

Antibacterial activity and phytochemical screening of *Hemidesmus indicus* L. B. BR.

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ABSTRACT

The paper deals with the significance of these roots in traditional medical systems with respect to their antimicrobial and photochemical. The qualitative analysis showed the presence of various phytochemicals like flavonoids, alkaloids, phenols, saponins, tannins. Methanol extracts of the dried root *Hemidesmus indicus* (L.) R.Br. (Indian sarsaparilla) were analysed for their physiochemical constituents. Further study some of these phytochemicals were also quantitatively estimated. The antibacterial properties of Methonal Extracts root material on agar plates was investigated in the present study. The antibacterial activity of Plant Extract was evaluated against on Gram Positive and negative bacteria strains.

Keywords: *Hemidesmus indicus*, Asclepiadaceous, Antibacterial activity, qualitative photochemical analysis.

INTRODUCTION

Hemidesmus indicus (L.) R.Br. Commonly known as Indian sarsaparilla. It belongs to the family Asclepiadaceous. It is a perennial climber and growing widely in upper Gangetic plains and Eastwards of Bengal and from Central to South India. The roots and woody portion has been used traditionally for curing various ailments like stomach pain, fever, venereal diseases, rheumatism and also act as blood purifier. It serves as an alternative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases and urinary infections. It is a perennial, slender, laticiferous, twining or prostrate, wiry, shrub with a woody root-stock, commonly called anantamul in Sanskrit, and is a well known medicinal plant, widely used in Ayurveda, Siddha and Unani systems of medicine, for a variety of diseases including rheumatism, leprosy, impotence and skin infections. The root has a sweet taste and pleasant smell due to the presence of an essential oil containing p-methoxy salicylic aldehyde as a major constituent. The roots of the plant are used extensively by aboriginals of Orissa state (India) for diarrhea and dysentery¹.

They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; any part of the plant body may contain active components² like bark, leaves, stem, root, flower, fruits, seeds etc. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds which are more frequent in some plant parts than in others³. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region¹. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product.

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Medicinal plants are well known natural sources for the treatment of various diseases since antiquity. Natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity⁴. This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents⁵. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant⁶. In the Present study was phytochemical analysis of antibacterial activity study of the methanol extracts of *Hemidesmus indicus* root was carried out to investigate its activity against several members of the Entero- bacteriaceae, the major causative organisms of bacterial-associated diarrhea.

MATERIALS AND METHODS

Plant Material collection and extract preparation.

Plant Material: Plant material *Hemidesmus indicus* (L.) R.Br was collected in Botanical Garden, Osmania University. A specimen was stored in the departmental herbarium. The shade dried plant material was crushed into fine powder (fig-1).

Preparation of Methanolic Extract of *Hemidesmus indicus* (L.) R.Br: Around 50 gm fresh roots shade dried plant material was powdered and wrapped in muslin cloth. It was extracted by Soxhlet apparatus with 250 ml of methanol. The concentrated extract was kept at 37 °C for the residual solvent to evaporate. The percolation process was continued until the extraction process was completed. The extract was allowed to cool and then poured in to a petri plate, left for drying. The dried extract was scratched and was collected in eppendorf tube and weighed, used for further antimicrobial activity and phytochemical screening.



Figure 1: A-D: A. *Hemidesmus indicus* (L.) R.Br plant, B. *Hemidesmus indicus* roots (up rooted tap root), C. Shade dried roots of *Hemidesmus indicus* D. Roots powdered of *Hemidesmus indicus*.

Antibacterial assay**Diffusion method:**

Antimicrobial activity was carried out using disk diffusion method. Petri plates were prepared with 20 ml of sterile nutrient agar media (NA media). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10min. The tests were conducted at three different concentrations of the crude extracts with three replicates. The loaded discs were placed on the surface of medium and left for room temperature for compound diffusion, negative control was prepared using respective solvent. Streptomycin (10ug/disc) was used as positive control. The plates were incubated for 10 hours at 37°C. Zone of inhibition was recorded in millimeters and the experiment was repeated by three replicates.

Phytochemical analysis**Qualitative analysis:**

Standard screening tests of plant extracts were carried out for different parts of *Hemidesmus indicus*. The crude extracts were screened for presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and phenols.

Test for identification of Alkaloids: The plant extract was prepared (0.5 gm of roots ground in 100 ml of water). It was dissolved in dilute HCl solution and clarified by filtration. The filtrate was tested with Drangendroff's and Mayer's reagent. The treated solution was observed for precipitation.

Test for identification of Flavonoids: Ethyl acetate (5 ml) was added to the plant extract (0.5 gm of roots ground in 100 ml of water). The mixture was shaken and allowed to settle. Production of yellow colour is taken as positive for flavonoids.

