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In vitro antibacterial activity on human pathogenic bacteria and larvicidal effect of root from *Hemidesmus indicus* (Linn.) on *Culex qinquifasciatus*

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Abstract

The present study was undertaken to evaluate the effect of extraction from *Hemidesmus indicus* roots on five different solvents activity against the pathogenic and non-pathogenic organisms also larvae of the Culex qinquifasciatus mosquito. *H. indicus* (L.) is one of the plants used in Ayurveda for several remedies it belongs to the family Asclepiadaceae. The experimental roots were tested for their phytochemical constituents and antimicrobial activity against 15 human pathogenic microorganisms using standard disc diffusion method. Moreover, the methanol and petroleum ether extracts were active against most of the tested organisms as they showed potential phytochemical constituents. The antimicrobial activities of the extracts were compared with their respective reference antibiotics as minimum inhibitory concentrations (MICs). Apart from petroleum ether, all other solvent extracts such as ethanol, methanol, chloroform and aqueous extract showed significant results. Among the 12 bacterial species maximum inhibition zone was 16.00±0.18, 10.65±0.19 and 16.3±0.20 observed the following bacteria such as *E.coli*, *P. mirabilis* and *S. typhimurium* respectively. The larvicidal effect of aqueous extracts of H. indicus roots were tested against *Culex quinquefasciatus* larvae at the concentrations of 1, 2, 3, 4 and 5% upto three days. Larval mortality was 100% with the use of 5% concentration of root aqueous extract after 2 days was observed. It is suggested that the aqueous extract of *Hemidesmus indicus* can be used as natural insecticide for mosquito control.

Keywords: *Hemidesmus indicus*, antibacterial, larvicidal activity, *Culex quinquefasciatus.*

Introduction

Herbal medicine represents one of the traditional medicines all over the world. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to the host cells are considered for developing new antimicrobial drugs [1]. Recently, [2] evaluated the plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics, there is very urgent to search a new infection fighting strategies [3].

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Hemidesmus indicus (L.) commonly known as Indian Sarsaparilla, belonging to the family Asclepiadaceae, is a slender laciciferous, twining, semi erect shrub occuring over the greater part of India. This is a common medicinal plant widely used in Indian and also an official drug in Indian pharmacopoeia and British Pharmacopoeia [4]. Roots are woody and aromatic [5]. Highly valuable medicinal plant, *H. indicus* roots are used as antipyretic, anti-diarrhoeal, astringent and tonic. Roots are also useful in blood diseases [6], biliousness [7] dysentery, respiratory disorders, skin diseases, syphilis [8]. Furthermore, [9] reported the antienterobacterial activity of the chloroform and methanol extracts of *Hemidesmus indicus* root was demonstrated using a variety of methods and different enterobacterial strains. In previously, [10] identified the 105 Indian plant species ant its antibacterial, antifungal activities against the pathogenic and non-pathogenic microorganisms. Since, there is an ample of works has been done, hence this research work have been designed the following objectives to study the antibacterial activity against the root extract of *H. indicus* with five various solvents against some important human pathogenic bacteria and the larvicidal activity of this valuable medicinal plant against *Culex quinquefasciatus*.

Materials and Methods

Plant materials

Hemidesmus indicus is widely seen in the tropical and sub tropical regions of the world and all over the India. Healthy rapidly growing plant's roots were collected from the tropical areas of Kanyakumari District (South India) and were identified by using the Herbarium of TBGRI (Tropical Botanical Garden Research Institute), Trivandrum and the flora of the presidency of Madras.

Preparation of solvent root extraction

The collected roots were thoroughly washed with water. Then, it was dried in shade for 20 days. The shade dried roots were finely powdered. The powdered plant materials were stored at room temperature for extraction. The dried 25g root was powdered and soaked separately in 100ml of petroleum ether, chloroform, methanol, ethanol and aqueous by increasing order of their polarity [11]. Each solvent in separate flasks with powdered sample were kept in a rotating shaker for 3 days. The extracts were filtered through cheese cloth and the extracts were reduced to half of its original volume. The organic solvents were concentrated in vacuum using a rotary evaporator, while aqueous extract was dried using water bath.

