Evaluation of phytochemical and antimicrobial activities of Boerhavia diffusa

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ABSTRACT

Boerhavia diffusa is a plant of Ayurvedic, traditional, ethnoherbalological and clinical- medicinal importance. Indigenous tribes of many countries have been reported to use different parts of the plant for food and Medicine. The aim of the present study was to evaluate the phytochemicals and antimicrobial activity of various solvent extracts of Boerhavia diffusa L. (Family: Nyctaginaceae). The antimicrobial activity of different solvent extracts of B. diffusa L. were tested against the Gram-positive and Gram-negative bacterial strains and fungus by observing the zone of inhibition. The Gram-positive bacteria used in the test were Staphylococcus aureus, Bacillus cereus, and Micrococcus luteus, and the Gram-negative bacteria were Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae. Fungus such as Aspergillus flavus, Aspergillus niger, and C. albicans were used. It was observed that ethanol, methanol, chloroform, hexane and acetone extract showed activity against Gram-positive and Gram-negative bacteria and fungus. The ethanol extract of B. diffusa L. roots showed more activity against Gram-positive (e.g. S. aureus, 11 mm) and Gram-negative bacteria (e.g. E. coli, 9 mm) when compared to other solvent extracts. The results confirmed the presence of antimicrobial activity of B. diffusa roots extract against various human pathogenic bacteria and fungi.

Key words: Boerhavia diffusa, solvent extracts, phytochemical analysis, Antibacterial activity, antifungal activity

INTRODUCTION

Boerhavia diffusa (Family Nyctaginaceae) is a herbal plant, which is common in the tropics in both dry and rainy seasons. It is found in India, Nigeria and many other countries. The herbalists, however, use the aqueous leaf extract to treat diabetes in man (unpublished data). The plant exhibits a somewhat periodic efficacy, with its maximum activity being noticed in the month of May [1]. B. diffusa is used in traditional medicine for its anti-inflammatory, antibacterial and cardiotonic properties [2]. It is used in the treatment of elefantiasis, night blindness and corneal ulcers [3]. Boerhavia diffusa Linn. (Family: Nyctaginaceae) is a plant known for its medicinal properties, employed in folkloric medicine in Nigeria, and Ayurvedic medicine system of India [4] It is a low growing, spreading vine with a tuberous tap root widely distributed in the tropical, subtropical and temperate regions of the world [5] The plant is consumed as vegetable as it is believed to be a rich source of vitamins, minerals, protein and carbohydrate [6]. It has been shown to contain a large number of compounds such as flavonoids, saponins, steroids and alkaloids [7] Traditionally, the plant has been evaluated for its hepatoprotective, anti-diabetic, diuretic, anti-inflammatory, antibacterial, antiviral and cancer chemopreventive properties [8].

Infectious diseases pose serious problems to health and they are main causes of morbidity and mortality worldwide [9]. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide many opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and immediate need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [10]. Therefore, scientists are increasingly turning their attention to for medicine, looking for new leads to develop better drugs against microbial infections [11]. The increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity [12].

Present days, secondary plant metabolites (phytochemicals), previously with unknown biological activities, have been extensively investigated as a source of medicinal agents [13]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficiency will be used for the treatment of bacterial infections. [14]. Since immortal, man has used various parts of the plants in the treatment and prevention of various ailments [15]. About 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine. Boerhavia diffusa is one of the most widely used plants.

The aim of the present study was to evaluate the preliminary phytochemical screening and antimicrobial potential of different solvent extracts of B. diffusa roots on several Gram-positive and Gram-negative microorganisms of clinical importance.

METHODS AND MATERIAL:

Plant Material
B. diffusa whole plant was collected, authenticated and deposited in the department itself. All the laboratory works are done in Microlabs, Institute of Research and Technology, Arcot, Tamil Nadu India. The plants washed with fresh water and dried under shade at room temperature, cut into small pieces and powdered in a mixer grinder. The roots were powdered and stored in sterile containers for further use. Then this powdered samples (100g/100ml) in ethanol methanol, hexane, chloroform and acetone for over night at room temperature. Soxhlet apparatus are used for this extraction [16]. The extract from three consecutive soaking are pooled and evaporated under pressure.

