International Journal of Chemical Studies

P-ISSN2349–8528
E-ISSN 2321–4902
IJCS 2015; 3(2): 76-82

Received: 20-06-2015
Accepted: 23-07-2015

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Identification of the therapeutic properties of Indian medicinal plant extracts with phytochemical screening & anti-microbial activity

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Abstract
Many forms of alternative medicines are available for those who cannot be helped by conventional medicine. 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 therapeutic properties of Indian medicinal plant extracts. It is a well-known that medicinal plants have disease curing properties and this is due to the compounds presents in the extracts used for the treatment. In the present work different compounds of *Hemidesmus indices* were identified which were used as medicine for antioxidant and antimicrobial activity. After isolating theory HPLC, different compounds present in ethanoic extract of *Hemidesmus indices* root were designed using Chemsketch software is compared with Mass finder. The ethanoic extract of it root was used for its anti-oxidant and antimicrobial activity. It root extract has very well anti-oxidant and anti-microbial activity. These compounds of the extract were checked for ADMET studies. The ethanoic extract of *Hemidesmus indices* was checked for anti-microbial activity against pathogenic bacteria such as *Bacillus magisterium*, *staphylococcus aureus*, *pseudomonas aeruginosa and Klebsila pneumonia*.

Keywords: *Hemidesmus indices*, Antioxidant activity, Anti-microbial activity, phytochemical screening.

1. Introduction
Hemidesmus indices commonly known as Indian Sarsaparilla, belonging to the family Asclepiadaceous, is a slender lactiferous, twining, sometimes prostrate or semi erect shrub, occurring over the greater part of India. Roots are woody and aromatic; stems numerous, slender, terete, thickened at the nodes; leaves opposite, short-petiole, very variable, elliptic-oblong to linear-lanceolate often variegated with white above, sometimes silvery white and pubescent beneath, flowers are greenish outside, purplish inside, crowded in sub sessile aeillary cymes; follicles are slender, four inches long, cylindrical, sometimes curved, divaricate; seeds numerous, black, flattened, with a silvery white coma. This is a common medicinal plant widely used in Indian Systems of Medicine (Anonymous, 1997) [7] and also an official drug in Indian Pharmacopoeia (Anonymous, 1996) [7] and British Pharmacopoeia (Anonymous, 2003) [8].

Various market samples are available in the name, identified as *H. indices*, *Decalepis hamiltoni* and *Cryptokpis buchanani* belonging to the family Asclepiadaceous; *lchnocarpta frutescent* and *Vallaris solanaceae* of the family Apocyanaceae. Apart from this *H. indices* exists with two variants namely var. *indices* and var. *pubescent*, which are not given much emphasis? Hence this review was carried out to enumerate the benefits of *H. indices*. (Anonymous 1986, 1997) [7]. The roots wall used as antipyretic, anti-diarrhoeal, astringent, blood purifier, diaphoretic, diuretic, refrigerant and tonic (Anonymous, 1986, 1997;) [7]. Roots are useful in biliousness, blood diseases, dysentery, diarrhoea, respiratory disorders, skin diseases, syphilis, fever, leprosy, leukoderma, leucorrhoea, itching, bronchitis, asthma, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism. Root bark is used to cure dyspepsia, loss of appetite, nutritional disorders, fever, skin diseases, ulcer, syphilis, rheumatism and leucorrhoea Stem of *H. indices* is used as diaphoretic, diuretic, laxative and in treating brain, liver and kidney diseases, syphilis, sleet, urinary discharges, uterine complaints, leucodenna, cough and asthma. Ethnobotanical studies: Ethnobotanical studies on *H. indices* revealed its benefits towards various ailments, like scorpion sting, snake bite, fever (Sharma *et al*., 1979) [37] and as a blood

purifier. It has cooling effect and used in venereal diseases including gonorrhea (Singh and Maheswari, 1983) [43]., stomach ulcer (Jain and Singh, 1994; Jain, 1996) [45]., diabetes and fever increases lactation in mothers, spennatorrhoea (Singh and Prakash, 1996) [46]., biliousness (Balasubriunanian and Prasad, 1996) [21] and headache.

