Assessment Of Antibacterial Activity and Antioxidant Screening of *Hemidesmus Indicus* Root Extracts

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ABSTRACT
The *Hemidesmus indicus* is used to cure leprosy, leucoderma, itching, skin disease, asthma, bronchitis, leucorrhoea, dysentery, piles, syphilis, and paralysis. The present study was aimed to investigate the antioxidant and antibacterial activity of *Hemidesmus indicus* root. The root extracts of *Hemidesmus indicus* were prepared using different solvents like Methanol, Ethanol, Chloroform, Petroleum Ether and Aqueous. The antibacterial activity of different extracts of *Hemidesmus indicus* root was tested by disc diffusion method. Zones of Inhibition produced by different extracts in a dose of 100 and 200 mg/ml against selected strains was measured and compared with those of standard drug Streptomycin. The highest zones of growth inhibition were exhibited by ethanol extract against all the microorganisms compared to other extracts. The antioxidant activity of the root extracts was performed. On the basis of the results, it can be concluded that the petroleum ether extract of *Hemidesmus indicus* root possess significant antioxidant activity against *in vitro* studies.

KEYWORDS: Antioxidant, Antibacterial, Hemidesmus indicus, Streptomycin.

INTRODUCTION

Medicinal Plants

Medicinal plants have been identified and used throughout human history. Plants make many chemical compounds for biological functions, including defence against insects, fungi and herbivorous mammals. Over 12,000 active compounds are known to science. These chemicals work on the human body in exactly the same way as pharmaceutical drugs, so herbal medicines can be beneficial and have harmful side effects just like conventional drugs. However, since a single plant may contain many substances, the effects of taking a plant medicine can be complex [1].

Hemidesmus indicus

Hemidesmus indicus, Indian sarsaparilla is a species of plant that is found in South Asia. It is a slender, laticiferous, twining, sometimes prostrate or semi-erect shrub [Fig 1]. Roots are woody and aromatic. The stem is numerous, slender, terete, thickened at the nodes. The leaves are opposite, short-petioled, very variable, elliptic-oblong to linear-lanceolate. The flowers are greenish outside, purplish inside, crowded in sub-sessile axillary cymes [2]. It occurs over the greater part of India, from the upper Gangetic plain eastwards to Assam and in some places in central, western and South India. *Hemidesmus indicus Linn*, commonly referred to as Indian sarsaparilla,

Anantamool or Nannari is a commonly available perennial climbing plant, used as the main ingredient in the preparation of the cool and refreshing drink Nannari sherbat. The root is a substitute for sarsaparilla (the dried root of the tropical species of Smilax, Smilacaceae; in India *Smilax aspera L*., and *Smilax ovalifolia Roxb.).* It should be distinguished from American Sarsaparilla *Smilax aristolochiifolia* and Jamaican *Sarsaparilla Smilax* ornate [3].

Fig. 1. Hemidesmus indicus plant

Medicinal Uses

H. indicus serves as an alternative tonic, demulcent, diaphoretic and traditionally been used to treat venereal diseases, skin diseases, urinary infections, negative emotions and impotence. It also prevents abdominal distention, arthritis, rheumatism, gout and epilepsy. According to practitioners of traditional Indian medicine, Ayurveda, this root can be administered in the fourth and ninth month of pregnancy to prevent miscarriage. They also claim its efficacy in treating ulcers, fever, loss of appetite, Gastritis, Anorexia nervosa cough, excessive thirst Menorrhagia, Diarrhea and Diabetes. It is also believed that the extracts from this root help in increasing semen count, purifies blood, neutralizes poisons, works as a diuretic and emetic, and has anti-inflammatory properties. Some experimental studies have displayed the beneficial effect of the extract of this root [4]. The alkaloid content present in it is Tylophorine and is anti-inflammatory, antispasmodic and anti-anaphylactic in nature. The other compounds present in it are coumarin, essential oil, starch, tannic acid and triterpenoid saponins. The roots and leaves of the plant possess medicinal properties [5][6].

MATERIALS AND METHODS

Collection of Plant Materials

Hemidesmus indicus roots was collected from Madurai district during the month of January - February in the year 2022.

Preparation of Powder from Plant roots

The roots were separated and cleaned well. Cleaned roots were then dried under shade. The drying process was continued until all the water molecules evaporated and roots became well dried for grinding. After drying, the roots were ground well using mechanical blender into fine powder and transferred into air tight container with proper labeling for further use.