The crude samples were subjected to phytochemical screening for the presence of amino acids, proteins, anthraquinones, saponins, triterpenoids, flavonoids, carboxydrates, alkaloids, phytosterols, glycosidal sugars, tannins, phenols and furanoids using the method of Harborne [18].

Phytochemical analysis:
The extracted samples were stirred with diluted HCl and filtered. This filtrate was tested carefully and used for compound analysis. In this Alkaloids (Mayer’s test), Carbohydrates and Glycosides (Molish test), Saponins (Chloroform and H2SO4 test), Protein and amino acid (Millon’s Test), Phytosterols (Libermann- Burchard’s Test), Phenolic compound and Tannin (Ferric chloride test and Lead acetate test) Adopting the Procedures Described by Stephen [19] and Parekh and Chanda [20] were analyzed.

Antimicrobial Activity:
The Antimicrobial activity was determined by well diffusion method. 21, 23, 24 Muller Hinton Agar (MHA) was (3.1 g/100 ml) weighed and dissolved in 100 ml of distilled water in a sterile conical flask and Potato agar (PDA) with Lawn culture using desire test organism. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into the sterile Petri plate. The inoculated plates were kept aside for few minutes, using well cutter. Two wells are made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 20 µl of different solvents with selected B. diffusa roots extract was added in to one well and in another well the same volume of correspondent controls.

The plate was incubated at 37°C for overnight. The microbial growth was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of roots (B. diffusa) extracts.
RESULT AND DISCUSSION: The results of qualitative analysis of phytochemical present in the ethanol, methanol, chloroform, hexane and acetone extracts of B. diffusa were presented in [Table 1].

In extracts obtained using ethanol showed a max activity against pathogen like Micrococcus luteus(10mm), Klebsiella pneumoniae(15mm), Pseudomonas aeruginosa(5mm), Bacillus cereus (13mm) and Staphylococcus aureus (10mm) and minimum activity against pathogen like E. coli. In hexane extract showed a maximum activity against Micrococcus luteus(10mm) minimum activity in Staphylococcus aureus (7mm) and Bacillus cereus(7mm) it has no activity against the pathogens like, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli.

Table 1: Preliminary phytochemical analysis of B. diffusa L.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Phytochemicals</th>
<th>Test performed</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Hexane extract</th>
<th>Chloroform extract</th>
<th>Acetone extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>Molish test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>Chloroform and H2SO4 test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>Millon’s Test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Proteins</td>
<td>Perfumed chloride test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phytosterols</td>
<td>Lieberman-Burchard’s Test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic compounds</td>
<td>Feric chloride test and Lead acetate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
<td>Noller’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Tannins</td>
<td>Neutral FeCl3</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

In the present study, preliminary phytochemical analysis revealed the presence or absence of various phytochemicals qualitatively in ethanol, methanol, chloroform and Acetone extracts except Hexane. Saponins were absent in methanol and hexane extracts. Glycosides were present in all solvent extracts. Proteins and amino acids were extracted in all solvents used except chloroform and hexane. Sterols were absent in hexane extract. Phenolic compounds were present in all solvent extracts. Flavonoids were found in all solvent extracts. Triterpenoids were extracted in ethanol, methanol and acetone solvent. Tannins were not found in hexane and methanol solvent extracts.

Their antibacterial potency was assessed by the presence or absence of inhibition zones and zone diameters (mm). The Acetone extract of B. diffusa showed the maximum antimicrobial activity against Gram-positive bacteria like S. aureus (11mm), Bacillus cereus (11mm) and M. luteus (19mm), and Gram-negative bacteria like E. coli (18mm), K. pneumoniae (20mm) and p. aeruginosa (21mm).

