Antibacterial and Phytochemical Screening of Ethanol Extract of *Hemidesmus indicus* roots

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**Abstract**

*Hemidesmus indicus* belongs to the family Asclepiadaceae, and is one of the rare medicinal and aromatic plants of India. The *Hemidesmus indicus* recommended for the treatment of various diseases namely skin disease, liver impairment, leprosy, dysentery, bronchitis etc by tribal people. The present study was aimed to investigate the phytochemical and antibacterial activity of ethanol extracts of *Hemidesmus indicus* root. The ethanol extract of *Hemidesmus indicus* root was prepared and phytochemical screening was performed. The findings indicate the presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins and terpenoids in extract. The antibacterial activity of various concentrations (25%, 50%, 75% and 100%) of ethanol extract was tested by disk diffusion method against *E. coli*, *S. aureus*, *S. epidermis*, *B. subtilis*, *P. aeruginosa* and *S. flexneri*. Zones of Inhibition produced by extract at various concentrations against selected strains were measured. The highest zones of growth inhibition were observed against *B. subtilis* compared to other microorganism. It produced a mean zone diameter of 23.5 mm at a dose of 100% on *B. subtilis* and lowest zone of growth inhibition was observed on *S. epidermis*, which gave a zone of inhibition measuring 9.8 mm.

**Keywords:** Hemidesmus indicus, Ethanol extract, Phytochemical screening, Antimicrobial activity

1 Introduction

The increasing prevalence of multidrug resistant strains of microbes and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable microbial infections and makes it an urgent task to the search for new infection-fighting strategies. For this reason many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals, and plants. Fungi are also responsible for a number of diseases in humans and a number of fungal species work in association with some bacteria species; among them *Candida albicans* is responsible for a number of diseases¹⁻³.

In addition, the increasing occurrence of opportunistic systemic mycoses associated with the use of immunosuppressive drugs and AIDS has led to new efforts in the search for novel antifungal compounds. At the same time, there is continuing interest in the discovery of antifungal agents, which are effective against plant pathogenic fungi, and the plant kingdom has proved to be a useful source of lead compounds of novel structure with antifungal activity⁴⁻⁶.

*Hemidesmus indicus* commonly known as ‘Indian Sarsaparilla’ is an aromatic medicinal twining shrub distributed in moist localities of India and Sri Lanka⁶. *Hemidesmus indicus* is a pharmacologically important plant belonging to the Asclepiadaceae family or the milk weed or calotropis family includes 320 genera and 1,700 species of world wide distribution but most abundant in the subtropics and tropics⁷. 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 disorders⁸. 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 properties⁹. Herbal medicines generally have fewer side effects than synthetic
compounds, and their effectiveness can be improved by modern pharmacological methods.

It mainly comprises saponins, tannins, hemidesmine, hemidesmol, hemidesterol, stearoptin, pregnane glycosides, β-sitosterol, indicusin, coumarin, volatile oils, triterpines, flavonoids. Syrup prepared from the roots is used as a flavoring agent and in the preparation of a sherbet which have cooling properties.

The present study was to evaluate the antibacterial activity of ethanol extracts of *Hemidesmus indicus* against several Gram positive and Gram negative bacterial strains in vitro i.e. *E. coli*, *S. aureus*, *S. epidermis*, *B. subtilis*, *P. aeruginosa* and *S. flexneri*.

2 Materials and Methods

2.1 Plant material

*Hemidesmus indicus* roots were collected from Sanjeevani botanial 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 ethanol. 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 ethanol 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 3ml 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 1cm 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 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml 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) Bomtrager’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 ammonical 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 a-napthol 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.

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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 flavonoids.

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

(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 microorganisms

The antibacterial activity of ethanol extracts was tested against different species of microorganisms: E. coli, S. aureus, S. epidermis, B. subtilis, P. aeruginosa and S. flexneri. 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 culture of a bacterial strain was mixed in the 10 ml of Nutrient broth medium and incubated at 37 °C overnight and the activated culture was used for streaking onto the agar plates for antimicrobial sensitivity.

2.4.2 Preparation of the media

The nutrient agar was used as a growth medium for microbes. 38g of the agar was dissolved in 1000ml of distilled water in a conical flask with swirling to homogenize. The flask was cotton plugged and sterilized by means of autoclave at 121 °C for about 15 minutes. 20 ml of the agar solution was dispensed into sterilized petridishes near a gas flame in order to prevent contamination after which plates were covered and allowed to gel.

2.4.3 Sensitivity test for ethanol extract

The prepared plates were inoculated with the test organisms (E. coli, S. aureus, S. epidermis, B. subtilis, P. aeruginosa and S. flexneri) by using pour plate technique in a laminar air flow chamber.

2.4.4 Antibacterial screening test

The crude extract was screened for antimicrobial activity using the disk diffusion method developed (Ericson et al 1960).

