

Original Article

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ACTIVE COMPOUNDS FROM *HEMIDESMUS INDICUS*

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ABSTRACT

**Objective:** Ayurveda and Herbal medicine are two important forms of alternative medicine that are widely available in India. This work is mainly concerned with the identification of the compounds by HPLC analysis, phytochemical analysis, and antioxidant and antibacterial properties of Indian medicinal plant *Hemidesmus indicus* root extracts.

**Methods:** In the present work the compounds of *Hemidesmus indicus* were identified from ethanolic extract, by HPLC. Different extracts (Hexane, Ethylacetate, and Ethanol) of its root were used for the determination of the antioxidant activity by using DPPH method. These extracts of *Hemidesmus indicus* were also checked for antibacterial activity using disc diffusion method against pathogenic bacteria such as *Bacillus megaterium*, *staphylococcus aureus*, *pseudomonas aeruginosa* and *Klebsiella pneumonia*.

**Results:** In the present work two (2-hydroxy-4-methoxy benzaldehyde and 2-hydroxy-4-methoxybenzoic acid) compounds of *Hemidesmus indicus* were identified from ethanolic extract, by HPLC technique which were used as medicine for antioxidant and antibacterial activity. The phytochemical analysis of *H. indicus* root extracts showed the presence of phenols, glycosides, flavonoids and steroids. All root extracts has very well anti-oxidant and antibacterial activity

**Conclusion:** *In vitro* study indicates that these plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses and presence of alkaloids and polyphenols in higher concentration than the other phytochemicals suggests that these compound can be responsible for the antibacterial activity.

**Keywords:** *Hemidesmus indicus*, Phytochemical screening, Fingerprinting, HPLC, Antioxidant activity, Antibacterial activity.

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INTRODUCTION

Plant synthesized a variety of compounds, including carotenoids, flavonoids, cinnamic acid, tocopherols, other polyphenolic compounds that prevent the oxidation of the susceptible substrate and act as a natural antioxidant. They have aroused intensified concerns towards the scientific evaluation of naturally occurring antioxidant and antimicrobial agent and their applications in medicine food and cosmetics industries. *Hemidesmus indicus* commonly known as *Indian Sarsaparilla* belonging to the family *Asclepiadaceae*. It is a lactiferous shrub, with aromatic and woody roots, slender stems and greenish flowers. This is a common medicinal plant widely used in Indian system of medicine [1] and also an official drug in Indian pharmacopoeia [2] and British pharmacopoeia [3]. Ethnobotanical studies on *H. indicus* revealed its benefits towards various ailments, like scorpion sting, snake bite, fever [4] and as a blood purifier [5]. It had a cooling effect and used in venereal diseases including gonorrhoea [6], stomach ulcer, diabetes and fever increases lactation in mothers, spennatorrhoea [7], biliousness and headache [8]. Microscopic studies on the root and root bark of *H. indicus* were carried. The highest concentration of glycosides, flavonoids, tannins and sterols in the entire plant during summer was reported it, frutescent and *C. buechanani* commonly used as Sariva were differentiated. Along with the above plants. The market samples were identified as *C. buechanani*, *D. hamiltoni* and *H. indicus* belonging to the family *Asclepiadaceae*; *I. frutescens* and *V. solanacrae* of the family *Apocyanaceae*. Austin *et al.* [9-11] tied out phannacognostical analysis of two varieties of *H. indicus* viz, var. *indicus* and var. *pubescens*. Both varieties have a similar habit and more or less, same morphological features. They differ by the nature of the stem and branching pattern and presence and absence of pubescent hairs. *H. indicus* possesses the reservoir of therapeutically bioactive phytochemicals, which belongs to the multiple pharmacological effects including antimicrobial activity against a microorganism. Many studies have documented the antioxidant activities of *H. indicus* [12]. Different root extracts of the *H.*

*indicus* performed as a protecting agent against radiation and DNA damage. Many phytochemical and chromatographic studies have been carried out on *H. indicus*. From the roots of *H. indicus*, hemidesmol, resin and glucoside, tannin and resin, lupeol, a and 13-amyrins, 11-sitosterol [13], lupeol,  $\alpha$ -amyrin, lupeol acetate,  $\beta$ -amyrin acetate, hexane triconate acid and lupeol octacosonate, a coumarino lignoid like hesmidemine, hemidesmin-1, and hemidesmin-2 [14] were isolated. So the present work is carried out for the antioxidant, antibacterial and phytochemical screening of the different extracts of the *H. indicus* root and developed an HPLC chromatogram of ethanol extract of *H. indicus*.