Preparation of Extract from Plant roots

The dried and powdered *Hemidesmus indicus* roots were extracted sequentially with Methanol, Ethanol, Chloroform, Petroleum Ether and Aqueous using Soxhlet apparatus. Each 50 g of dried powdered roots were defatted with Petroleum ether by immersing the extracts in Petroleum ether and kept for 24 hours incubation. After incubation excess petroleum ether was decanted and kept for drying. The dried samples were wrapped in muslin cloth and were kept for soxhlet extraction in 300 ml of solvent at boiling point of increasing polarity. Solute thus separated were collected in a centrifuge tube and used for further studies.

Antibacterial Screening Test Using *Hemidesmus indicus* root Extract against Bacterial Test Organisms

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective. Interpretative criteria of NCCLS are developed based on international

collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

Culture and Media Preparation

The five different solvent extracts of the *Hemidesmus indicus* root samples were tested for antimicrobial activity using diffusion method. The microbial strains used for current study are *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris* and *Streptococcus mutans*. The microorganisms were collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory by periodic subculture.

Antibacterial Assay

Sterilized discs were soaked in *Hemidesmus indicus* root extracts (Methanol, Ethanol, Chloroform, Petroleum Ether and Aqueous) and kept overnight in room temperature. Then soaked discs were dried aseptically to ensure evaporation of solvents. The prepared Muller-Hinton Media was poured in each petridish and allowed to cool. Cotton swabs charged with each test bacterial suspension were inoculated on Muller-Hinton agar plates and were spreaded over agar surface to make a lawn.

Then the plates were allowed to dry for 20 minutes. The sterile dried antimicrobial discs impregnated with crude Hemidesmus indicus root extracts of 25 mg/disc were carefully dispensed with uniform distances placed on Muller-Hinton agar plates and incubated for 18-24 hours at 37° C. The assay was carried out in triplicate. Control discs with solvents alone also maintained. The zone of inhibition was measured from the centre of disc to the clear zone in millimeter and the results were recorded.

In vitro Antioxidant Activity

Antioxidants are molecules that slow or prevent the oxidation of molecules by eliminating free radical intermediates. They can also oxidize themselves, thus inhibiting other oxidation reactions. Antioxidants may be hydrophobic or hydrophilic. In general, the former reacts with oxidants in the cell cytosol and blood plasma, while the latter defend cell membranes from lipid peroxidation. The antioxidant potential of Hemidesmus indicus root extracts were evaluated by analysing free radical scavenging activity on nitric oxide radical scavenging activity and hydrogen peroxide scavenging activity.

Nitric oxide radical scavenging activity

The nitric oxide scavenging activity of the extract was evaluated as per the Sreejayan and Rao (1997) procedure. 10 mM sodium nitroprusside (3 ml) was dissolved in 0.2 M PBS solution (phosphate buffered saline - pH 7.4). This was further mixed with solvent extracts taken at different concentrations and then incubated at room temperature for 150 minutes. Incubation was followed by addition of 0.5 ml Griess reagent (1% sulphanilamide and 0.1% naphthylethylene diamine dihydrochloride dissolved in 2% H_3PO_4). Measurement of absorbance was done at a wavelength of 546 nm.

Percentage of radical scavenging activity of the extract was determined as follows:

The analysis was repeated thrice. A graph of inhibition percentage versus sample concentration was plotted.

$$\%\ \text{NO radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{Control OD}} \times 100$$

Hydrogen peroxide scavenging activity

A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Extract at various concentrations were added to hydrogen peroxide solution (0.6 ml). For each concentration, a separate blank sample was used for background subtraction. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide by UV visible spectrometer Shimadzu, UV-1800, Japan. The percentage inhibition of H_2O_2 scavenging activity was calculated using the following

$$\%\ \text{inhibition of Hydrogen Peroxide Scavenging Assay} = \frac{(1 - \text{Absorbance (test)})}{\text{Absorbance (blank)}} \times 100$$

Where, Absorbance (Test): Absorbance of the test (With extract) and Absorbance (Blank): Absorbance of the control (Without extract).

RESULTS AND DISCUSSION

Antimicrobial Activity

The root extracts of *Hemidesmus indicus* were NM (Methanol), NE (Ethanol), NCL (Chloroform), NPE (Petroleum Ether) and NAQ (Water) were tested for antimicrobial activity against bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris* and *Streptococcus mutans*. The plates were incubated in an incubator at 37˚C for 24hours and the activity was evaluated by measuring the zone of inhibition. The resulting zone of inhibition were tabulated in Table 1 and shown in Fig 2. The zone of inhibition was measured in 'mm'.