Petri plates containing 20 ml of agar medium were seeded with a 24 h culture of the bacterial strains. In each plate, hole of 6-mm diameter was made using a sterile borer. The discs (6mm in diameter) were impregnated into plant extracts at various concentrations (25%, 50%, 75% and 100%) separately and placed on the inoculated agar. The inoculum size was adjusted so as to deliver a final inoculum of approximately 108 colony-forming units (CFU)/ml. Incubation was performed for bacteria and fungus at 37 °C for 24 h and 37 °C for 72 h respectively. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well. The zone of inhibition was measured by using transparent plastic ruler, and the mean values were tabulated.

3 Results

3.1 Phytochemical screening

The qualitative chemical test of Hemidesmus indicus root powder of ethanol extract 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 (table 1). The ethanol extract contains maximum number of...
phytoconstituents along with flavonoids, phenolic content, sapoins etc and it imparts antibacterial activity. Hence ethanol extracts were selected for antibacterial activity\textsuperscript{17}.

Table 1 Qualitative analysis of phytochemical constituents of ethanol extract of *Hemidesmus indicus*

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Ethanol Extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>-</td>
</tr>
</tbody>
</table>

- = Negative; + = Positive

3.2 Disc diffusion antibacterial bioassay

The antimicrobial activity of ethanol extract was reported in this investigation, we report that the ethanol extracts are potential antimicrobial agents that can be purified and employed as broad spectrum antibiotics. The extracts were active (weak to moderate) against all the microbes tested. The highest zones of growth inhibition were observed against *B. subtilis* compared to other microorganism. It produced a mean zone diameter of 23.5±2.31mm at a dose of 100% on *B. subtilis* and lowest zone of growth inhibition was observed on *S. epidermis*, which gave a zone of inhibition measuring 9.8±1.73 mm (Table 2).

The ethanol extract of *Hemidesmus indicus* roots showed varying amounts of inhibitions against *E. coli*, *S. aureus*, *S. epidermis*, *B. subtilis*, *P. aeruginosa* and *S. flexneri* and compared with each other.

The antibacterial activity of *Hemidesmus indicus* root extracts might be due to the presence of β-sitosterol, α and β amyrins, lupeol, tetracyclic triterpenes and tannins. The findings of antibacterial activity of *Hemidesmus indicus* root exhibited that the *B. subtilis* are more sensitive to the secondary metabolites present in the extracts.

Table 2: Sensitivity test on organisms of ethanol extracts at four different concentrations

<table>
<thead>
<tr>
<th>Concentration of ethanol extract</th>
<th>Zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td><em>S. epidermis</em></td>
</tr>
<tr>
<td>25%</td>
<td>1.8±1.25</td>
</tr>
<tr>
<td>50%</td>
<td>3.4±1.93</td>
</tr>
<tr>
<td>75%</td>
<td>7.1±2.06</td>
</tr>
<tr>
<td>100%</td>
<td>9.8±1.73</td>
</tr>
</tbody>
</table>

4 Discussions

The ethanol extract of *Hemidesmus indicus* roots shows varying amounts of inhibitions against *E. coli*, *S. aureus*, *S. epidermis*, *B. subtilis*, *P. aeruginosa* and *S. flexneri* and compared with each other. The antibacterial activity of *Hemidesmus indicus* root extracts might be due to the presence of β-sitosterol, α and β amyrins, lupeol, tetracyclic triterpenes and tannins. The findings of antibacterial activity of *Hemidesmus indicus* root exhibited that the *B. subtilis* are more sensitive to the secondary metabolites present in the extracts. Antibacterial activity was carried out by Naovi et al. (1991)\textsuperscript{19} and Prasad et al. (1983)\textsuperscript{20} and reported antibacterial activity against these bacteria’s.

Ahmad and Beg (2001)\textsuperscript{21} reported ethanol extract shows antimicrobial activity against yeast *Candida species*. These observations may be attributed to the nature of biologically active components tannins, flavonoids, terpenoids, glycosides and phlobatannins which could be more enhanced in the presence of ethanol as that of water. These micro-organisms are causative agents for several illness for which the plant is traditionally reported to remedy.

5 Conclusions

The present study indicates the *Hemidesmus indicus* root extracts have great potential antimicrobial compounds against microorganisms. The presence of the most general phytochemicals...
might be responsible for their antimicrobial effects. The inhibitory activity of these extracts suggests that the traditional medicinal use of *Hemidesmus indicus* should be continued and scientific evaluation of its active constituents given serious considerations. Thus, the study ascertains the value of plants used in Ayurveda, which could be of considerable interest to the development of new drugs.

6 Competing interests

There are no conflicts of interests.

7 Author's contributions

The present research work was carried out by equal contribution of both the authors. PP, RTB, PS and SK designed the experimental protocol, carried out literature review and draft the manuscript. SK participated in collection of data through experimental work. The final draft of the manuscript was reviewed and edited under the guidance of RTB. All authors read and approved the final manuscript.

8 References


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