Test for identification of Saponins: Roots (0.5 gm) were ground with 100 ml of distilled water and transferred to a test tube. The test tube was shaken vigorously for about 30 sec and allowed to stand in vertical position and observed for 30 min. If a honey comb froth above the surface of the liquid persists after 30 min, it indicates the presence of saponins.

Test for identification of Tannins: The leaf extract was prepared (by grinding 0.5 gm of leaves in 100 ml of water) and clarified by filtration. 10% ferric chloride solution was added to the clear filtrate, and it was observed for a change in colour to blue.

Test for identification of Phenols: The plant extract was taken in a test tube (0.5 gm of roots ground in 100 ml of water) and warmed. To this 2 ml of ferric chloride was added and observed for formation of green or blue colour (Table:II).

RESULTS AND DISCUSSION

Crude methanolic extract of *Hemidesmus indicus* (L.) R.Br were screened for their *in vitro* antimicrobial and Phytochemical analysis. Antimicrobial activity was determined by using agar well diffusion assay. Our results indicated a potent antimicrobial activity of methanolic root extract of *Hemidesmus indicus* (L.) R.Br. As shown in (Table:-I). In the present study methanolic extracts of plant have been tested against resistant bacteria. The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter. The results of antimicrobial activity of *Hemidesmus indicus* (L.) R.Br was encouraging and that the plant extract showed significant antimicrobial activity against different bacterial strains. In the (figure:II), the antimicrobial activity of methanolic extracts of *Hemidesmus indicus* (L.) R.Br (root) against two bacterial strain *Gram Positive is Staphylococcus aureus* at different concentration (5µg/5µl, 10µg/10µl, 15µg/10µl, 20µg/20µl) was found in the following increasing order 9>11> 12>13 of Zone of Inhibition on Gram Positive bacteria determination of antibacterial activity by agar well diffusion assay showed *and Gram Negative E.coli* that methanolic extract of *Hemidesmus indicus* (L.) R.Br root exhibited the 4>7>9>10 Increasing zone of inhibition antibacterial effect against pathogenic as well as non-pathogenic test bacteria. Methanol extract significant effect on growth inhibition of gram positive more than gram negative bacteria was noticed. It was noted that among all tested organisms the gram positive bacterial strain registered maximum susceptibility to the methanolic extract at 20 µg/20µl of the entire *Hemidesmus indicus* (L.) R.Br root used, with the maximum inhibitory zone of 13mm and 17mm and study compared with reference Drug (AMP) Ampicilline (20µg/20µl) respectively.

These differences may be attributed to the fact that while the cell wall in Gram-positive bacteria consist of a single layer that of Gram-negative is a multi-layered and quite complex structure. The results provided evidence that the studied plant might indeed be potential sources of natural antimicrobial agents. Based on these results similar to 7, 8, it is possible to conclude that methanolic extracts of *Hemidesmus indicus* (L.) had different levels of Phytochemicals and antimicrobial activity. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antimicrobial activity. Qualitative analysis of plant extract indicated the presence of alkaloids, flavonoids, phenols and saponins in the roots of *Hemidesmus indicus* and tannins in leaves. These results are similar to 9, 10, 11. Aqueous extracts in the present study were positive for alkaloids in contrast to 9. Review on *Hemidesmus indicus* also confirmed the presence of these compounds 10. *Hemidesmus indicus* is a climbing slender plant with twining woody stem and opposite petiolate leaves, entire, smooth shiny, varying in shape and size according to their age. Flowers are small, in axillary sessile racemes. The root is long, rigid and cylindrical. The study comprises phytochemical analysis studies to develop a good protocol (Table:-II).