Root decoction is useful for curing high fever and skin diseases (Sudhakar and Rao, 1985; Vyas, 1993) [49]. The rind of the root is chewed for sore mouth (Prasad *et al.*, 1964, 1996) [21]. Fresh root paste with neem oil applied on scalp of children for development of skull bones to enable carrying head-loads in adult age.The root is used to make sweet smelling drink, which is used in the place of coffee and tea (Prasad *et al.*, 1996) [21]. The dried entire plant is used for skin diseases. Along with *Piper; coven, and P. nigrum*, it is used for postpartum recovery and also in diarrhoea and to improve appetite. This is also used for impotency and to reduce body heat, as a stimulant and as food. This is said to increase blood circulation and acts as a cure for diarrhoea (Jain and Singh, 1994) [7]. The roots are peeled and eaten raw as a blood purifier and as cooling beverage and for treating skin diseases (Arseculeratne *et al.*, 1985) [19]. Along with Mimosa pudica, it is taken orally, during the menstrual period to treat leucorrhoea and also as a body tonic with black pepper, it is used for fevers of long duration and with milk is taken for anemia (Singh and Prakash, 1996) [46]. This is used as ananti-venom (Selvanayahgarn *et al.*, 1994) [35]. Decoction taken thrice daily checks menorrhagia. It is also used as anti-rheumatic, diuretic. Anti-inflammatory and to treat snake bite (Alam *et al.*, 1994) [2]. Externally it is applied to provide relief from scorpion stings (Singh and Ali, 1992) [24]. This is also used for venereal diseases and as blood purifier (Reddy *et al.*, 1992) [30], to cure stomach pain and diarrhoea. A decoction with *Elephant opus: caber, H. indices and P. nigrum* are used for gonorrhea (Sahu, 1984) [33]. This is also used for skin affections, syphilis and as a tonic. With honey, the fresh roots are used for health and vitality. Along with the woods of *Acacia sundra* and *Cinnarnomum seylanicum*, it is used to make a soft, nourishing beverage to promote youthfulness, health and vitality.

2. Objectives

Keeping in view, the present work was undertaken with the following objectives.

- To isolate compounds from *Hemidesmus indices* root extract.
- To determine anti-oxidant and anti-bacterial activity of isolated compounds.

2.1 Review of Literature

Satoskar *et al.* (1962) [34] found out that alcoholic and steam distilled extracts of roots of *H. indices* had no significant diuretic activity, whereas aqueous extract caused a slight increase in urinary flow in rats, but not in dogs.

Dutta *et al.* (1982) [27] Found that the ethyl acetate extract of roots of *H. indices* exhibited significant anti-inflammatory activity in both acute and subacute inflammation as revealed by significant inhibition of inflammation induced by carrageenan, bradykinin, S-hydroxyl tryptamine, employing granuloma pouch and cotton pellet implantation methods in rats. However, it was found less active than phenyl butanone or P-methadone, against granuloma pouch and cotton pellet implantation. It is ineffective in dextran induced inflammation.

Arseculeratne *et al.* (1985) [19] investigated the toxicity studies of H. indices the dried stem was fed to albino rats at a dose of 25% in their diet for ten days. Histopathological studies showed hepatotoxic activity. There were diffuse hydropic degeneration and focal hepatocellular necrosis. Toxicity was seen in the liver, but not in the lungs or kidney.

Atal *et al.* (1986) [15] carried out quantitative toxicity assessment by oral route for LD. It was found to be 2500 mg kr'. *Anoop Austin and Jegadeesan (2002, 2003a)* alined out toxicity studies on *H. indices* var. indices and var. pubescent. They also established the influence of seasonal variation and maturity of the parts. LD was found to be 915.21 and 853.7 mg kg^{r} cluing vegetative and flowering seasons of var. indices. Hepatomegaly and scleroses glomeruli were observed, which were confirmed by biochemical parameters. This contradictory observation among the other studies as a hepatoprotective drug might be due to the fact of the aqueous alcoholic extract used for this study. Vegetative samples were safe compared to flowering seasonal samples. The reason for the changes in the difference in the qualitative and quantitative change in the biological active compound is due to genetic, climatic and development phase of the medicinal plants. Var. pubescent possessed only nonspecific hepatomegaly which might be reversible, but care should be taken in liver diseases. LD was found to be 848.3 and 813.7 mg kg among seasonal variations.