MATERIALS AND METHODS

Preparation of extracts

Fresh roots of *H. indicus* were collected from the Department of horticulture, SHIATS, Allahabad, India. The plant materials were cleaned with tap water, dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with different solvents like hexane, ethyl acetate and ethyl alcohol by using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvents. The extracts were filtered using Whatman filter paper then concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The extracts were then kept in sterile bottles, under refrigerated conditions, until further investigation.

Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literature.

## HPLC analysis

### Preparation of sample solutions

Accurately weight 7 mg of powdered ethanol extract of *H. indicus* was taken into a 10 ml volumetric flask. The extract was dissolved into 7 ml of the HPLC grade methanol then the volume made up to 10 ml and the sample solution was sonicated using ultrasonicator for 10 min.

### HPLC conditions

The HPLC analysis was performed using a Shimadzu Model LC210 A<sub>HT</sub> Auto Sampler (UV-VIS Detector) with ChemSketch software. The compounds were separated out on a column Hypersil BDS C18 (250 x 4.6 mm 5 Micron), with the flow rate 1 ml/min. Ethanol extract was weighed and dissolved in methanol. 20 µl concentration of extract in methanol was injected onto HPLC column at a temperature 30 °C. and the peaks were recorded at a wavelength 254 nm.

### Antioxidant studies

#### DPPH radical scavenging activity assay

The hydrogen atom or electron donation capability of the extracts of *Hemidesmus indicus* was examined on the basis of bleaching of the purple-colored methanol solution of (1, 1-diphenyl-2-picryl hydroxyl) DPPH radical. This spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentration of the different extracts (hexane, ethyl acetate, and ethanol) of *H. indicus* (6.25, 12.5, 25, 50, 100, 200 µg/ml) was added to 4 ml of 0.004 % (w/v) methanol solution of DPPH. The absorbance was read after a 30 min incubation period at room temperature, against blank at 517 nm. The scavenging ability of these extracts to scavenge DPPH radicals was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

Where:  $A_{\text{control}}$  is the absorbance of the control reaction (containing all reagents except the test compound), and  $A_{\text{sample}}$  is the absorbance of the test sample. Tests were carried out in triplicate. IC<sub>50</sub> values for hexane, ethyl acetate and ethanolic extracts of *Hemidesmus indicus* and BHT as a standard were calculated by plotting a graph concentration (µg/ml) vs. (%) percent of the scavenging activity. IC<sub>50</sub> value the concentration of the extracts which is required to scavenge 50 % of DPPH free radicals.

### Antibacterial studies

*Bacillus megaterium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* were chosen based on their clinical and pharmacological importance. The bacterial strains were obtained from Department of Biotechnology, Global Institute of Biotechnology, were used for evaluating antimicrobial activity. The bacterial stock cultures were incubated for 24 h at 37 °C on nutrient agar medium, following refrigeration storage at 4 °C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37 °C (the bacteria were grown in the nutrient broth at 37 °C and maintained on nutrient agar slants at 4 °C. The prepared sterile whatman no: 1 filter paper discs of 6 mm diameter were impregnated with the extracts (20 µl concentration) and shaken thoroughly and this test plates incubated for a period of 48 h, in BOD at 37 °C for the development of inhibitory zones and the average of two independent readings for each organism in different extracts were recorded. The control Petri plates were also maintained above respective cultures. The inhibition zones (ZI) were lead after 1 d at 37 °C for bacteria. The diameter (in mm) of the inhibition zone was measured and recorded.

## RESULTS AND DISCUSSION

### Phytochemical screening

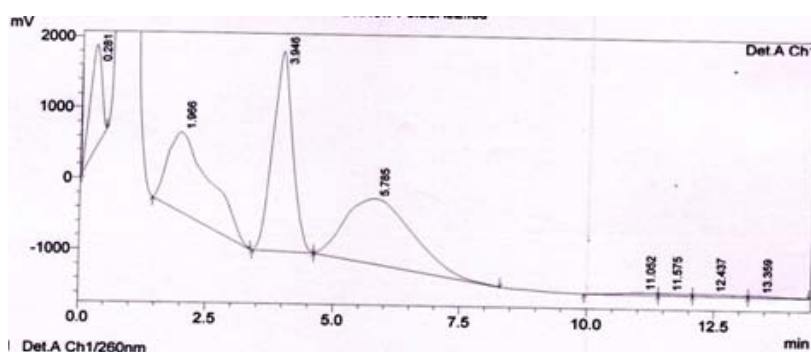
The percentage yield of the hexane, ethyl acetate and ethanol extracts of *H. indicus* root were found to be 20.5%, 21.0% and 23.0% (w/w) respectively. All the extracts were examined for phytochemical analysis showed in table 1.

