ANTIBACTERIAL EFFECTS OF AERIAL PART OF BARLERIA PRIONITIS WITH ETHANOL EXTRACT ON PATHOGENIC STRAINS

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ABSTRACT

This study was planned for the investigation of antimicrobial effects of Barleria prionitis leaves. Therefore, the preliminary successive solvent extraction and chemical test revealed the presence of secondary metabolites in various extracts. Microbial inhibitory effects can provide us clue for further investigation. Ethanol extract was used for investigation of inhibition on five pathogenic bacterial strains and done by broth dilution methods with triplicate. Nutrient media was used for bacterial culture. Tetracycline was used as positive control and double distilled water was used as Negative control. Statistical analysis has been done by Mean ± Standard Deviation with range, in which n=3.

There were five bacteria were tested, out of which drug has been given best results against P. aeruginosa while average effect against B. thurengenesis, C. diphtheria, S. typhi, and C pneumonia and the plant leaves have shown the presence of alkaloids, flavanoids, saponins and tannins. As we calculate that Barleria leaf ethanol extract diluted with 2.5 mg/ml to get 214.4 activities against B. thurengenesis, S.typhi and C. diphtheria but in 5 mg/ml dilution 107.2 activities has been get against, C.pneumoniae while for P. aeruginosa 1.25 mg/ml dilution given 428.8 Total activities. The highest activity of our study is 428.8. In the present study, we found that Barleria prionitis Leaf ethanol extract was given the very best inhibitory effect against all tested pathogenic bacteria. But B. thurengenesis, C. diphtheria and Salmonella typhi were very sensitive with Barleria leaves. Barleria leaves with ethanol extract given the very best results against Pseudomonas aeruginosa. It has been given the presence of flavonoids, alkaloids, saponins and tannins, which is responsible to show
antimicrobial, relieving in toothache and whooping cough. *Barleria* leaves have high level of activity against tested pathogenic strains.

**KEYWORDS:** *Barleria prionitis*, secondary metabolites, antimicrobial activity, Ethanol, Total activity, statistical analysis, Phytoconstituents.

**INTRODUCTION**

Ayurveda has been popular and unbroken medical tradition in the world. Among 17000 plant species, 1000 species are used over several centuries in traditional systems. Nearly 25 hotspots have been recognized worldwide, which harbor 44% of all endemic plant species. According to the World Health Organization (2002), 80% of the world population is dependent upon plants for primary health care. India has 2.4% of the world’s area with 8% of global bio-diversity. National studies have shown 120 medicinal plants are rare or endangered in India. Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs of higher efficacy or as models for pharmacologically active compounds (Mukherjee et al., 2001). Medicinal plants having therapeutic properties for healing various disease naturally and have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali et al., 1998; Barbour et al., 2004; Yasunaka et al., 2005), (Kalemba and Kunicka et al., 2003).

*Barleria prionitis* L. (Acanthaceae) is an Ayurvedic herb distributed in the tropical Asia, Africa and Yemen. The whole plant or its specific parts (leaf, stem, bark and flower) have been used for the treatment of toothache, catarrhal affections, whooping cough, inflammation, glandular swellings, urinary infection, jaundice, fever, gastrointestinal disorders and as diuretic and tonic. Among all extracts, the ether extract showed strongest antibacterial activity (Shukla et al., 2011).

In developing countries, plant resources can cure a wide range of disease, as allopathic medicine, but it is expensive than herbal medicines.(Kala CP, 2005) Traditional medicine, mostly plant drugs, have been used by 60% of world population and 80% of the population of developing countries for their primary health care needs(Shreshtha PM, 2003). Knowledge about the medicinal plant species carried and transmitted by indigenous people (Bhat JA, 2013). Antibacterial activity as a objective can explore environmentally safe alternatives for
disease control. There was various plant extracts have also been examined for their antibacterial activity. (Gracelin et al., 2011).

**MATERIALS AND METHODS**

**Collection of plant**
The whole plant was collected from Govt. Nursery of Modinagar, Dist. Ghaziabad, U.P, India, and has been authenticated by NBRI, Lucknow, India (Voucher/Specimen no. is NBRI-SOP-202) a voucher specimen was deposited in the department.

**Extraction**
Leaves were separated from each other and washed carefully under running tap water followed by distilled water. These were shade dried under room temperature for one week and pulverized to a fine powder, using a sterilized mixer grinder. The 50 gm powder of plant part extracted by the Soxhlet method using Chloroform, as solvent on the basis of their polarity of 4.1.