Atal *et al.* (1986) [15] used ethanoic (95%) extract for its delayed type cutaneous hypersensitivity stimulation effects. The aqueous extract of *H, indices* was given orally at a concentration of 2% of diet in mice was active against *Mycobacterium leprae*.

Waffler *et al.* (1988) [12] carried out a comparative clinical study with beldame siesta and Mahatiktaka &rite for peptic ulcer, where *H. indices* is a major ingredient in Mahatiktaka Arita.

Sharma *et al.* (1994) [38]. carried out studies on *Sariva ghanasatva*, which contains *H. indices* as a major ingredient was found to be effective against allergic conjunctivitis. It was also found that *H. indices* was effective in advanced cases of malignancies like multiple myeloma, adenocarcinoma, squamous cell carcinoma, Hodgkin's lymphoma *etc.* though not a cure.

Alain and Gomes (1998) [50] found that 2-hydroxy-4- methoxy benzoic acid, isolated and purified from the methanol extract possessed potent anti-inflammatory, antipyretic and antioxidant properties. It neutralized inflammation induced by *Vipera russelli* venom in male albino mice and reduced cotton pellet-induced granuloma. In addition, it also produced a significant fall in body temperature in yeast-induced pyrexia and did not change nomothetic body temperature. The compound effectively neutralized viper venom-induced changes in serum phosphatase and transaminase activities and neutralized free radical formation as estimated by TBAPS and superoxide dismutase activities, which helps in neutralizing the venom. The study was further continued in Rabbits.

Alain and Gomes (1998) [50] studied the venom neutralizing capacity of this antiserum in rabbits immunized with Vipera russelli venom which showed positive adjuvant effects as evident by the higher neutralization capacity (lethal and hemorrhage) when compared with the antiserum raised with venom alone. It potentiated the lethal action neutralization of venom by commercial equine polyvalent snake venom antiserum in experimental models. These observations raised the possibility of the use of chemical antagonists (from herbs) against snake bite, which may provide a better protection in presence of antiserum, especially in rural parts of India.

3. Materials and Methods

3.1 Plan of work

The research work will be carried out in Hyderabad. The plan of work includes

3.1.1 Scheme (3.1):-Column with stopcock – approx. 0.7 cm diameter, 10 cm length
Ring stand and clamp for holding column.
Equilibration Buffer (Potassium Phosphate, EDTA, ammonium sulfate, pH 6.5)

3.1.2 Nutrient Agar medium (NAM)

Beef extract	--	500mg
Sodium chloride	--	500mg
Peptone	--	1gm
PH	--	7.0 – 7.2
Distilled water	--	100ml
Agar	--	2gm

3.2 Preparation of Plant Extract

3.2.1 Extraction

The extraction of the plant root was carried out using known standard procedures. The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials was initially defatted with ethyl alcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. 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 ethyl alcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2 to 4 °C. This crude extracts of ethyl alcohol was used for further investigation for potential antimicrobial properties.

3.3 Column Chromatography

Chromatography is a process by which individual components of a mixture can be separated. In this technique there is mobile phase and a stationary phase (solid). The mixture components interact differentially with the mobile and stationary phases to effect the separation of components.