The antibacterial activity of *Hemidesmus indicus* root extracts were tested against four bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris* and *Streptococcus mutans*. In this, *Bacillus subtilis, Streptococcus mutans* and *Staphylococcus aureus* are gram positive bacteria whereas *Escherichia coli, Klebsiella pneumonia* and *Proteus vulgaris* are gram negative bacteria. The result revealed that *Hemidesmus indicus* root extracts ethanolic root extracts show better activity than other extracts. The water extract has no antibacterial activity against all bacterial pathogens. Thus, it confirmed that *Hemidesmus indicus* water root extracts have no antibacterial activity. The plates were shown in Fig. 1.

Table. 1. Antibacterial activity of *Hemidesmus indicus* root extracts

Strains	Zone of inhibition in 'mm'					
	NM	NE	NCL	NPE	NAQ	+ve
Staphylococcus aureus	NZ	9	NZ	NZ	NZ	22
Streptococcus mutans	11	11	NZ	NZ	NZ	28
Bacillus subtilis	8	11	NZ	NZ	NZ	24
Proteus vulgaris	10	9	NZ	NZ	NZ	26
Klebsiella pneumoniae	10	10	NZ	10	NZ	19
Escherichia coli	9	9	NZ	13	NZ	12

NZ – No Zone

The root extracts of *Hemidesmus indicus* were indicated as NM (Methanol), NE (Ethanol), NCL (Chloroform), NPE (Petroleum Ether) and NAQ (Water). The Ethanolic root extracts of *Hemidesmus indicus* shown the better antibacterial activity against *Streptococcus mutans* and *Bacillus subtilis*. The zone of inhibition appeared in both *Streptococcus mutans* and *Bacillus subtilis* strains were 11 mm. In methanolic root extracts of *Hemidesmus indicus* the *Proteus vulgaris* and *Klebsiella pneumoniae* have the better antibacterial activity and the zone of inhibition were 10 mm. The chloroform and aqueous root extracts of *Hemidesmus indicus* have no antibacterial activity against all six bacterial strains. In case of root extracts of *Hemidesmus indicus*, petroleum ether two zone were noted in *Klebsiella pneumoniae* and *Escherichia coli* with the zone of inhibition as 10 mm and 13 mm.

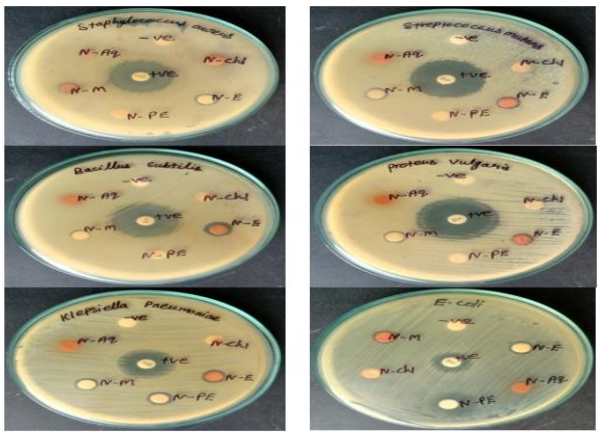

Fig. 2. Antibacterial activity of *Hemidesmus indicus* root extracts against various bacterial pathogens.

Antioxidant Activity of *Hemidesmus indicus* roots

The antioxidant potential of root extracts of *Hemidesmus indicus* were carried out by free radical scavenging activity on nitric oxide radical scavenging activity and hydrogen peroxide scavenging activity.

Nitric oxide radical scavenging activity

The absorbance of the sample was measured at 517 nm. Percentage radical scavenging activity of the sample was calculated. The nitric oxide radical scavenging activity were calculated and the results were tabulated in Table 2.

Table. 2. Nitric oxide radical scavenging activity of *Hemidesmus indicus* root extracts

S.No	Sample	Percentage activity (%)
1	NM	6.49 ± 0.29
2	NE	3.69 ± 0.45
3	NCL	15.55 ± 0.45
4	NPE	21.7 ± 0.45
5	NAQ	7.57 ± 0.45

The extracts providing antioxidant property were calculated and the values were tabulated. The Petroleum Ether extract shows the better antioxidant result. In case of Nitric oxide radical scavenging activity ethanolic extract have low antioxidant value as 3.69 ± 0.45. Rest of the three extracts show gradually higher inhibition value than ethanol as 15.55 ± 0.45 (chloroform), 6.49 ± 0.29 (Methanol) and 7.57 ± 0.45 (water). *Hemidesmus indicus* root extracts show better antioxidant activity in Nitric oxide radical scavenging activity.