**Yield:** The percentage yields were calculated using following formula: (Panchal P, 2015)

\[
\text{Yield in } \% = \left( \frac{\text{Quantity of extraction in gm}}{\text{Quantity of dry powder of plant in gm}} \right) \times 100
\]

**ANTIMICROBIAL ACTIVITY**

**Collection of strain**

**Preparation of Bacterial strains and culture conditions**
The obtained culture of *S. typhi, C. pneumoniae, B. thurengensis, B. anthracis, P. aeruginosa* were maintained on nutrient by making slants and the stock cultures were transferred at monthly intervals. A single colony was transferred in sterile 100 ml of nutrient broth /potato dextrose media and incubated at 37° C in a shaker at 140 rpm for 14 hrs. Bacterial culture was centrifuge and suspended with sterile distilled water; the concentration of the pathogen was optimized by maintaining optical density (OD) to 0.1 at 600 nm.
Screening of bioactive compounds against various microbes

The method was used to screen plant extracts before determination of MIC, was agar disc diffusion method, in which 10 ml of nutrient agar media poured in a sterile Petri dish, 100 µl of test organisms were spread on the surface of media, wells were prepared with help of sterile borer and wells were aseptically filled by 50 µl of plant extract along with positive (antibacterial compound tetracycline at 50 µg/ml and negative control (autoclaved distilled water). Plates were incubated aerobically at 37°C for 72 hrs. The diameter of zones of inhibition was measured. The initial concentration of MIC was 10 mg/ml for bacterial strains.

Minimum Inhibitory Concentration (MIC) against bacterial strains

3 ml of nutrient broth (for bacterial cultures) was taken in 5 test tubes, sterilized by autoclave. Plant extracts with final concentration of 100 mg/ml (for bacterial pathogens) was mixed with another extra 3 ml of liquid media (Nutrient broth). It was added to first test tube, mixed property, and after mixing, 3 ml of the solution was taken from the first test tube add was added to the second test tube. The same was repeated till the 5th test tube, followed by removal of extra 3ml solution from last test tube to keep the media volume constant.

30 micro liter of fresh culture was added to all of the test tubes and was incubated at 37°C for overnight. The inhibition of growth of pathogens was recorded in terms of optical density at 600nm. The concentration of plant extract just before the test tube showing growth was recorded as MIC. (Panchal P. 2016)

Total Activity (TA) determination

Total activity is the volume at which the test extract can be diluted with the ability to kill microorganisms. It is calculated by dividing the amount of extract from 1 g plant material by the MIC of the same extract or compound isolated and is expressed in ml/g. (Singariya P, 2012)

Total Activity = \( \frac{\text{Extract per gram dried plant part}}{\text{MIC of extract}} \)

Statistical Analysis

Statistical analysis has been done on triplicate, and the data are expressed as the mean ± Standard Deviation with range. Analysis of this study has been done by MS Excel 2007. One way ANOVA followed by Turkey’s honestly significant difference post hoc test was used to compare the data, in which p values were considered significant at 95% confidence intervals (p<0.05).
RESULTS AND DISCUSSION

In this study, ethanol extracts of leaf of Barleria prionitis were evaluated for the presence of phytochemical constituents as well as their antimicrobial activity. Antimicrobial activity results given in the Table-1 analyze statistically, i.e. mean ± standard deviation with range (min-max).

Table 1: By Dilution method, minimum inhibitory Concentration (MIC) of Barleria Leaves with ethanol extract and it’s Optical Density (OD) at 600 nm by (mg/ml).

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of strains</th>
<th>20 mg/ml</th>
<th>10 mg/ml</th>
<th>5 mg/ml</th>
<th>2.5 mg/ml</th>
<th>1.25 mg/ml</th>
<th>Negative control</th>
<th>Positive control</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Salmonella typhi</td>
<td>0.0±0.0002</td>
<td>0.02±0.0005</td>
<td>0.01±0.0004</td>
<td>0.01±0.0002</td>
<td>0.12±0.0003</td>
<td>0.65</td>
<td>NG</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>Chlamydia Pneumoniae</td>
<td>0±0.0003</td>
<td>0.01±0.0002</td>
<td>0.0±0.0004</td>
<td>0.13±0.0005</td>
<td>0.24±0.0061</td>
<td>0.61</td>
<td>NG</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus Thurengensis</td>
<td>0±0.0002</td>
<td>0±0.0001</td>
<td>0.02±0.0001</td>
<td>0.02±0.0003</td>
<td>0.18±0.0004</td>
<td>0.68</td>
<td>NG</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>Pseudomonas aeruginosa</td>
<td>0±0.0002</td>
<td>0±0.0001</td>
<td>0.02±0.0005</td>
<td>0.01±0.0003</td>
<td>0.02±0.0005</td>
<td>0.61</td>
<td>NG</td>
<td>1.25</td>
</tr>
<tr>
<td>5</td>
<td>Chlamydia dipherthiae</td>
<td>0.02±0.0004</td>
<td>0.01±0.0007</td>
<td>0.01±0.0003</td>
<td>0.04±0.0001</td>
<td>0.21±0.0004</td>
<td>0.72</td>
<td>NG</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* ± value= ± standard deviation with Range (min-max) where n=3, Negative control- Double Distilled water, Positive control- Tetracyclin.