3.4 Procedure

- Plant sample (1g) was ground with pastle and mortar.
- Placed weighed sample into the mortar and added enough equilibration buffer to cover the beet. Ground with Pestle as much as possible getting the smallest pieces of plant.
- Placed ground liquefied plant material in a 2 mL centrifuge tube.
- Centrifuged at 500-1000 rpm for 5 minutes to form pellet of plant solids plus liquid on top (supernatant).
- Transfer the supernatant (the top liquid, solid plant material should be stuck at bottom) into a fresh 2 ml tube. Discarded tube with pellet and then centrifuged (under 500-1000 2pm) for 5min to form pellet I solid plant a supertitle on top.
- A series of clean empty micro centrifuge tubes were set up in a rack and numbered them in the order they will be used.
- Positioned the column on a stand above the test tube rack.
- Closed the column stopcock; add 4ml of equilibrium buffer. Open the stopcock as you add ~ 2 mL of your Phenyl Sepharose or Sand slurry or Silica powder, depending on your assignment. The bed height should be about 3-4 cm high. If you run out of Equilibration Buffer, just add more to keep slurry/sand wet/silica powder.
- Continued to run equilibration buffer through the column until the solid material is completely level and settled down into compact bed. Discard outflow.
- Once the meniscus of the equilibration buffer has reached the top of the bed, close the stopcock and add 500 mL of plant extract/sample/material. Drizzle this extract gently and slowly down the side of the column so that you do not disturb the bed.
- Opened stopcock and allow the sample to flow into the bed. When the top meniscus of the sample touches the top of the bed, close the stopcock. Discard outflow. The compounds bind to column in presence of equilibrium buffer.

(Clean up. If you have sand slurry just dump the sand from the column into trash. If using Phenyl Sepharose, which is very expensive, "wash" the column with 5mL of 8mM NaOH and then collect and save the phenyl Sepharose slurry.)

4. Results and Discussion

4.1 Identification of Compounds of Root extract

The TLC of root exotic was run in solvent system Acetone: Hexane (1:3) with standard showed 3 spots visible by naked eye and R_{AF} .60 by spraying with 10% H_2SO_4. Graph showing the result of extract. It showed the respectively natural compounds in plant extract.

In the present study, HPLC was performed at 254nm, the time which showed that as the column size increases it effects on retention time. HPLC profile of ethanol extract of *Phyllanthus indofischeri* has characteristics peaks at different retention time. These peaks showed that there are different compounds and characteristic fingerprints for each drug to judge in an herbal formulation. These normalized fingerprints are principal markers that can check purity or impurity of drug at very low concentration. From the HPLC of plant extract the peaks and graphs observed were seen in following figures.

