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Screening of phytochemical and antibacterial activity of *Hemidesmus indicus* (L.) and *Vetiveria zizanoides* (L.)

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ABSTRACT

The screening and study of selected Indian medicinal plants Hemidesmus indicus (L.) and Vetiveria zizanoides (L.) were selected for phytochemical screening and antibacterial studies. The solvents used for the extraction of plant roots were ethanol,methanol and distilled water. The invitro antibacterial activity was performed by agar well diffusion method. The most susceptible Gram-Postive and Gram-negative bacteria were tested. The extracts of plant Hemidesmus indicus (L.) and Vetiveria zizanoides (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 Tanins. The Hemidesmus indicus (L.) and Vetiveria zizanoides (L.) could be used in treating diseases caused by the test organisms. The results are discussed in detail.

Keywords: Medicinal plants, Phytochemicals, Antibacterial activity, *Vetiveria zizanioides* (L.), *Hemidesmus indicus* (L.), and Pathogens.

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 [15]. 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 [7]. 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 [11, 13]. The following plants were selected for investigation present *Vetiveria zizanioides* (L.) and *Hemidesmus indicus* (L.)

Vetiveria zizanioides (L.) belonging to the family Poaceae and commonly known as 'Khas-Khas' in Bangladesh and India. It is a perennial grass with thick fibrous adventitious roots [6]. This species is native of Indian subcontinent and has been introduced in many tropical countries. Roots are stimulant, tonic, cooling, stomachic, diuretic, and antispasmodic and emmenagogue, and used in fevers, inflammations and irritability of stomach. Essence of the root is used to check vomiting in cholera. Smoke of grass is inhaled to relieve headache [6]. Apart from its use as insect repellent and soil erosion management tool, vetiver grass has numerous traditional uses such as root paste for headaches and leaf paste for rheumatism and sprains. Commercial uses of vetiver grass mainly pertain to the extraction of vetiver oil through distillation of the roots.

Hemidesmus indicus (L.) (Family: Asclepiadaceae), commonly known as Indian sarsaparilla or Anantmool is a slender, laticiferous and twining shrub, occurs over the greater part of India [1]. 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, antiinflammatory, immunomodulatory, antidiabetic etc. have been reported from various root extracts [3, 14].

MATERIALS AND METHODS

Plant collection

The following medicinal plants were selected for the study from the local area based on their basic information in the available. The Medicinal Plants *Hemidesmus indicus* (L.) and *Vetiveria zizanoides* (L.) were collected from follow land in and arround orathanadu, Thanjavur (Dt) brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags 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

1 g of the powdered dried leaves of each 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

1 g of each powdered sample 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

1 g of each powdered 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**:** 2.5 ml 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
1 g of powdered sample of each specimen was separately boiled with distilled water and 10 ml 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 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 (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur 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^{o}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^{0} 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.

RESULTS AND DISCUSSION

In the present investigation, the antibacterial properties and preliminary phytochemical analysis of two medicinal plants viz.., *Hemidesmus indicus* (L.) and *Vetiveria zizanoides* (L.) were 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 plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served a valuable starting material for drug development [5]. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by [9]. [10] reports alkaloids in 12 leafy vegetables studied. [2,8] had earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension.

In the present investigation, screening of these two different plant roots species namely *Hemidesmus indicus* (L.) and *Vetiveria zizanoides* (L.) 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 plants (Table-1).

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 [12].

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

The methanol extract of *Hemidesmus indicus* (L.) showed maximum zone of inhibition against *E.coli* (10mm) *Klebseilla pnemoniae,* (12mm), *Salmonella typhi.* (10 mm) *Staphylococcus aureus* (11mm) and *Vibrio cholerae*. (10mm). The aqueous extract of *Hemidesmus indicus* (L.) does not showed any activity against selected pathogenic bacteria respectively (Table-2& Fig-1)

Antibacterial activity of *Vetiveria zizanoides* (L.)