**Table 1: Phytochemical screening of Hexane, Ethyl acetate, and Ethanolic extracts of *Hemidesmus indicus***

S. No.	Secondary metabolites	Hexane	Ethyl acetate	Ethanolic
1	Steroids	+	++	++
2	Triterpenes	-	+	+
3	Saponins	-	-	-
4	Triterpinoidal saponins	-	-	-
5	Alkaloids	+	++	++
6	Carbohydrates	-	-	+
7	Flavonoids	+	+	++
8	Tannins	+	++	+
9	Glycosides	+	++	++
10	Polyphenols	+	++	++

All of the extracts of the roots of the *H. indicus*, contained alkaloids, flavonoids, phenolic compounds, steroids, and tannins. The chloroform extracts of the roots, stem, and leaves of *O. sanctum* also contained all of the investigated constituents (alkaloids, flavonoids,

phenolic compounds, steroids, and tannins). These various phytochemical constituents have many bioactive actions and could contribute to the antioxidant, and antibacterial activities of the plant parts investigated below [15-17].



**Fig. 1: HPLC Chromatogram of ethanolic extract of *Hemidesmus indicus***

### HPLC analysis

In the present work HPLC analysis was performed for the fingerprints of the different compounds present in the ethanolic extract of the *H. indicus* root. These normalized fingerprints are the principal markers that can check the purity or impurity of different constituents compounds at very low concentration. From Fig.1 the HPLC of plant extract the peaks and graph observed were seen in the following fig.

From the HPLC-Chromatogram of the ethanolic extract, the peaks were seen at Rt-value 11.8 min and 13.3 min by using solvent system acetonitrile: acetic acid. The peaks which could be identified from the graph were 2-hydroxy-4-methoxy benzaldehyde and 2-hydroxy-4-methoxybenzoic acid respectively. The identification of these compounds was performed by comparison with the compounds chromatogram reported in the literature [18]. Recently the presences of amyrin triterpene, p-amyrin triterpene, and benzaldehyde, 2-hydroxy-4-methoxyl benzenoid in the root of *H. indicus* have been reported [19]. Nagarajan and Rao 2003 isolated 2-hydroxy-4-methoxybenzaldehyde from the roots of *D. Hamiltonii* and *H. Indicus*, which is responsible for its aromatic nature, was found to be >90% in their volatile oil, which was isolated from both, fresh and dried roots of different origin. The unusual aromatic aldehyde 2-hydroxy-4-methoxy benzaldehyde is responsible for the

fragrance in roots. HPLC method possesses the ability to quantify the marker compounds in plants by comparison of their chromatogram using similarity analysis and chemometric method [21]. Hence, this chromatogram can be used as fingerprints for the 2-hydroxy-4-methoxy benzaldehyde and 2-hydroxy-4-methoxybenzoic acid compounds obtained from the ethanolic extract of *H. indicus* root.

### Antioxidant activity

#### DPPH radical scavenging activity

The antioxidant activity of plant extracts cannot be assessed by using a single method due to the complex nature of phytochemicals. Therefore, to measure the antioxidant activity of the plant extract, it is essential to use commonly accepted assays. One of the most universally accepted methods of antioxidant activity is DPPH radical scavenging activity assay [22]. DPPH is a stable free radical with an odd electron that imparts deep violet color in methanol solution illustrated by a strong absorption band centered at 517 nm. The principle behind the DPPH assay is that the free radical scavenging antioxidants donate hydrogen to the stable DPPH radical, and reduce the stable DPPH radical to a yellow-colored, non-radical form of para-hydroxyl-derivatives of 1, 1-diphenyl-2-picrylhydrazine (DPPH-H).