Table 2: Antibacterial activity of Boerhaavia diffusa extracts against selected bacterial pathogens

<table>
<thead>
<tr>
<th>Bacterial pathogens</th>
<th>Control</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>No zone</td>
<td>9.0±0.50</td>
<td>7.3±0.31</td>
<td>7.0±0.15</td>
<td>7.2±0.25</td>
<td>7.7±0.25</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>No zone</td>
<td>10.3±0.20</td>
<td>13.0±0.50</td>
<td>9.7±0.31</td>
<td>11.3±0.25</td>
<td>10.7±0.25</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>No zone</td>
<td>12.0±0.50</td>
<td>19.7±0.45</td>
<td>9.9±0.45</td>
<td>5.7±0.12</td>
<td>18.5±0.50</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>No zone</td>
<td>11.7±0.28</td>
<td>15.3±0.61</td>
<td>No zone</td>
<td>No zone</td>
<td>20.0±0.50</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>No zone</td>
<td>18.0±0.50</td>
<td>15.0±0.60</td>
<td>No zone</td>
<td>No zone</td>
<td>20.8±0.29</td>
</tr>
<tr>
<td>E. coli</td>
<td>No zone</td>
<td>4.8±0.41</td>
<td>6.7±0.25</td>
<td>No zone</td>
<td>No zone</td>
<td>17.7±0.25</td>
</tr>
</tbody>
</table>

In Table II The Boerhaavia diffusa extracts showed varied in the exploitation of antibacterial activity of zone of inhibition from 5-21 mm against all tested bacteria. In methanol extract showed a maximum activity against Pseudomonas aeruginosa(18mm) Micrococcus luteus (12mm), Klebsiella pneumoniae(11mm), Bacillus cereus (10mm) and Staphylococcus aureus (9mm) minimum activity in E. coli.

In extracts using methanol showed a max activity against pathogen like Micrococcus luteus(20mm), Klebsiella pneumoniae (15mm), Pseudomonas aeruginosa(15mm), Bacillus cereus (13mm) and Staphylococcus aureus (10mm) and minimum activity against pathogen like E. coli. In hexane extract showed a maximum activity against Micrococcus luteus(10mm) minimum activity in Staphylococcus aureus (7mm) and Bacillus cereus(7mm) it has no activity against the pathogens like, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. coli.

Table 3: Antifungal activity of Boerhaavia diffusa extracts with different solvents

<table>
<thead>
<tr>
<th>Fungal pathogens</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>6.0±0.50</td>
<td>6.1±0.36</td>
<td>No zone</td>
<td>No zone</td>
<td>No zone</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>No zone</td>
<td>3.9±0.45</td>
<td>4.0±0.15</td>
<td>7.0±0.50</td>
<td>4.5±0.50</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>6.0±0.20</td>
<td>7.1±0.36</td>
<td>5.0±0.50</td>
<td>5.0±0.50</td>
<td>10.0±0.50</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>15.0±0.40</td>
<td>20.0±0.50</td>
<td>No zone</td>
<td>No zone</td>
<td>8.1±0.36</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>5.0±0.20</td>
<td>8.0±0.20</td>
<td>No zone</td>
<td>No zone</td>
<td>7.0±0.20</td>
</tr>
</tbody>
</table>

Different superscripts are significantly different at p<0.05 Level (least significance Difference) mean followed by ± S.D.

In Table III, The Boerhaavia diffusa extracts are obtained from five different solvents like methanol, ethanol, hexane, chloroform and acetone and various anti fungal activities was observed. In Methanol extract, the maximum activities were seen in Aspergillus flavus (15mm) and Aspergillus niger (7mm) minimum activities are seen in C. albicans (5mm) and Aspergillus fumigatus (6mm) no activities in C. tropicalis. In Ethanol extracts the maximum activity is seen Aspergillus flavus (20mm) and Aspergillus fumigatus (8mm) minimum activities are seen in C. albicans (6mm), C. tropicalis (4mm), Aspergillus niger (7mm).

Comparison of controls with the solvent extracts of B. diffusa revealed that the plant extracts are more effective towards pathogenic organisms. The results of present antimi- crobial study revealed that acetone, methanol and ethanol extract showed more activity towards human pathogenic organisms.

Stainer et al [28] stated that the some of the solvent extracts that were ineffective in their study did not possess antibiotic properties or the plant extracts might have contained antimicrobial constituents just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in some solvents. The present study also agreed with above said statement and the antibacterial activity of B. diffusa plants. The results were correlated with the other medicinal plants antimicrobial activities [29, 30].

Conclusion Based on the results of the present study it is concluded that the B. diffusa plants have potent antimicrobial activity against various Bacteria and fungi which might be due to the phytochemicals present in the plants. Also, there is further scope to study the identification and purification of active compound(s) involved in this antimicrobial activity of B. diffusa.

REFERENCES

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