agar (MHA) medium. Medium was poured in to sterilized Petri plates and allowed to solidify for 5 – 10 min. A cork borer (6 mm) 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. Extracts were assayed in triplicate and the mean values were observed. All plates were incubated at 37˚C for 24 h. Antibacterial activities were interpreted from the dimension of the diameter of inhibition zone calculated in millimetres as observed from clear zones neighbouring the wells.

Minimum inhibitory concentrations (MICs)

MIC was done by using modified two fold serial dilution method[33]. Sterilized nutrient broth was poured uniformly into all test tubes. Bacterial cultures were prepared by Mcfarland's turbidity standard scale number 0.5 was poured in each test tubes containing broth with normal saline and incubated for 6 h at 37˚C for making a turbid suspension of the bacterial culture. After incubation, dilution of the culture in DMSO was ready until it matching with the turbidity (1.5 x 10^6 cfu/mL) of the Mcfarland's scale. Solution of MeOH extract was serially diluted with broth to obtain the following concentrations 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/mL. From the above suspension equipped in DMSO, 0.1 mL was dispensed into the different concentration of the extract in nutrient broth. The test tubes were observed for turbidity after incubating at 37˚C for 24 h. The lowest concentration of the extract in the broth which showed no turbidity determined the MIC.

Phytochemical Screening

All extracts were subjected to identify the chemical nature of phytochemical constituents present in extracts[34,35].

Test for Alkaloids

The test solution gave brown precipitate with the Dragendroff's reagent. The presence of brown precipitate showed the presence of alkaloid.

Test for Flavonoids

2-5 drop of 1% NH_3 solution is added to the extract in a test tube. A yellow colour is observed that indicate the presence of flavonoids.

After addition of conc. HCl in MeOH extract, a red colour appeared which indicated the presence of flavonoids.

Test for Steroids

Extract was mixed with 3 mL CHCl3 (chloroform) and 2 mL concentrate H_2SO_4 (sulphuric acid) was added from side of test tube. Colour of the ring at connection of two layers was noted. If a red colour observed it confirmed the presence of steroids.

Test for Resins

An aliquot of 10 mL of diluted extract and 10 mL of 1% cupper acetate solution was added and the mixture was shaking vigorously. A separate green colour indicated the presence of resin.

Test for Saponins

Extracts was diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15 min. If 1 cm layer of foam produced it indicates the presence of saponins.

Test for Tannins

1% $FeCl_3$ was added in the extract and colour was observed. A bluish black colour was appeared which disappeared after addition of dilute H_2SO_4 following a yellow -brown precipitate indicates the presence of tannins.

Test for Phenols

2 mL of extract added in alcohol with one drop of neutral Fe_2Cl_3 (5%) solution. Formation of blue colour indicates the presence of phenols.

Test for Lignins

Phloroglucinol with HCl were added in the test solution. Formation of pink colour indicates the presence of lignins.

Test for Terpenoids

In a test tube 5 mL of H_2O extract was mixed with 2 mL of $CHCl_3$ further 3 mL of concentrated H_2SO_4 is added to the test tube containing extract to form a layer. If terpenoids are present an interface with a reddish brown colouration is formed.

Test for Glycosides

1 mL of conc. H_2SO_4 is prepared in test tube 5 mL the extract and mixed with 2 mL of glacial CH_2CO_2H

Table 1: Diameter of inhibition zone all extracts of *B. lupulina*.

Pathogen	Inhibition zone (in diameter)				Erythromycin (positive control)
	PET	ACE	MeOH	H_2O	
S. pneumoniae	11.33±0.55	11.78±0.26	13.40±0.54	12.34±0.30	22.56±0.95
S. aureus	10.88±0.22	14.73±0.80	17.34±0.78	9.70±0.52	29.42±0.73
H. influenzae	10.11±0.31	9.44±0.32	13.24±0.70	10.39±0.41	21.68±0.43
P. aerugienosa	12.63±0.91	15.54±0.52	14.98±0.28	9.71±0.61	16.91±0.19
S. pyogenes	10.93±0.59	13.80±0.35	11.2±0.57	10.04±0.23	25.01±0.97

Where, given values cork borer diameter (6 mm), all values are mean of three replicates, PET = Petroleum ether, ACE= Acetone, MeOH = Methanol, H_2O= Aqueous.

