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Screening of phytochemical and antibacterial activity of *Hemidesmus indicus* (L.) medicinal plant

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Abstract
The present paper deals the Phytochemical and antimicrobial study of *Hemidesmus indicus* L. medicinal plant. The solvents used for the extraction of plant roots were ethanol, methanol and distilled water. The in-vitro antibacterial activity was performed by agar well diffusion method. The most susceptible Gram-Positive and Gram-negative bacteria were tested. The extracts of plant *Hemidesmus indicus* (L.) inhibited the growth of the bacterial strains investigated. The most active extracts were compared with the standard antibiotics, penicillin, Streptomycin and Ampicillin 100mg/disc). The results obtained in the present study suggest that preliminary phytochemical analysis detected the presence of Alkaloids, Aminoacid, Flavonoids Saponins and Tannins. The *Hemidesmus indicus* (L.) could be used in treating diseases caused by the test organisms. The results are discussed in detail.

Keywords: medicinal plants, phytochemicals, antibacterial activity, *Hemidesmus indicus* (L.), and Pathogens

1. Introduction
Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. According to World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (WHO, 2008) [1]. In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infectious diseases. These plants are ingested as decoctions, teas or juice preparations (Gonzalez, 1980) [2]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Making antibacterial therapy effective, safe and affordable has been the focus of interest during recent years. There is several reports on antimicrobial activity of different herbal extracts (Nair, *et al.* 2005 and Varsh, *et al.* 2009) [3, 4].
Hemidesmus indicus (L.) (Family: Asclepiadaceae), commonly known as Anantmool is a slender, laticiferous and twining shrub, occurs over the greater part of India (Anonymous, 1997) [5]. It is widely recognized in folk medicine and as 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 root is said to be tonic, diuretic, and alterative. Root decoction helps in skin diseases, syphilis, elephantiasis, loss of appetite, blood purification and for kidney and urinary disorders. Several biological activities like hepatoprotective, antioxidant, antithrombotic, anti-ulcerogenic, anti-inflammatory, immunomodulatory, antidiabetic etc. have been reported from various root extracts (Baheti, *et al.* 2006 and Wadkar, *et al.* 2008) [6, 7].

2. Materials and methods
Plant collection
The *Hemidesmus indicus* is selected for the study from the local area based on the basic information is the available. It is collected from follow land in and around Kothi Compound Rewa (M.P.) brought into the laboratory for further processes. The collected sample was carefully stored in sterile polythene bag and used for the further study.

Sterilization of Plant Materials
The disease-free roots were selected for this investigation. About 2gm dried roots were taken. Then, surface sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again the materials were washed thoroughly with distilled water.

Preparation of Plant Extracts
Two grams of sterilized roots were kept in the 10 ml organic solvents such as Ethanol, Methanol and Aqueous. Then these are grind with the help of mortar and pestle. The grind plant material was subjected to centrifugation, for 10-15min (at 10,000rpm). The supernatant was collected and stored for further purposes.

Preliminary Phytochemical screening
Chemical test were carried out on the aqueous extract and on the powdered specimen using standard procedure to identify the constituents.

Test for flavonoids
1g of the powdered dried leaves of the specimen was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20% sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colorless

solution depicted the presence of flavonoids.

Test for tannins
1g of powder was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10% ferric chloride were observed for any formation of precipitates and any color change. A bluish-black or brownish green precipitate indicated the presence of tannins.

Test for saponins
1g of powder dried stain was separately boiled with 10ml of distilled water for 10minutes. The mixture was filtered while hot and allowed to cool. The following tests were then carried out. Demonstration of frothing**:** 2.5ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2minutes (frothing indicated the presence of saponin in the filtrate).

Test for alkaloids
1g powder sample of specimen was separately boiled with distilled water and 10ml hydrochloric acid on a water bath and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7. A very small quantity of the following reagents was added separately to about 0.5 ml of the filtrate in a different test tube and observed. Picric acid solution. 10% tannic solution. Mayer's reagent (Potassium mercuric iodide solution). The test tubes were observed for coloured precipitates or turbidity.

Test for amino acids
To 2ml of sample added 2ml of ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of aminoacids in the sample.

Selection of microorganisms
Totally five human pathogenic bacteria were selected for the present investigation. Among them, five bacterial strains such as, *Staphylococcus aureus. E. coli, Klebseilla pnemoniae*, *Salmonella typhi* and *Vibrio cholerae.* The human pathogenic bacteria were originally obtained from Microbial Germ Plasm Culture Collection unit Biotech Department of Awadhesh Pratap Singh Univeersity, Rewa (M.P.) and used for present investigation.

Preparation of microbial inoculums
The young microbial inoculum culture was prepared and used during the research period. The nutrient broth (NB) was prepared and poured into several sterilized test tubes. The pure microbial cultures were inoculated. After these tubes were incubated at 37°C for 24-28 hrs. After incubation the cultures were used for the experiments.

Media Preparation
Composition of nutrient agar medium

Peptone	-	5gm
Beef extract	-	3gm
NaCl	-	5 gm
Agar	-	15gm
Distilled water	-	1000ml
pH	-	6.8

Preparation of nutrient agar medium
The ingredients (peptone - 5g; beef extract - 3g; Nacl -15g) were weighed and taken in a conical flask contains 1000ml distilled water. Then pH of the medium was adjusted to 6.8 using a pH meter by the addition of either acid (or) alkali. The flask were sterilized in an autoclave at 121ºC for 15 lbs pressure for 15 minutes and allowed to cool.