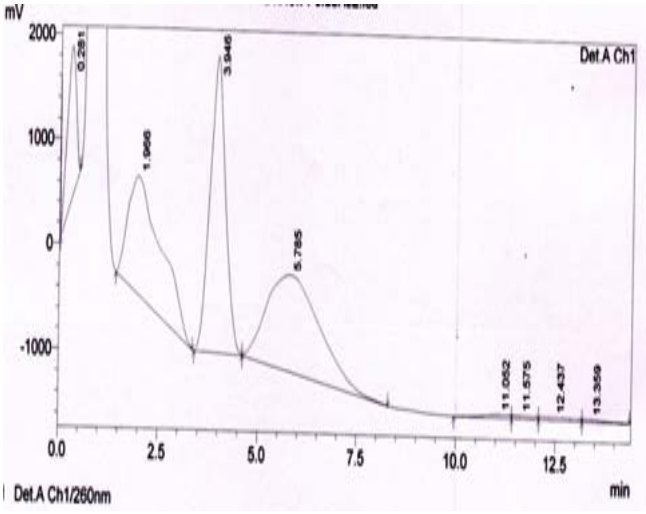


Fig 4.1: HPLC Chromatogram of ethanoic extract of *Hemidesmus indices*

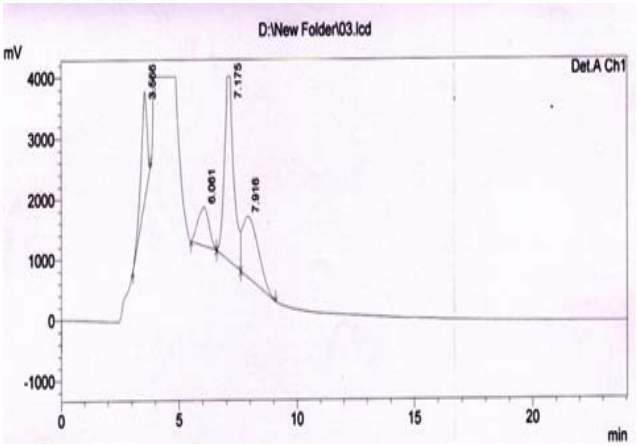


Fig 4.2: HPLC Chromatogram of ethanoic extract of *Hemidesmus indices*

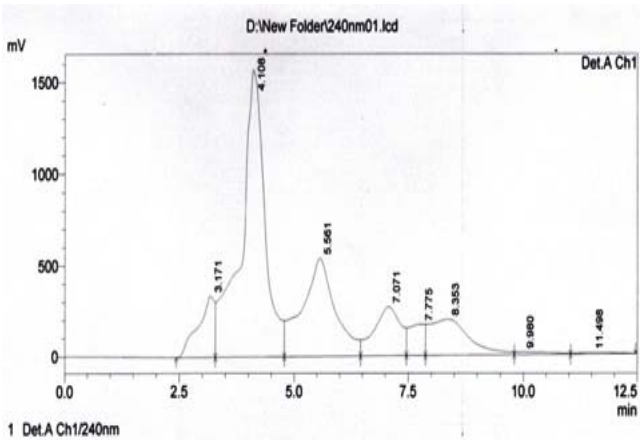
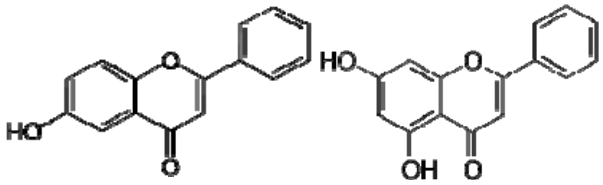
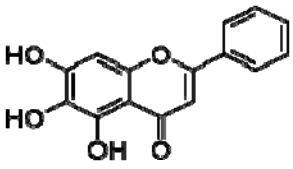


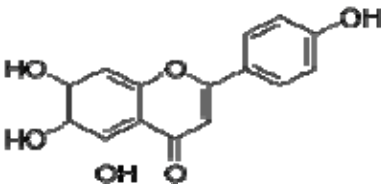
Fig 4.3: HPLC Chromatogram of ethanoic extract of *Hemidesmus indices*



(a) 6-HYDROXY-2-PHERYLCHROMEN-4-ONE



(b) Chrysin



(c) Scutella rein

Fig 4.4: Structures of the compounds identified from *Hemidesmus indices*

4.2 ADME Studies

ADMET stands for Absorption, Distribution, Metabolism, Excretion and Toxicity. The prediction of the ADMET properties plays an important role in the drug designing process because these properties account for the failure of about 60% of all drugs in the clinical phases. Traditionally ADME tools were applied at the end of the drug development pipeline, nowadays ADME is applied at an early phase of the drug development process, in order to remove molecules with poor ADME properties from the drug development pipeline it leads to significant savings in research and development costs. These studies to identify compounds with better gratuities. From the molinspiration the properties and bioactivity studies were for compounds identified in ethanoic extract of root powder of Hemidesmus in odious tabulated in table 3.

The properties and bioactivity studies for the extracts compounds by molinspiration results

Table 4.1: Phytochemical Screening of *Hexane, Ethyl acetate, Ethanoic and Aqueous extract of Hemidesmus indices*

