Assessment of antibacterial activity and phytochemical screening of *Hemidesmus indicus* root extracts

Prasanna Purohit¹, Ritu Thakur Bais¹, Pratibha Singh¹, Shagufta Khan²

¹Department of Botany, Sarojini Naidu Govt. Girls P.G. Autonomous College, Shivaji Nagar Bhopal (M.P.)
²Grow Tips Biotech, Bhopal (M.P.)

Abstract

The *Hemidesmus indicus* is used to cure leprosy, leucoderma, itching, skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis, and paralysis. The present study was aimed to investigate the phytochemical and antibacterial activity of *Hemidesmus indicus* root. The root extracts of *Hemidesmus indicus* were prepared using different solvents like petroleum ether, ethanol and distilled water. The phytochemical screening of the root extracts was performed. The presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids were indicated by the test conducted. The antibacterial activity of the ethanol and aqueous extract of *Hemidesmus indicus* root was tested by agar diffusion method. Zones of Inhibition produced by both extract in a dose of 100 and 200 mg/ml against selected strains was measured and compared with those of standard drug ciprofloxacin (10 μg/ml). Both extract recorded significant activity against all the test bacteria. The highest zones of growth inhibition were exhibited by ethanol extract against all the microorganisms compared to be aqueous extract.

1 Introduction

Over the past 2 decades, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. The indigenous system of medicine namely Ayurvedic, Siddha and Unani has been in existence for several centuries¹. This system of medicine supports the need of more than 70% of population residing in the rural areas. Besides the demands made by these systems as their raw materials, the demands of medicinal plants made by the modern pharmaceutical industries have also increased manifold². Since a long period of time, plants have been a valuable source of natural products for maintaining human health and infections control because, microbial infections pose a health problem throughout the world, and plants are a possible source of antimicrobial agents. Many of the herbs and spices used by humans to season food yield useful medicinal compounds. Microbial infections pose a health problem throughout the world, and plants are a possible source of antimicrobial agents. Medicinal plants contain active principles which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections³.

There is a continuous and urgent need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action because there has been a threatening increases in the incidence of unknown and reemerging infectious diseases. Another big concern is the development of resistance to the antibiotics in existing clinical use⁴. The complication of microbial resistance is flourishing and the outlook for the use of antimicrobial drugs in the future is still unconvincing. For that reason actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, by natural.

The concluding goal is to offer appropriate and competent antimicrobial drugs to the patient⁵.

In a constant attempt to improve the quality of life, men have used plants as a source of food, shelter, clothing, medicine, cosmetics,
and for seeking relief from hardship of life. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug. The search for new antibacterial compounds which have different mechanisms of action from those in current use is an alternative way for solving this problem.

*Hemidesmus indicus* R. Br. (Family: Asclepiadaceae), commonly known as Indian sarsaparilla or Anantmool is a slender, laticiferous and twining shrub, occurs over the greater part of India. It is widely recognized in folk medicine and as the ingredient in Ayurvedic and Unani preparations against disease of biliousness, blood diseases, diarrhea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism and gastric disorders. The roots served as the remedy for leprosy, syphilis, leucoderma, asthma, dysentery, fever and blood, kidney and urinary diseases and root extracts have been found to exhibit various pharmacological properties. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. The present study was attempted to substantiate the antibacterial activity and phytochemical screening of *Hemidesmus indicus* root against some Gram-positive and Gram-negative bacteria.

### 2 Materials and method

#### 2.1 Plant material

*Hemidesmus indicus* roots were collected from Sanjeevani botanical garden Bhopal, Madhya Pradesh in the month of January 2012. The collected plant material was identified by the Grow Tips Biotech Research Centre, Bhopal. Roots were washed under the running tap water and dried under shade, and then homogenized to fine powder and stored in air tight container till further use.

#### 2.2 Preparation of extracts

The shade dried, and powdered roots (500g) were subjected to successive extraction in a soxhlet extractor using petroleum ether, ethanol and distilled water. The extracts were filtered, and the filtrates were concentrated under reduced pressure to obtain the extracts as solid residues.

#### 2.3 Preliminary Phytochemical studies

Preliminary phytochemical tests of various extracts of roots powder of *Hemidesmus indicus* were performed for phytochemical analysis of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids.

### 2.3.1 Test for alkaloids

(a) Dragendorff’s test: To 1 ml of the extract, add 1 ml of dragendorff’s reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer’s test: To 1 ml of the extract, add 1 ml of mayer’s reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) Hager’s test: To 1 ml of the extract, add 3 ml of Hager’s reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner’s test: To 1 ml of the extract, add 2 ml of wagner’s reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

### 2.3.2 Test for saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1 cm layer of foam indicates the presence of saponins.

### 2.3.3 Test for Glycosides

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet test: To 1 ml of the test extract, add 1 ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1 gm of powdered drug is extracted with 10 ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10 ml of water and 0.5 ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5 ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3 ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2 ml of concentrated sulphuric acid. A reddish brown layer
forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager’s test: Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonial layer shows the presence of anthraquinone glycosides.

