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Research Paper

ANTIBACTERIAL ACTIVITY OF ROOTS EXTRACTS OF *BARLERIA LUPULINA*

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ABSTRACT

Antibacterial activity of aqueous, acetonic, methanolic and ethanolic root extracts of *Barleria lupulina* was investigated against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas* spp. The antibacterial activity was performed by agar disc and agar well diffusion methods against 6 bacterial species (3gram positive and 3 gram negative).Aqueous extracts has profound effect on *Staphylococcus aureus* and *Eschereschia coli*. Acetonic, methanolic and ethanolic extracts shown their antibacterial effect on *Bacillus subtilis* and *Pseudomonas* spp. No activity was seen on *Klebsiella pneumoniae*. The significant antibacterial activity was compared with standard antibiotics ampicillin and streptomycin. The results obtained in the present study suggest that they can be used in treating diseases caused by the test organisms.

Keywords: Antimicrobial, *Barleria lupulina* root extracts

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INTRODUCTION

India is an exquisite example of biodiversity and explored the possibility of such a diversity for human welfare and the most conspicuous exploration in this field has lead to the discovery of so many indigenous medicinal plants that were scripted mainly in Vedas (1500 BC) that contain rich materials on herbal lore of that time. Similar to the plant diversity of India the same kind of diversity exist in the world of scriptures delineating the miracle of medicinal plant (Gayatri R *et al 2008*). Medicinal plants can form an excellent source for deviation of lead compounds or newer drugs (Balick J.M *et al 1996*).In last three decades number of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens (Cohen ML 1992).Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Barbour E.K *et al* 2004*)*. Many infectious microorganisms an are resistant to synthetic drug, hence, an alternative therapy is very much needed and attract the attention of many researchers all over the world (Mohanan PV*et al* 1998). Plants were synthesized many compounds with complex molecular structures by a secondary metabolism.have antimicrobial properties such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins,glycosides, terpenes and phenolic compounds(Simoes CMO*et al 1999).* Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharvaveda, Charaka, Sushrutaetc(Farhana AR *et al* 2009).In recent years, secondary plant metabolites (photochemical), previously with unknown activities, have been extensively investigated as a source of medicinal agents(Krishnaraju AV*et al 2005*). A wide range of medicinal plant parts is used to extract as raw drugsand they possess varied medicinal properties (Uniyal SK *et al 2006*).Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have studied by a very large number of researchers in different parts of the world (Reddy PS et al 2001;Ates DA and Erdogul OT 2003).The present Indian medicinal plant *Barleria lupulina* was belongs to the Family Acanthaceae which is commonly called as hophead .Preliminary phytochemical analysis of the roots and leaves extracts indicates the presence of carbohydrates, cardiac lycosides, phytosterols, phenols, falvonoids, diterpenes and saponins. The aim of this present study was to evaluate the activity of different extracts against Gram positive and Gram negative test bacterial strains.

MATERIALS AND METHODS

Bacterial Strains

The microbial strains are identified andbacterial strains studied are Bacillus cereus NCIM 2157, *Bacillus subtilis* NCIM 2063, *Staphylococcus aureus* NCIM 2901, Escherichia coli NCIM 2065,Klebsiellapneumoniae NCIM 2883 and *Pseudomonas* sp. NCIM 2207.

Plant Material

The plant *Barleria lupulina* was collected from Bangalore region. The plant was taxonomically identified. The fresh plant parts were collected. The roots were detached and washed with clean water. Roots were air dried on a clean sheet for one week at room temperature. Dried root material of *Barleria lupulina* was powdered and passed through sieve #10.

Solvent Extraction

Ten grams of air dried root powder was placed in100 ml of organic solvents (Acetone, Methanol and Ethanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 175 – 200 rpm for24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15min. The supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume. It was stored at 4º C in airtight bottles for further studies.

Aqueous Extraction

For aqueous extraction, 10 g of air – dried root powder was placed in distilled water and boiled for6 h. At intervals of 2 h it was filtered through 8layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant was collected. After 6h, the supernatant was concentrated to make the final volume one – fourth of the original volume. It was then autoclaved at 121º C and 15 lbs pressure and stored at 4º C.

Media Preparation and Antibacterial Activity

The antimicrobial assay of root was performed by two methods viz., agar disc diffusion method (Parekh J et al 2005)for aqueous extract and agar well diffusion method (Bauer AW et al 1996) for solvent extract..A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 h to activate the strain. Muller Hinton Agar (MHA) (3.8g/100ml) was weighed and dissolved in 100 ml of distilled water in a sterile conical flask. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The molten Muller Hinton Agar was inoculated with 200 µl of the inoculum and poured into the Petri plate. For Agar disc diffusion method, the disc (0.6 cm)was saturated with 50 µl of the compound, allowed to dry and was introduced on to the upper layer ofthe medium with bacteria. Antibiotic paper(streptomycin, ampicillin) discs

were used as positive control. These test discs was placed on MHA plate swabbed with the culture of microorganisms. The plates were incubated at 37°C for overnight. For agar well diffusion method, seven wells were prepared in the plates with the help of a cork-borer(0.6 cm). 50 µl of each test extract was introduced into well. Antibiotics were added to the wells. The plates were incubated overnight at 37° C.

Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube(bacteriostatic concentration). The Vollekova *et al* (2001) method modified by Usman*et al*(2007) was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration in sterile distilled water and serially diluted (two-fold)to a working concentration using nutrient broth and later inoculated with 0.2 ml suspension of bacterial strains. After 18 hours of incubation at 37º C, thetest tubes were observed for turbidity. The least where no turbidity was observed was determined and noted as the minimum inhibitory concentration(MIC) value.

Minimum Bacterial Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed(bacteriocidal concentration). This was determined by the broth dilution resulting from the MIC tubes by sub-culturing to antimicrobial free agar as described by Vollekova *et al*(2001) and Usman*et al (2007)*. In this technique, the contents of the test tubesresulting from MIC was streaked using a sterilewire loop on agar plate free of bacteria andincubated as 37º C for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

RESULTS AND DISCUSSION

The antibacterial activity of *Barleria lupulina* plant root extracts was assayed against 6 bacterial species (3 gram positive and 3 gram negative).Table 1 & 2 shows the microbial growth inhibition of aqueous, acetonic, methanolic and ethanolic extracts of the plant species. The acetonic, methanolic and ethanolic extract has showed the effect on gram positive *Bacillus subtilis* and *Pseudomonas sp*., (gram negative). Aqueous extract shown its effect on *Bacillus cereus* and *Eschereschia coli.* Acetonic extract has its effect on only *Bacillus cereus*. The rest were resistant to the extracts. The significant antibacterial activity has compared

with the standard antimicrobial antibiotic sampicillin and streptomycin (50 µL). MIC values were also represented in the table 3 & 4. No profound effect was shown in MBC. Phytomedicine is a great relief to the present situation of medical treatments. The development of antimicrobials from the plant species will lead to the development of newer green medicines against microbes. Green medicines can serve the purpose with lesser side effects than the synthetic antimicrobials.

Table 1: Antimicrobial activity of root extracts of *Barleria lupulina* against various Gram positive bacteria

Zone of Inhibition (cm)

Gram positive bacteria	**Aqueous extract**	**Acetonic extract**	**Methanolic extract**	**Ethanolic extract**	**Streptomycin**	**Ampicillin**
Bacillus cereus	–	0.7	–	–	3.1	–
Bacillus subtilis	–	1.1	0.8	0.8	2.9	2.9
Staphylococcus aureus	0.8	–	–	–	2.0	1.1

" – "= no activity

Table 2: Antimicrobial activity of root extracts of *Barleria lupulina* against various Gram negative bacteria

Gram negative bacteria	**Aqueous extract**	**Acetonic extract**	**Methanolic extract**	**Ethanolic extract**	**Streptomycin**	**Ampicillin**
Eschereschia coli	0.7	–	–	–	1.5	2.3
Klebsiella pneumoniae	–	–	–	–	1.9	1.9
Pseudomonas spp.,	–	1.5	1.3	1.7	1.9	1.8

" – "= no activity

Table 3: Minimum Inhibitory concentration (MIC) of root extracts of *Barleria lupulina* against various Gram positive bacteria

Extract dilution

Bacillus cereus	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**
Aqueous	–	–	–	–	–	–	–	–
Acetonic	–	–	–	–	+	–	–	–
Methanolic	–	–	–	–	–	–	–	–
Ethanolic	–	–	–	–	–	–	–	–
Bacillus subtilis								
Aqueous	–	–	–	–	–		–	–
Acetonic	–	–	–	–	+	–	–	+
Methanolic	–	–	–	–	–	–	–	–
Ethanolic	–	–	–	–	–	–	–	–
						–		
S. aureus								
Aqueous	–	–	–	–	–	–	+	–
Acetonic	–	–	–	–	+	–	–	–
Methanolic	–	–	+	–	–	–	–	–
Ethanolic	–	–	–	–	–	–	–	–

S. aureus = Staphylococcus aureus
– = turbidity
+ = least concentration showing no turbidity

Table 4: Minimum Inhibitory concentration (MIC) of root extracts of*Barleria lupulina* against various Gram negative bacteria

Extract dilution

Bacillus cereus	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**
Aqueous	+	–	–	–	–	–	–	–
Acetonic	–	–	–	–	–	–	–	–
Methanolic	–	–	–	–		–	–	–
Ethanolic	–	–	–	–	–	–	–	–
					–			
Bacillus subtilis								
Aqueous	–	–	–	–	–		–	–
Acetonic	–	–	–	–	+	–	–	+
Methanolic	–	–	–	–	–	–	–	–
Ethanolic	–	–	–	–	–	–	–	–
						–		
S. aureus								
Aqueous	–	–	–	–	–	–	+	–
Acetonic	–	–	–	+	-	+	–	–
Methanolic	–	–	–	–	–	–	+	–
Ethanolic	–	+	–	–	–	–	–	–

– = turbidity
+ = least concentration showing no turbidity

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