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ANTIMICROBIAL ACTIVITY AND QUALITATIVE ESTIMATION OF PHYTOCHEMICALS PRESENT IN *HEMIDESMUS INDICUS*

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ABSTRACT

Hemidesmus indicus – Anantamool, is a plant species of Apocynaceae family commonly found in India, specially in different areas of West Bengal. It is a slender, laticiferous, semi-erect endangered shrub. Roots are woody and aromatic as it contain camphor like substances and it is known for its immense medicinal values. Antimicrobial activity of different plant part extracts were checked against *Escherichia coli*, *Bacillus* sp., *Aspergillus niger* and *Curvularia lunata*. The present study was conducted to quantitatively evaluate the marked presence of different phytochemicals available from acetone and ethyl acetate extracts of leaf, stem and root parts of *H. indicus* which were tested according to standardized protocols and showed to contain large amount of Flavonoid, Steroid, Phenol, Terpenoid, Anthraquinone, Cardiac glycoside, Amino acid, Tannin in *H. indicus* leaf, stem and root; which probably supports its antimicrobial and antifungal activity.

KEYWORDS: Antimicrobial activity, *Hemidesmus indicus*, Phytochemicals.

INTRODUCTION

Hemidesmus indicus – Anantamool, is a plant species of Apocynaceae family commonly found in India, specially in different areas of West Bengal.[1] It is a slender, laticiferous, semi-erect endangered shrub. Roots are woody and aromatic as it contain camphor like substances and is known for its immense medicinal values. Traditional uses of *H. indicus* for dyspepsia, appetiser, diarrhoea, epilepsy, bronchitis, leprosy, lucoderma, skin diseases, anthelmintic property and ayurvedic references are reason for taking up this study on *H. indicus*.[2]

MATERIALS AND METHODS

Plant collection

The plant selected for the study was collected from the area of Golapbag, Burdwan. The collected samples were carefully stored in sterile polythene bags and used for the further study.

Sterilization of Plant Materials

The disease free roots, stems and leaves were selected for this study. About 1 gm of dried roots, stems and leaves were taken. Then, surface sterilized with Tween 20 for 5 min, followed by 70% ethanol for 30 sec and 0.1% mercuric chloride for two minutes. Again the materials were washed thoroughly with distilled water.

Preparation of Plant Extracts

One gm of sterilized roots, stems and leaves respectively were dissolved in 10 ml distilled water, methanol, acetone and ethyl acetate. Then these were grind with the help of mortar and pestle. The grind plant material was subjected to centrifugation for 10 min at 10,000 rpm. The supernatant was collected and stored for further studies.

Screening for Antibacterial Activity

The antibacterial activities of the roots, stems and leaves were tested against the selected bacterial strains *E. coli* and *Bacillus* sp. Nutrient agar medium was poured into each sterile petriplates and allowed to solidity. After solidification, using a sterile cotton swab, fresh bacterial culture with known population count was spread over the plate by spread plate technique. One well of 1 cm size made in the agar plates with the help of sterile cork borer, the wells were loaded with 400µl of distilled water, methanol, acetone and ethyl acetate solvent extracts. All the plates were incubated at 37ºC for 24 h. After incubation, the plates were observed for zone of inhibition around the wells, indicating the presence of antibacterial activity.[3], [4]

Screening for Antifungal Activity (Poisoned food technique)

The antifungal activities of the roots, stems and leaves were tested against the selected fungi *Aspergillus niger* and *Curvularia lunata*. Potato dextrose agar medium was poured into each sterile petriplates and allowed to solidity. After solidification, using a sterile inoculating loop, fungi were respectively inoculated in plate by poisoned food technique with 1ml plant extract. All the plates were incubated at 30ºC for 96 h.

Preliminary screening of phytochemical content

Qualitative estimation of phytochemicals present in *H. indicus* roots, stems and leaves ethyl acetate and acetone extracts were done according to the standardized protocols.