Hydrogen peroxide scavenging activity

The absorbance of the sample was measured at 230 nm. Percentage scavenging activity of the sample was calculated. The hydrogen peroxide radical scavenging activity were calculated and the results were tabulated in Table 3.

Table. 3. Hydrogen peroxide scavenging activity of *Hemidesmus indicus* root extracts

S.No	Sample	Percentage activity (%)
1	NM	92.37 ± 0.56
2	NE	76.00 ± 0.43
3	NCL	76.30 ± 0.35
4	NPE	94.18 ± 0.51
5	NAQ	55.74 ± 0.57

The extracts providing antioxidant property were calculated and the values were tabulated. The petroleum ether extract shows the better antioxidant result. In case of hydrogen peroxide scavenging activity aqueous extract have low antioxidant value 55.74 ± 0.57. Rest of the three extracts show gradually higher inhibition value than water as 76.00 ± 0.43 (ethanol), 76.30 ± 0.35 (chloroform) and 92.37 ± 0.56 (methanol). *Hemidesmus indicus* root extracts show better antioxidant activity in hydrogen peroxide scavenging activity.

CONCLUSION

The selected plant for the present study *Hemidesmus indicus* roots was collected and the roots were extracted using varying solvents such as methanol, ethanol, chloroform, petroleum ether and aqueous. The antibacterial activity for the extract of *Hemidesmus indicus* roots such as methanol, ethanol, chloroform, petroleum ether and aqueous against *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Proteus vulgaris* and *Streptococcus mutans*. The result revealed that *Hemidesmus indicus* root extracts ethanolic root extracts show better activity than other extracts. The water extract has no antibacterial activity against all bacterial pathogens. Thus, it confirmed that *Hemidesmus indicus* water root extracts have no antibacterial activity.

On the basis of above results, it can be concluded that the petroleum ether extract of Hemidesmus indicus root possess significant antioxidant activity against in vitro studies and ethanolic root extract of *Hemidesmus indicus* possess significant antimicrobial activity against bacterial pathogens [7]. The study also provides strong evidence for the use of *Hemidesmus indicus* root extracts to treat for various diseases as a therapeutic agent [8-10]. The activity may be due to the presence of one or more phytochemical constituents present in the extract. However, further studies have to be extended for other pharmacological studies.

REFERENCES

1. Kirtikar K.R., and Basu B.D., Indian medicinal plants, Indian Medicinal Plants., 1918,

2. Austin A., and Herbals R., A review on Indian sarsaparilla, Hemidesmus indicus (L.) R. Br, Journal of biological sciences, 2008, 8, 1-12.
3. Aneja V., Suthar A., Verma S., and Kalkunte S., Phyto-pharmacology of Hemidesmus indicus, Pharmacognosy Reviews, 2008, 2, 143.
4. George S., Tushar K., Urmikrishnan K., Hashim K., and Balachandran I., Henúdesmus indicus (L.) R. Br. A Review, Journal of Plant Sciences, 2008, 3, 146-156.
5. Alam M., Auddy B., and Gomes A., Isolation, purification and partial characterization of viper venom inhibiting factor from the root extract of the Indian medicinal plant sarsaparilla (Hemidesmus indicus R. Br.), Toxicon, 1994, 32, 1551-1557.
6. Miller H.E., Rigelhof F., Marquart L., Prakash A., and Kanter M., Antioxidant content of whole grain breakfast cereals, fruits and vegetables, Journal of the American College of Nutrition, 2000, 19, 312S-319S.
7. Rios J., and Recio M., Medicinal plants and antimicrobial activity, Journal of ethnopharmacology, 2005, 100, 80-84.
8. Patnaik J., and Debata B., Micro-propagation of Hemidesmus indicus (L.) R. Br. through axillary bud culture, Plant Cell Reports, 1996, 15, 427-430.
9. Alam M., Auddy B., and Gomes A., Viper venom neutralization by Indian medicinal plant (Hemidesmus indicus and Pluchea indica) root extracts, Phytotherapy Research, 1996, 10, 58-61.
10. Das S., and Singh Bisht S., The bioactive and therapeutic potential of Hemidesmus indicus R. Br.(Indian Sarsaparilla) root, Phytotherapy Research, 2013, 27, 791-801.