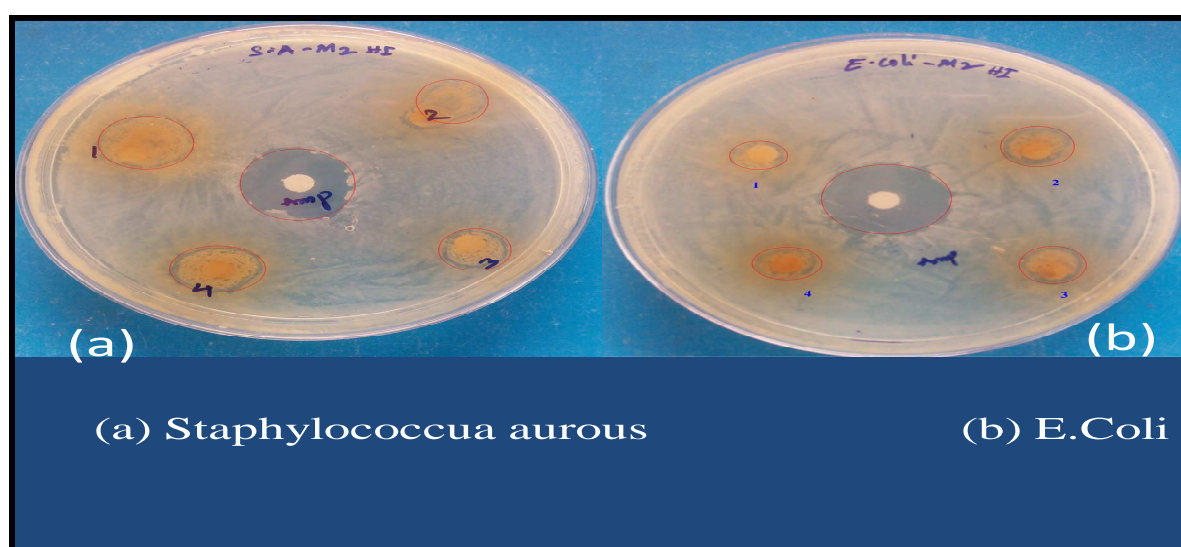


Figure 2: Antimicrobial activity of methanol extracts from *Hemidesmus Indicus* Root. A. *Staphylococcus*

Table:1 Zone of Inhibition Gram Positive and Negative on Methanol Extract from *Hemidesmus Indicus* Root

S.No.	Name of Bacteria s	5 ($\mu\text{g}/\mu\text{l}$)	10 ($\mu\text{g}/\mu\text{l}$)	15 ($\mu\text{g}/\mu\text{l}$)	20 ($\mu\text{g}/\mu\text{l}$)	Ampillicine (10 $\mu\text{g}/\mu\text{l}$)
1	<i>Staphylococcus aureus</i>	9	11	12	13	17
2	<i>E.Coli</i>	4	7	9	10	20

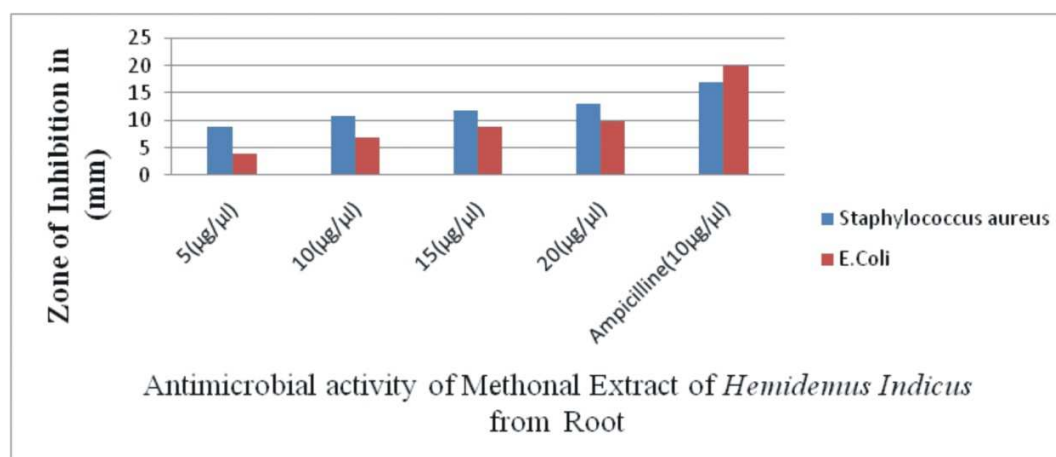


Figure 3: Zone of Inhibition Gram Positive and Negative on Methanol Extract from *Hemidesmus Indicus* Root

Table 2. Qualitative analysis of phytochemical constituents of *Hemidesmus indicus*

S. No.	Plant part	Phytochemical	Presence(+) / Absence(-)
1	Roots	Alkaloids	+
2	Roots	Flavonoids	+
3	Roots	Tannins	+
4	Roots	Saponins	-
5	Roots	Phenols	+

+= Present, - = absent

CONCLUSION

The antimicrobial and phytochemical analysis of *Hemidesmus indicus*. The Present study as situation has forced to search new antimicrobial substances in various sources like medicinal plants. The plant *Hemidesmus indicus* roots shown antimicrobial activity in methanol and aqueous solutions. The methanol solvent shows more inhibition in *Staphylococcus aureus* (gram positive) than *Escherichia coli* (gram negative). Physiochemical analysis of the *Hemidesmus indicus* roots revealed the presence of significant Phytochemicals such as flavonoids, steroids, phenols, alkaloids, and tannins that are therapeutically important. Flavonoids and Alkaloids are the potential phytochemicals which boost the immune system and in nature and particularly useful in maintenance of healthy circulations. The presence of tannins indicates the astringent potential of the roots that protect internal organs of body. Cardiac glycosides supports and strengthens the function of the heart. triterpenoids are used as strong expectorants. Steroids and Saponins are used in hormonal activity. The present study emphasizes the need for intensive research for the development of antimicrobial activity and phytochemicals analysis. pharmaceutical industry for preparation of drugs and stress the need for more intensive research since they play a great role in healthcare.

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