RESULTS AND DISCUSSION

The maximum zone of inhibition was seen against *E. coli* in ethyl acetate extract of leaf, stem and root of *H. indicus* and *Bacillus* sp. against acetone extract of leaf, stem and root (**Figs. 1 and 2)**. Whereas no fungal growth was observed for *C. lunata* in ethyl acetate extract of leaf, stem and root compared to control. Also after 96 h of observation, no remarkable growth observed on *A. niger* against leaf, stem and root methanolic extract separately and *A. niger* had shown to loss its spore forming capacity for transmission (**Table 1**).

Table 1. Antimicrobial activity present in different plant extracts (Distilled water extracts of all *H. indicus* leaves, stems and roots showed negative result)

***H. indicus* extracts**	***E. coli***	***Bacillus* sp.**	***A. niger***	***C. lunata***
Methanol leaves	+	+	+	+
Methanol stems	-	-	+	-
Methanol roots	-	-	-	+
Acetone leaves	-	+	-	-
Acetone stems	-	+	-	-
Acetone roots	+	+	-	-
Ethyl acetate leaves	+	-	-	+
Ethyl acetate stems	+	-	-	+
Ethyl acetate roots	+	-	+	+

The qualitative estimation of phytochemicals showed to contain large amount of Flavonoid, Steroid, Phenol, Terpenoid, Anthraquinone, Cardiac glycoside, Amino acid, Tannin in *H. indicus* leaf, stem and root; which probably conferring its antimicrobial activity (**Table 2**).[5]

Table 2. Qualitative estimation of phytochemicals present in leaf, stem and root extracts of *H. indicus* (where - = absent, + = little amount, ++ = moderate amount, +++ = higher amount)

Plant extract	**Fixed Oil (Spot test)**	**Flavonoid (10% NaOH test)**	**Protein (Biuret test)**	**Anthraquinone (Borntrager test)**	**Cardiac Glycoside (Keller-Killiani test)**	**Steroid (Salkowski test)**	**Terpenoid (Salkowski test)**	**Phenol ($FeCl_3$ test)**	**Tannin (Braymer test)**	**Amino acid (Ninhydrin test)**
Ethyl acetate leaf	-	+++	+	-	++	++	-	-	++	+
Ethyl acetate	-	+	-	-	+	+	+	-	+	++

stem										
Ethyl acetate root	+	-	-	++	+	-	+	-	-	++
Acetone leaf	-	+++	++	-	+++	++	-	-	++	+
Acetone stem	-	++	-	-	+	+	-	-	+	++
Acetone root	-	+	-	+	++	-	+	+	-	++

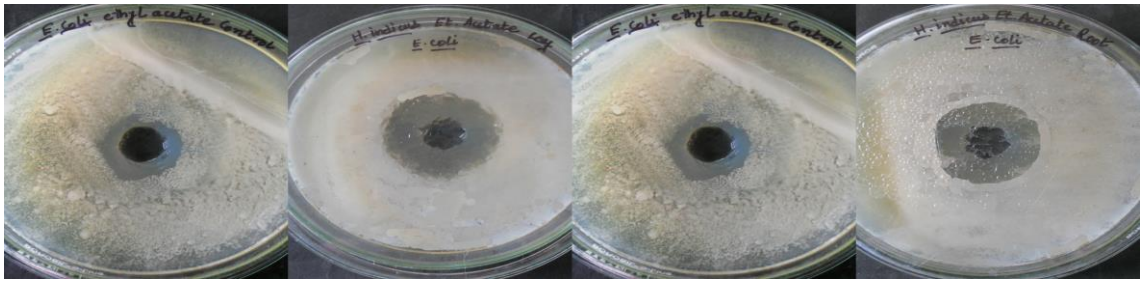

Fig 1. Antimicrobial activity of Ethyl acetate leaf, stem and root extracts respectively against *E. coli*, with control growth on left.