Screening for antibacterial activity assay (Agar - well diffusion method)
The antibacterial activities of the roots were tested against the selected bacterial strains. The petriplates were washed and placed in an autoclave for sterilization. After sterilization, nutrient agar medium was poured into each sterile petriplates and allowed to solidity in a laminar air flow chamber. After solidification, using a sterile cotton swabs, fresh bacterial culture with known population count was spread over the plate by spread plate technique. One well of 5mm size made in the agar plates with the help of sterile cork borer, the wells were loaded with 200µl of solvent extract (Ethanol, Methanol and Aqueous) of these root extracts. All the plates were incubated at 37°C for 24-48 hours. After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

Antibiotic sensitivity test on microbes (Positive control)
The antibiotic sensitivity test using standard antibiotics (Tetracycline, Erythromycine and Chloramphenical) were analysed.

Antibacterial effects of solvents (Negative control)
The antimicrobial activities of Ethanol and Methanol solvents were tested against the selected bacterial strains.

3. Results and discussion
In the present investigation, the antibacterial properties and preliminary phytochemical analysis of medicinal plant *Hemidesmus indicus* (L.) is tested against five human pathogenic bacteria. The antibacterial properties of the extracts were also comparatively analyzed against standard antibiotics by antibiotic sensitivity test.

Preliminary phytochemical screening
The most important of these bioactive constituents of plant are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plant in all facet of life have served a valuable starting material for drug development (Edoga, *et al.* 2005) [8]. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa, *et al.* (2007) [9]. Mensah *et al.* (2008) [10] reports alkaloids in 12 leafy vegetables studied. Ayiety-Smith and Addae-Mensah (1977) and Kindra and Satayanaraya (1978) [11, 12] had earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension.
In the present investigation, screening of this plant roots species for phytochemical constituent was performed using generally accepted laboratory technique for qualitative

determinations. The study indicated that Alkaloids, Aminoacid, Flavonoids Saponins and Tanins were present in the plant (Table-1).

Table- 1: Primilinary Phytochemical analysis of *Hemidesmus indicus* (L.)

S. No.	Phytochemicals	Reactions
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+
5.	Aminoacid	+

Antibacterial activity of *Hemidesmus indicus* (L.)

Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use (Sofowora (1993) [13].

The ethanol extract of *Hemidesmus indicus* (L.) exhibited maximum zone of inhibition against *E. coli* (13mm) *Klebseilla pnemoniae*, (11mm), *Salmonella typhi.* (12mm) *Staphylococcus aureus* (10mm) and *Vibrio cholerae*. (12mm).

The methanol extract of *Hemidesmus indicus* (L.) showed maximum zone of inhibition against *E. coli* (11mm) *Klebseilla pnemoniae*, (13mm), *Salmonella typhi.* (9 mm) *Staphylococcus aureus* (11mm) and *Vibrio cholerae*. (12mm). The aqueous extract of *Hemidesmus indicus* (L.) does not showed any activity against selected pathogenic bacteria respectively (Table-2 & Graph-1)

Table 2: Antibacterial activity of *Hemidesmus indicus* (L.)

S. No.	Test organisms (Bacterial pathogens)	Zone of inhibition (diameter in mm)		
		Ethanol	Methanol	Aqueous
1.	*E. coli*	13	11	-
2.	*Klebseilla pnemoniae*	11	13	-
3.	*Salmonella typhi*	12	9	-
4.	*Staphylococcus aureus*	10	11	-
5.	*Vibrio cholera*	12	12	-

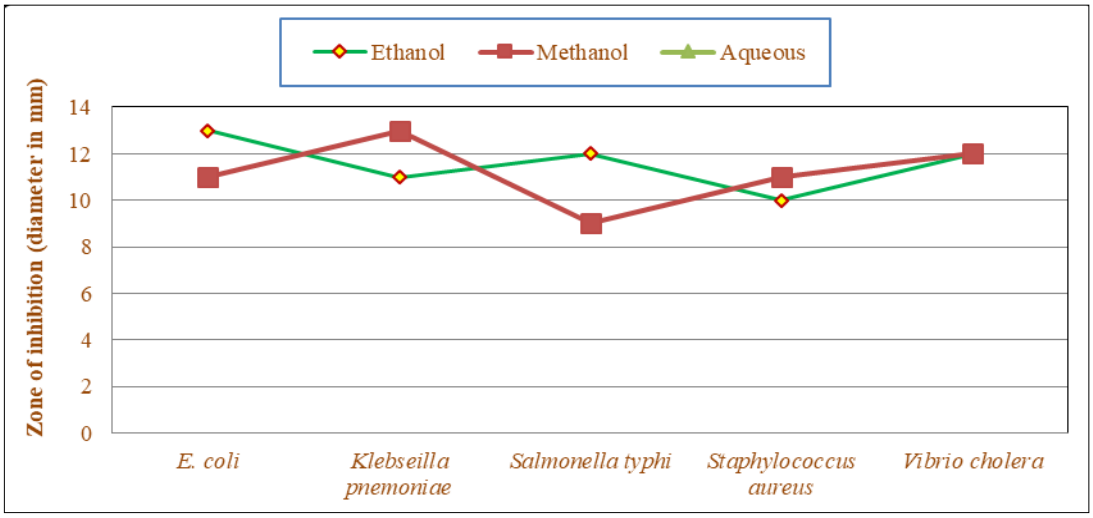

Fig 1: Graphics analysis of Antibacterial activity of *Hemidesmus indicus* (L.)

Antibiotic sensitivity test (Positive control)

The antibiotic sensitivity test using standard antibiotics viz., ampicillin, penicillin and streptomycin were tested against pathogenic bacteria studied. Similarly, when compared to the standard antibiotics, the solvent extracts of *Hemidesmus indicus* (L.) showed lesser antibacterial activity against bacteria.

Antibacterial effect of solvents (Negative control)

The result of antimicrobial effect of ethyl ethanol, methanol and aqueous extract solvents revealed no activity against pathogenic bacteria.

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