The observation of the present study is presence of secondary metabolites such as alkaloid, glycosides, flavonoids, saponins, tannins, steroids and anthraquinone. Hence the phytochemical screening reveals that extracts show high no. of secondary metabolites (Table-2). The plant leaves have shown the presence of alkaloids, flavanoids, steroids and anthroquinone, which may be responsible for antibacterial and immunomodulatory effect.
Table-2 Phytochemical screening on *Barleria prionitis* Leaves ethanol extract.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>S.M</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Hager’s method and Tannic acid</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>Saponins</td>
<td>Foam test</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>Salkowski reaction</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>Shindona test and Sodium hydroxide test</td>
<td>P</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Lead Acetate test</td>
<td>P</td>
</tr>
<tr>
<td>6</td>
<td>Cd. Glycosides</td>
<td>Kellar Killani test and Baljet's test</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>Anthroquinone</td>
<td>Confirmation test from kokate.(Pharmacology Hand Book)</td>
<td>NA</td>
</tr>
</tbody>
</table>

The following table describes the presence of alkaloids, saponins, flavonoids and tannins in the extract. Thus the preliminary screening analysis is required to analyse the bioactive components for the discovery and development of new drugs. In the case of *Barleria prionitis* Leaf ethanol has proved a highly effective extract than ethanol as per previous study. (Panchal *et al.*, 2015).

The percent inhibition of bacterial cells has been shown in graphs. In which, Graph-1 represents the percent inhibition on *Salmonella typhi*. As a minimum inhibitory concentration, consideration will be effective on more than 80% inhibition of bacteria. So 1.2 mg/ml of *Barleria* leaf extract is a MIC against *S. typhi*. Graph-2 represents the percent inhibition of *Chlamydia pneumonia* and again 5 mg/ml consider as MIC. Graph-3 represents the percent inhibition of *Bacillus threngenesis* and 2.5 mg/ml consider as MIC but 1.25 mg is also able to inhibit near about 70% of bacterial cells. Graph-4 represents the percent inhibition of *Pseudomonas aeruginosa* and again 2.5 mg/ml consider as MIC. In Graph-5, the percent inhibition of *Cornybacterium diphtheriae*, 1.25 mg/ml considers as MIC.
In Table -3, Total activity represents the amount of drug to inhibit the concerned bacterial strain, which can be dilute in one ml. As we have calculated, *Barleria Leaf ethanol* extract diluted with 2.5 mg/ml to get 214.4 activities against *B. thruengenesis*, *S.typhi* and *C. diphtheria* but in 5 mg/ml dilution 107.2 activities has been get against, *C.pneumoniae* while for *P. aeruginosa* 1.25 mg/ml dilution given 428.8 Total activities. which is the highest activity in this study.

**Table-3 Total activity of Barleria Leaves Ethanol extract**

<table>
<thead>
<tr>
<th>S.N</th>
<th>Strains</th>
<th>TA</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>S. typhi</em></td>
<td>214.4</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td><em>C. pneumoniae</em></td>
<td>107.2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td><em>B. thruengenesis</em></td>
<td>214.4</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td><em>P. aeruginosa</em></td>
<td>428.8</td>
<td>1.25</td>
</tr>
<tr>
<td>5</td>
<td><em>C. diphtheria</em></td>
<td>214.4</td>
<td>2.5</td>
</tr>
</tbody>
</table>
CONCLUSION
In the present study, we found that Barleria prionitis Leaf Ethanol extract was given the very best inhibitory effect against all tested pathogenic bacteria. But B. thurongensis, C. diphtheria and Salmonella typhi were very sensitive with Barleria leaves. Barleria leaves with ethanol extract given the very best results against Pseudomonas aeruginosa. It has been given the presence of flavonoids, alkaloids, saponins and tannins, which is responsible to show antimicrobial, relieving in toothache and whooping cough. Barleria leaves have high level of activity against tested pathogenic strains. According to Gracioso JS, The preliminary phytochemical analysis indicated the presence of flavonoids, sterols, glycosides, saponins. These secondary metabolite classes are related to gastro protective activity. Lewis DS observed in his study that leaves of the plant also contain saponins. Saponins exhibit ulcer protective effect by selective inhibition of prostaglandin F2x and by protection of gastric mucosa. So, Barleria prionitis leaves might be used to modulate the Ayurvedic medicines.

ACKNOWLEDGEMENT
The authors are thankful to Institute of Biotechnology, Mangalayatan University, Aligarh, for providing the laboratory facilities and other necessary support.

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**Books**

**Citation Links**
- macro/su.edu/howto/solvents/polarityindex.htm.