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

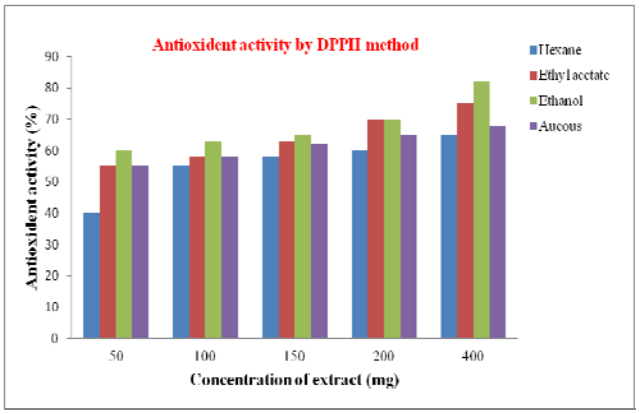


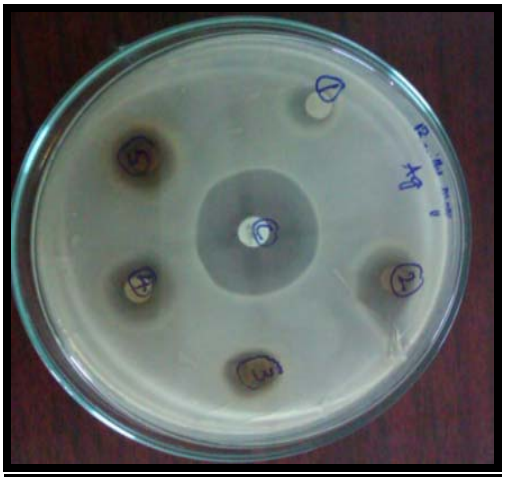
Fig 4.5: DPPH method of Antioxidant property of *Hemidesmus indices* root extract

4.3 Antibacterial Activity

The isolated extracts were found extremely active bacteria 4 bacterial species at a concentration of 20µl of methanol extract, Gram positive bothering Bacillus *magisterium*, *Staphylococcus aureus*, gram negative, *Pseudomonas aeruginosa*, *Klebsila Pneumonia*. The results are shown in table 4.8.

The seed extract of *Hemidesmus indices* were found suitable for simple and rapid extraction of phytochemical compounds by chromatography within 5- 10 min. This is a very simple and rapid method of isolation of compounds which can be useful in various biomedical and biotechnological applications.

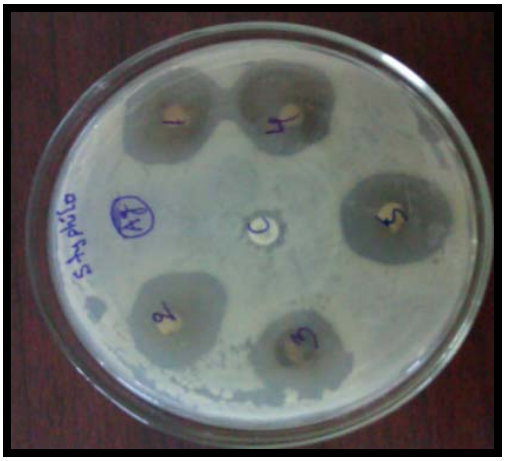
Compound: 1



Bacillus magisterium

Fig 4.6: plates of extract (1) showing best inhibition on bacterial growth

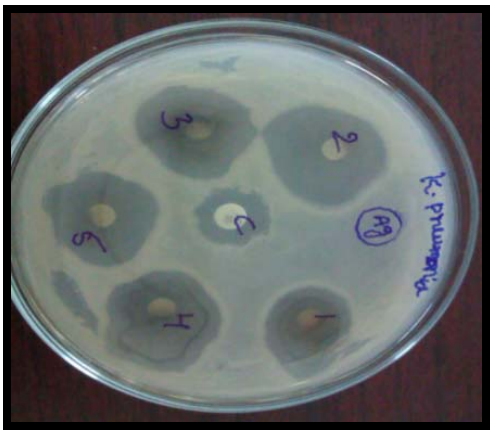
Compound: 2



Staphylococcus aureus

Fig 4.7: plate of extract (2) showing best inhibition on Staphylococcus aureus (Bacteria)

Compound: 3



Pneumonia

Fig 4.8: plate of extract (3) showing best inhibition on *pneumonia* (Bacteria)

5. Summary & Conclusion

The results of the present study clearly indicated that the ethanoic extract of *Hemidesmus indices* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. *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. Ethanoic extract of the roots of *H.indicus* exhibited antibacterial activity against tested bacterial strains. Presence of alkaloids and polyphenols in higher concentration than the other phytochemicals suggests that these phytochemicals could be responsible for the antibacterial activity.

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