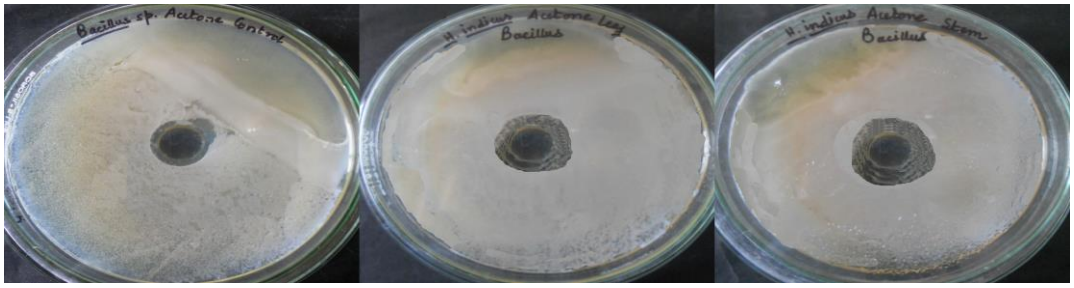

Fig 2. Antimicrobial activity of Acetone leaf and stem extracts respectively against *Bacillus* sp. with control growth on left.

After incubation at 30ºC for 96 h, the plates were observed for no or less fungal growth, indicating the presence of antifungal activity (**Figs. 3 and 4**).

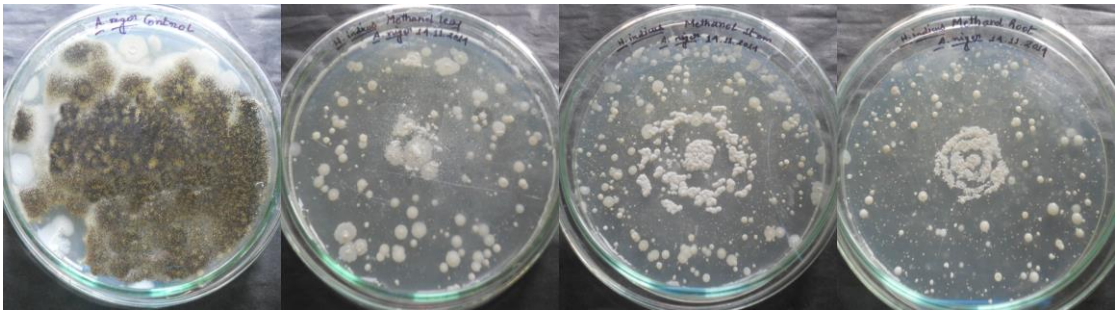

Fig 3. Antifungal activity of Methanol leaf, stem and root extracts respectively against *A. niger* with control growth on left

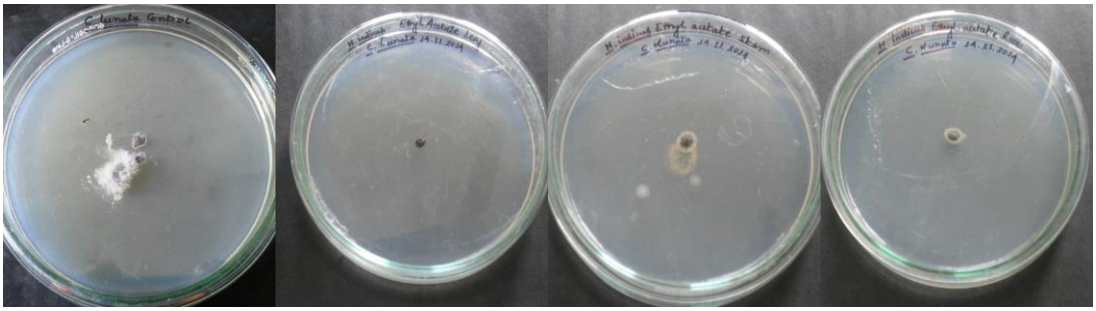

Fig 4. Antifungal activity of Ethyl acetate leaf, stem and root extracts against *C. lunata* with control growth on left

CONCLUSION

In the present study, the qualitative estimation of different phytochemicals present in polar solvent treated extracts of *H. indicus* leaf, stem and root which showed to possess antimicrobial and antifungal properties.

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