Test strains for in vitro antimicrobial activity:

The test microorganisms like *Esherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella paratyphi* (MTCC 735), *Salmonella typhi* (MTCC 733), *Salmonella typhimurium* (MTCC 2957), *Shigella boydii* (MTCC 1457), *Shigella sonnnei* (MTCC 2957), *Stapylococcus aureus* (MTCC 737), *Streptococcus faecalis* (MTCC 459) and authentically identified clinical isolates of Citrobacter sp., were obtained from culture repository of Best Biotech culture collection, Bangalore, India. The organisms were inoculated into NB (Nutrient Broth) medium, (0.5% Peptone, 0.5% Sodium Chloride, 0.15% Yeast extract; pH 7.4) and incubated at 37°C for overnight. The bacterial cells were harvested by centrifuging at 5000rpm for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer. The bacterial cells were diluted to approximately 105 CFU mlG1 before use.

Determination of antibacterial activity

The antimicrobial activity of the root extracts was determined using agar well diffusion method by following the published procedure with slight modification. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8mm diameter) were punched in the agar and filled with plant extracts. Control wells containing

antibiotic solution chloromphenicol of Hi-Media Laboratories were filled for comparative efficacy. The plates were incubated at 37^{o}C for 18hr and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drug chloromphenicol.

Statistical analysis

The resultant clear zones around the discs were measured in mm. The antibacterial activity of *Hemidesmus indicus* root extracts was indicated by clear zones of inhibition. Data of three independent experiments represented by six replicates were maintained. The means were analyzed by one way analysis of variance (ANOVA).

Collection of *Culex quinquefasciatus* **mosquito larvae**

C. quinquefasciatus mosquito larvae were collected from water stagnate area, and identified in Zonal Entomological Research Laboratory. They were then maintained under suitable temperature and humidity.

Larvicidal effect of *Hemidesmus indicus*

Twenty larvae of the *C. quinquefasciatus* were placed in each of the three 150 ml sterile beaker containing 90 ml of water. After adding the larvae to the beaker, 10 ml of aqueous root extracts of *H. indicus*, was added in each of the beakers, separately. Then the beaker containing the larvae were kept in the growth room maintained at room temperature. The larvicidal effects of the extracts were monitored by counting the number of dead larvae each day up to three days. Each test was repeated thrice, the percentages of larval mortality and standard error were calculated for each concentration of aqueous extracts of all the three plants.

Result

Antimicrobial activity

H. indicus extracts (1mg/ml) inhibited the growth of *S. aureus* and *K. pneumonia* (13.70mm) and *P. aeruginosa* (15.54mm). Remaining other microbes such as bengalensis. Typhimurium, *S. boydii* and *S. aureus* were also showed a similar order of antimicrobial activity against the tested organisms. Among the tested pathogens Proteus mirabilis showed *H. indicus* extracts exhibited moderate inhibition with the MIC ranging from 6.20 to 10.65mg against tested bacterial pathogens (Table1). Standard antibiotics amphicillin, tetracycline and chloromphenicol exhibited marked inhibition with the MIC values ranging from 9.5 – 21.6mg/ml.

Six different solvent extracts of *Hemidesmus indicus* (L.) roots were assessed for antimicrobial activity by using the agar well diffusion method by measuring the diameter of growth inhibition zones with 100µl concentrations (Table 1). The results showed that among the four solvent extracts (viz., petroleum ether, chloroform, methanol and ethanol) methanol and ethanol showed significant result of antimicrobial activity. When compared to both, chloroform showed minimum level of inhibition. Even though, antimicrobial activity was not observed in case of petroleum ether extracts against this experimental tested all pathogens. Citrobactor species having maximum MIC shown in Ethanol extract compared with methanol treated bacteria. Among the 12 bacterial species maximum inhibition zone was 16.00±0.18, 10.65±0.19 and 16.3±0.20 observed the following bacteria such as *E.coli*, *P. mirabilis* and *S. typhimurium* respectively. For instance, Ethanol treated bacterial organisms expressed Minimum Inhibition.