[4] have reported the antibacterial properties of tannins. Phlobatannins, cardiac glycosides, combined anthraquinones, free anthraquinones carotenoids and steroids are absent in all the seeds. The ethanol extract of *Vetiveria zizanoides* (L.) exhibited maximum zone of inhibition against *E.coli* (11mm) *Klebseilla pnemoniae,* (10mm), *Salmonella typhi.* (13 mm) *Staphylococcus aureus* (10mm) and *Vibrio cholera* (10mm).

The methanol extract of *Vetiveria zizanoides* (L.) showed maximum zone of inhibition against *E.coli* (9mm) *Klebseilla pnemoniae,* (10mm), *Salmonella typhi.* (8.5 mm) *Staphylococcus aureus* (10mm) and *Vibrio cholerae*. (10mm). The aqueous extract of *Vetiveria zizanoides* (L.) does not showed any activity against selected pathogenic bacteria respectively (Table-3 &Fig-2).

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.) and *Vetiveria zizanoides* (L.) howed 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.

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

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

Table -2: Antibacterial activity of *Vetiveria zizanoides* (L.)

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

Fig-1: Antibacterial activity of *Vetiveria zizanoides* (L.)

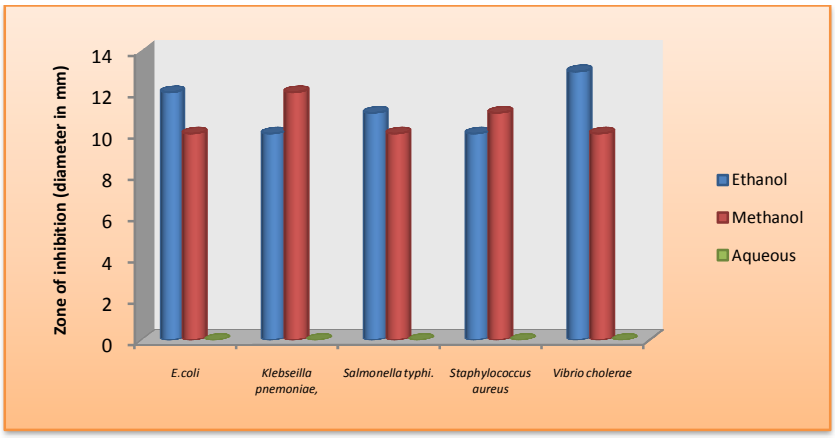

Fig-2: Antibacterial activity of *Hemidesmus indicus* (L.)

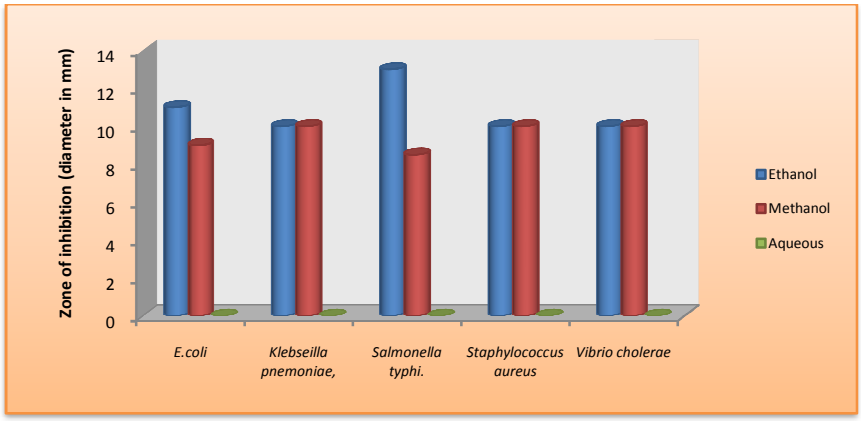

Table -3: Antibacterial activity of *Hemidesmus indicus* (L.)

S.No.	Test Organisms (Bacterial pathogens)	Zone of inhibition (diameter in mm)		
		Ethanol	**Methanol**	**Aqueous**
1.	*E.coli*	11	9	-
2.	*Klebseilla pnemoniae,*	10	10	-
3.	*Salmonella typhi.*	13	8.5	-
4.	*Staphylococcus aureus*	10	10	-
5.	*Vibrio cholerae*	10	10	-

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