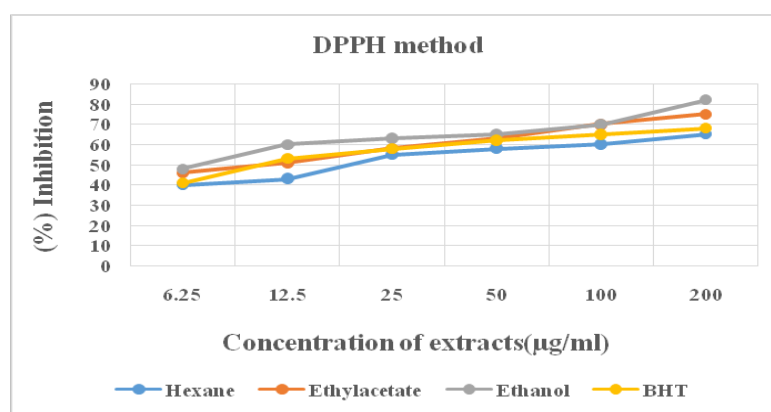


Fig. 2: DPPH method of Antioxidant property of *Hemidesmus indicus* root extracts

From the fig 2: it had been found that the hexane, ethyl acetate and ethanolic extracts of *Hemidesmus indicus* exhibited strong scavenging activity. Among these three extracts, ethanolic extract ( $IC_{50}$  6.510 µg/ml) showed highest antioxidant activity followed by the ethyl acetate ( $IC_{50}$  6.793 µg/ml) and hexane extract ( $IC_{50}$  14.53 µg/ml) as compared to that of standard BHT ( $IC_{50}$  7.621 µg/ml). The DPPH radical scavenging activity indicated that these plants extracts may contain compounds that donate hydrogen to terminate the odd electrons of the DPPH radicals, that are responsible for free radical

reactivity. The scavenging ability of these plant extracts to forage DPPH radical could indicate that these plant extracts could be used to treat radical-related pathological disturbances.

#### Antibacterial studies

The antibacterial activities of the different extracts of the *H. indicus* root were tested *in vitro* by using disc-diffusion method [23]. All the extracts were found to be extremely effective against bacterial species at a concentration of 20 µl of each extract.

Table 2: Antibacterial activity

Microorganisms	Zone of inhibition (mm)			
	Hexane	Ethyl-acetate	Ethanol	Control
<i>Bacillus megaterium</i>	16	22	24	26
<i>Staphylococcus aureus</i>	9	12	22	24
<i>Pseudomonas aeruginosa</i>	24	21	23	28
<i>Klebsiella pneumonia</i>	15	16	26	27

According to the result is shown in table 2, the ethanol extract of the *H. indicus* root showed higher activity against *Bacillus megaterium*, *staphylococcus aureus*, *pseudomonas aeruginosa* and *Klebsiella pneumonia* (with a zone of inhibition 24, 22, 23&26 mm diameter) than the other hexane and ethyl acetate extracts. Hexane extract shows minimum activity against *S. aureus* with inhibition zone 9 mm. It is also found from that table that the ethyl-acetate extract shows maximum activity against *B. magaterium* with inhibition zone

22 mm and minimum activity against *S. aureus* with inhibition zone 12 mm and moderately active against *P. aeruginosa* and *K. pneumonia* with inhibition zone 21 and 16 mm respectively. Ethanol extract shows maximum activity against *Klebsiella pneumonia* with inhibition zone 26 mm comparable with that of control (ampicillin) with inhibition zone 27 mm diameter. The control (ampicillin) with concentration 10 µg/ml shows inhibition zone 26, 24, 28, and 27 mm against *Bacillus megaterium*, *staphylococcus aureus*,

*pseudomonas aeruginosa* and *Klebsilla pneumonia* respectively. Overall the ethanolic extract of *H. indicus*, which contains all the phytochemicals that were investigated like alkaloids, flavonoids, tannins, steroids, and phenols, exhibited higher antibacterial activity among the other hexane and ethyl acetate extracts.

#### CONCLUSION

*Hemidesmus indicus* is a plant of great medicinal importance. A large numbers of activities are reported for that and because of so many uses of this plant; this has been taken for the study. *In vitro* study indicates that these plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The root extract of *H. indicus* exhibited antibacterial activity against tested bacterial strains. The presence of alkaloids and polyphenols in higher concentration than the other phytochemicals suggests that these compounds can be responsible for the antibacterial activity. The birds eye point view to establishing specific bioactive molecules, which might be responsible for these actions. Therefore, the cultivation, collection, and further pharmacological exploration of *H. indicus* are essential.

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#### CONFLICT OF INTERESTS

Declare none

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