Table 1 Antibacterial activity

Name of the Bacteria	Zone of inhibition in mm (Mean±SD)					
	M	E	C	PE	AQ	CHL
Esherichia coli	16.00±0.18	15.23±0.19	08.23±0.20	-	08.50±0.18	13.3±0.50
K. pneumoniae	13.70±0.00	13.21±0.00	12.50±0.34	-	11.70±0.25	14.5±0.40
Proteus mirabilis	10.65±0.19	09.50±0.19	06.20±0.25	-	10.50±0.19	09.5±0.40
P. aeruginosa	15.54±0.16	16.30±0.19	07.00±0.60	-	11.24±0.19	13.6±0.30
S. paratyphi	14.23±0.00	12.00±0.00	08.65±0.40	-	06.20±0.18	17.6±0.30
S. typhi	17.69±0.40	14.26±0.00	10.54±0.50	-	12.30±0.27	20.5±0.20
S. typhimurium	16.3±0.20	13.62±0.19	09.31±0.18	-	11.50±0.20	15.5±0.20
Shigella boydii	13.02±0.30	12.35±0.00	07.24±0.40	-	09.25±0.23	19.1±0.60
S. sonnnei	16.26±0.20	15.23±0.44	9.35±0.27	-	15.50±0.19	17.5±0.40
S. aureus	16.67±0.44	12.51±0.00	07.00±0.23	-	08.25±0.18	21.6±0.30
S. faecalis	14.42±0.60	12.25±0.25	10.26±0.40	-	11.40±0.23	17.5±0.50
Citrobacter sp.	14.00±0.19	13.49±0.00	11.00±0.50	-	08.35±0.48	14.4±0.20

Among methanol and ethanol extracts, methanol extracts recorded significant antibacterial activity followed by extract with ethanol. Furthermore, *Salmonella typhii* found highly susceptible to methanol extract, where as Proteus mirabilis was less susceptible to both methanol and ethanol extracts. However, *P. aeruginosa* was found highly susceptible to ethanol extract. Methanol extract exhibited similar antibacterial activity against *E. coli*, *P. aeruginosa*, *S. paratyphi* and *S. sonnei* where it was around 16mm and 14mm zone of inhibition. Similar result was absorbed for ethanol against Citrobacter sp., *S. paratyphi*, *S. typhimurium*, *S. aureus* and *S. faecalis*.

The chloroform extract showed highest activity against *K. pneumonia*. Inhibition zone of 10mm and above was observed in *S. typhi*, *S. faecalis* and Citrobacter sp. Antibacterial activity of aqueous extract varied greatly among the different test pathogenic bacteria. Highest antibacterial activity was observed against *S. sonnei* followed by *S. typhi*, even though antibacterial activity was observed against other pathogenic bacteria also it was not found significant. Inhibition zone more than 10mm noted against *Klebsiella pneumoniae*, *Pseudomonas aeroginosa*, *Salmonella typhimurium* and *Streptococcus faecalis*. Both methanol and ethanol showed significant antibacterial activity against *Escherichia coli* and *P. aeruginosa* compared to chloromphenicol. The inhibition zone of methanol extracts of Citrobacter sp. was equal to that of the chloromphenicol. Aqueous extract recorded significant antibacterial activity against

Table 2: Mortality rate of Culex quinquefasciatus mosquito larvae at different concentrations of aqueous extracts of *Hemidesmus indicus* root. (n = 3 ± SE).

Root extract Concentration (%)	Mortality rate (24hrs) (in %)	Mortality rate 48hrs (in %)	Mortality rate 72hrs (in %)
Control	0	0±0.00	0±0.00
1	24.5	29.5±0.0	30.1±0.18
2	28.6	43.2±0.30	55.2±0.25
3	36.2	54.3±0.40	69.7±0.19
4	58.3	85.0±0.18	96.3±0.29
5	82.1	100±0.00	100±0.00

Larvicidal activity

The larvicidal activity of aqueous extracts of *H. indicus* root against the larvae *C. quinquefasciatus* mosquito was given in Table 2. The larvicidal activity on aqueous extract of *H. indicus* roots showed 28, 55, and 65% of death with the use of 1, 2, and 3% concentrations, respectively, after 3 days. The third day 4% concentration revealed mortality rate was higher (95%) when compared to other treated larvae. Finally, 100% mortality has been observed on higher percentage (5%). Among the three days experiment the optimum level of mortality seen on the third concentration such as 36.2, 54.3 and 69.7.