2.3.4 Test for carbohydrates and sugars

(a) Molisch’s test: To 2ml of the extract, add 1ml of α-naphthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling’s test: To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars

(c) Benedict’s test: To 5ml of Benedict’s reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.3.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.3.6 Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda’s test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

(e) The extract is treated with concentrated H₂SO₄, formation of yellow or orange colour indicates flavones.

2.3.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H₂SO₄. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.3.8 Test for triterpenoids

Noller’s test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids.

2.4 Test microgranism:

The antibacterial activity of extracts of plants was tested against four species of microorganisms: Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonos aeruginosa.

2.5 Culture medium and inoculum preparation

High sensitivity testing agar (Hi-Media) was used for checking antibacterial activity of ethanol and aqueous extracts of Hemidesmus indicus roots against Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonos aeruginosa. The microbial strains were cultured on the slants in the sterilized Laminar Air Flow from the pure culture. These cultured slants were incubated at 37 °C for bacterial growth for 2–3 days. High sensitivity testing agar was mixed at a concentration of 23.4 g/1000 ml in distilled water and autoclaved at 121 °C for 15 min. A loop full from pure
The antibacterial activity of the ethanol and aqueous extracts of Hemidesmus indicus roots were determined by Agar well diffusion assay. 2.34 gm of high sensitivity testing agar was dissolved in 100 ml of distilled water and autoclaved at 121 °C for 15 min. Before transferring this medium in sterilized petri plates, it was allowed to cool and then was poured into the petri plates and allowed to solidify. After this, it was inoculated with activated culture using sterile cotton swabs. And the wells were created using sterile agar borer and the wells were filled by adding 25 μl of each extracts using micropipette and were incubated at 37 °C for 12–24 h. Three replicates were carried out for extract against each of the test organisms. Simultaneously, addition of the respective solvents instead of extracts was carried out as controls, while Ciprofloxacin (10 μg/ml) was used as a positive control. After incubation, the diameters of the zones of inhibition (ZOI) were measured in millimeters, and the mean values were tabulated.

3 Results and Discussion

The qualitative chemical test of Hemidesmus indicus root powder of various extracts demonstrated the presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids.

The preliminary phytochemical screening of ethanol extract exhibited the presence of alkaloids, glycosides, carbohydrates, polyphenol and saponin. Above mentioned secondary metabolite in ethanol extract were present in aqueous extracts except alkaloids. Petroleum ether extract shows positive results for steroids and terpenoids (table 1).

The ZOI of ethanol and aqueous extracts of Hemidesmus indicus root on gram positive and negative bacteria at different concentrations, by agar diffusion method, was determined to access their antibacterial effect. The ethanol and aqueous extracts of the root of Hemidesmus indicus exhibited moderate to significant antibacterial activity against four tested bacterial organisms as compared to the standard ciprofloxacin (table 2). The highest zones of growth inhibition were exhibited by ethanol extract against all the microorganisms compared to be aqueous extract. The ethanol extract produced a highest mean zone diameter of 19.35±3.28 mm at a dose of 200 mg/ml on S. aureus and lowest zone of growth inhibition was observed on P. aeruginosa, which gave a zone of inhibition measuring 5.34±2.38 mm at a dose of 100 mg/ml. The study revealed that ethanol extract of the crude drug was very much effective at E. coli and B. subtilis (Gram positive bacteria) and moderately effective at P. aeruginosa and S. aureus (Gram-negative bacteria). The aqueous extract of the crude drug was very much effective against B. subtilis and S. aureus and moderately effective at P. aeruginosa and E. coli. Thus assuming the results it is inferred that the ethanol extracts of Hemidesmus indicus root had in-vitro antibacterial property. The findings from this work may add to the overall value of the medicinal potential of Hemidesmus indicus root. Further phytochemical studies are required to determine the purified bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents. The observed activity can confirm the traditional use of the plant in the treatment of infectious diseases.

Table 1: Preliminary Phytochemical screening of different extracts of Hemidesmus indicus root

<table>
<thead>
<tr>
<th>Test</th>
<th>Pet. Ether extract</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+= present, = absent

4 Conclusion

The present study indicates the Hemidesmus indicus root extracts have been great potential as antimicrobial compounds against microorganisms. The presence of the most general phytochemicals might be responsible for their therapeutic effects. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.
Table 2: Antibacterial activity of ethanol and aqueous extracts of *Hemidesmus indicus* root

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Aqueous extract (100 mg/ml)</th>
<th>Ethanol extract (100 mg/ml)</th>
<th>Aqueous extract (200 mg/ml)</th>
<th>Ethanol extract (200 mg/ml)</th>
<th>Ciprofloxacin (10 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>4.37±2.34</td>
<td>4.34±2.38</td>
<td>8.23±3.37</td>
<td>9.52±5.64</td>
<td>19.64±3.47</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>9.16±4.51</td>
<td>10.35±2.71</td>
<td>13.27±5.24</td>
<td>16.39±3.14</td>
<td>27.15±2.78</td>
</tr>
</tbody>
</table>

Values are mean inhibition zone (mm) ± S.D of three replicates

5 References

16. Taylor RSL, Manandhar NP, Hudson JB, Towers GHN. Screening of selected medicinal plants of Nepal for...