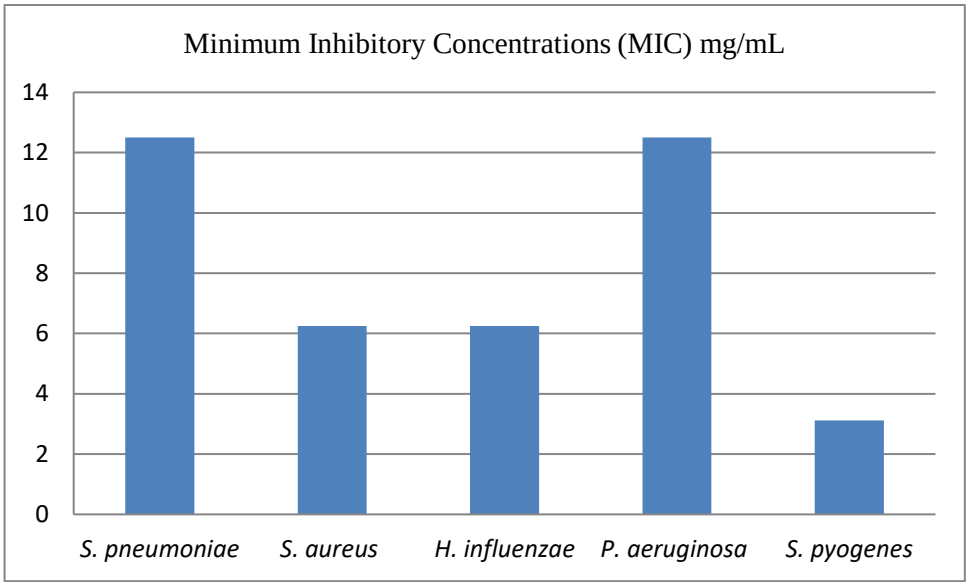

Figure 1: Showing minimum inhibitory concentration values for *S. pneumoniae* (12.5 mg/ml), *S. aureus* and *H. influenzae* (6.25 mg/ml), *P. aeruginosa* (12.25 mg/ml), *S. pyogens* (3.12 mg/ml).

containing 1 drop of $FeCl_3$. The above mixture is carefully added to 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture.

50 mg of extract was dissolved in 5 mL of distilled water and filtered through the Whatman filter paper no. 1. The filtrates of each extract were subjected to the following tests.

RESULTS AND DISCUSSION

The data presenting to antibacterial activity of aerial parts extracts of *B. lupulina* are shown in table 1. *B. lupulina* showed promising potential against test pathogens. MeOH extract was found most active followed by ACE, PET and H_2O while maximum inhibition was reported against *S. aureus* followed by *P. aeruginosa*, *S. aureus*, and *S. pyogens*. In a similar study Chomnawang *et al*., (2005) reported strong inhibitory activity *B. lupulina* against acne-inducing bacteria[20]. Moin *et al*., (2012) examined the antibacterial activity of MeOH extract of *B. lupulina* against *Staphylococcus aureus* and *Bacillus pumilus.* MIC values of 0.375 mg/ml and 0.500 mg/ml against *S. aureus* and *B. pumilus*, respectively[36]. Doss *et al*., (2011) reported antibacterial efficacy of MeOH extract of *B. lupulina* against *S. aureus* and *E. coli*[37]. The antibacterial activity of ACE, MeOH, and H_2O extracts of both leaf and stem of *B. lupulina* were evaluated against some human pathogens (*S. typhi*, *E. coli*, *P. aureginosa*, *K. pneumonia* and *S. aureus*). The highest antibacterial activities were exhibited at 100% concentration of all the extracts against the tested pathogens. ACE-soluble leaf and ACE-soluble stem extracts caused the maximum zone of inhibition against *P. aureginosa*, and MeOH-soluble leaf and MeOH soluble stem extracts against of *S. typhi*. In another similar study the EtOH and H_2O extracts of *B. lupulina* leaves displayed antibacterial activity against human bacterial pathogens (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhi K. pneumoniae*). The EtOH extract was inhibitory than the H_2O extract against all the test pathogens, which caused the maximum growth inhibition of *P. aeruginosa* at 100% concentration. MIC value of EtOH extract was 2.5 mg/mL against *E. coli*, *S. aureus* and *P. aeruginosa*, and 10.0 mg/mL against *S. typhi* and *K. pneumonia*[38]. It was found that the MeOH extract (100 mg/ml. and 200 mg/ ml.) diluted in 70% of methanol and extract of succulent leaves can induce 12 mm, 13 mm and 14 mm diameter zone of inhibition comparable with 24 mm of Ceftriaxone against *E. coli.* The zone of inhibition against *S. aureus* were 13 mm, 14 mm, 15 mm and 25 mm and against *S. enteritides* were 12 mm, 14 mm, 15 mm and 28 mm correspondingly. The fresh extract of the plant showed antimicrobial efficacy in the concentration of 16.5%[39].

Phytochemical study revealed the crude fractions of extract showed the presence of alkaloids, tannins, saponins, glycosides and phenolic compounds showing in

Table 2: Phytochemicals screening of crude extracts of *Barleria lupulina*.

S.No	Tests \ Extracts	Petroleum ether (PET)	Acetone (ACE)	Methanol (MeOH)	Aqueous (H_2O)
1.	Alkaloids	+	-	+	-
2.	Glycosids	+	+	+	+
3.	Lignins	+	+	-	-
4.	Tannins	-	-	-	+
5.	Saponins	+	-	+	-
6.	Steroids	-	+	+	+
7.	Terpenoids	-	+	-	-
8.	Phenols	+	+	+	+
9.	Flavanoids	-	+	+	+
11.	Resins	-	-	+	-

where + = present and - = absent

table 2. MICs values were showed between 12.5 mg/ml to 3.12 mg/ml for *S. pneumoniae* and *S. pyogens* respectively, showing in figure 2. Nag *et al*., (2013) assayed the phytochemical analysis of *B. lupulina*. They found the presence of alkaloids, starch, tannins, glycosides, reducing sugars, proteins, amino acids, flavonoids [40]. Phytochemicals found in the leaves of *B. lupulina* include barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester, ipolamiidoside and iridoid glucosides[19]. Several phytochemicals including barlerin alkaloid is derived from *B. lupulina* which possess antimicrobial and anticancerous properties. Iridoid glucosides, bataine and alkaloids have also been reported from *B. lupulina* [41,37].

CONCLUSION

This study supports the traditional use of *B. lupulina* areal parts contain some important bioactive compounds that inhibit the pathogenic microbes. It may be conclude that this plant will be helpful for many respiratory diseases.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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