Discussion

In the recent years development of multi drug resistance in the pathogenic bacteria and *Pseudomonas aeruginosa* compared to chloromphenicol, where as against other test pathogens it was not significant. The overall results clearly showed the extract of this medicinal plant root was detected with inhibition zone size ranged from 10.65 to 17.69 for methanol, 0.9 to 16.30 for Ethanol, and 0.7 to 12. 50mm for Chloroform. Some other problems such as toxicity of certain antimicrobial drugs on the host tissue triggered interest in searching of new antibacterial drugs of plant origin. Hence the present investigation reveals the screening and scientific evaluation of plants and suggests that this plant could be exploited in the management of diseases caused by these bacteria in human systems [12]. It is interesting to note that antibacterial activity was highly pronounced in solvent extract compared to aqueous extract of *H. indicus*. It is also important to note that susceptibility of the pathogens was varied to solvent extracts and aqueous extract [13]. If a new plant substance, which has never been used before and about which nothing is known has been developed then the requirements are the same as those required for a new synthetics. This kind of similar results has been observed on another plants based experiment by Aqil et al. [14] and Gayathri and Kannabiran, [15] demonstrated the time kill assay with most promising fractions of this plant extracts concentration-dependent killing of MRSA within 9-12hrs of incubation. Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of ethanolic extracts of four medicinal plants namely *Acorus calamus* (rhizome) *Hemidesmus indicus* (stem) *H. antidysenterica* (bark) and *Plumbago zeylanica* (root), were detected with inhibition zone size ranged from 11 to 44 mm and minimum inhibitory concentration (MIC) varied. from 0.32 to 3.25 mg/mL

It was reviewed that the methanolic extracts of *H. indicus* possessed inhibitory activity against *S. typhymurium, E. coli* and *S. flexneri* in in vitro cultures by agar well diffusion method [9, 12,]. For instance, [4, 16] established the helicobactericidal activity of various extracts against *Helicobacter pylori*, which is comparable to standard antibacterials. The comparative efficacy with chloromphenicol is also highly encouraging. *E.coli* and *P. aeruginosa* are two important pathogens causing urinary track infection. Both these microorganisms were highly susceptible to methanol and ethanol extract of *H. indicus* compared to chloromphenicol. Thus, the present study records the scientific validation of this plant for use as an antibacterial agent.

The current investigation also gives importance for the larvicidal effect of *H. indicus* of aqueous extract. A commercial saponin mixture extracted from Q. saponaria showed increasing toxicity in *Anopheles aegypti* and *Culex pipiens* when both saponin concentration and the duration of the experiment were increased [17]. Aluminium chloride, known for its phenolic complexing activity, obtained from alder leaf also reported to have the larvicidal activity against *A. aegypti* [18]. Monoterpene hydrocarbons showed a marked mosquito larvicidal activity against *C. pipiens* which is obtained from the fresh leaves of *Anthemis melampodina* and *Pluchea dioscoridis* [19]. A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito

larvae of *C. pipiens* [20]. Similarly an alkaloid derived from the tropical vine Triphyophyllum peltatum (Dioncophy-llaceae), was found to have larvicidal activity against the malaria vector *Anopheles stephensi* [21]. The comparative study of larvicidal activity of root extract of *H. indicus*, leaves extracts of *G. sylvestre* and *E. prostrata* was established against *C. quinquefasciatus* mosquito [22]. Here, this result showed that the plant *H. indicus* having high larvicidal activity, so it is suggested that the aqueous extract of this medicinal plant can be used as ecofriendly and sustainable insecticide to control mosquito. Several reports are available in support of antibacterial activity of several phytochemicals present in plant extracts [23,24]. Antibacterial activity of tannins and saponnins isolated from plant species are well documented [25]. Larvicidal (activity was reported for saponin isolated from fruit mesocarp of Balanites aegyptiaca). However, further studies are needed to evaluate the antibacterial activity of isolated phytochemicals such as tannins and saponins from these plants against pathogenic bacterial strains. In conclusion, aqueous extracts of the roots of *H. indicus* exhibited significant antibacterial activity against the tested bacterial strains. Presence of tannins and saponins in higher concentration than the other phytochemicals suggest that these phyto-chemicals could be responsible for the anti-bacterial activity. However, further studies are needed to establish that these plant extracts could form effective antimicrobial therapy against common bacterial diseases.

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