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# Bioactivity of *Hemidesmus indicus* (L.) on Human Pathogenic Bacteria and *Culex qinquifasciatus* (Diptera: Culicidae)

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## ABSRACT

The aim of this study was to identify the antibacterial and mosquito larvicidal activity of the *Hemidesmus indicus* (Linn.) root extract with five diferent solvents. 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 twelve human pathogenic bacteria 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% up to three days also after two days, 100% larval mortality has been observed at 5% concentration of root extract with aqueous solution (dist.water). From this results clearly showed solvent extracts of *H. indicus* (Linn.) root could be act as an antibacterial as well as larvicidal activity.

**Key words:** *Hemidesmus indicus*, antibacterial, larvicidal activity, *Culex quinquefasciatus,* therapeutic agent, medicinal value

## 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. Hemidesmus indicus is commonly known as Anantmool or sariva belongs to the family Asclepiadaceae and its well known during the Ayurvedic system of medicine (Kainthla *et al*., 2006; Thatoi *et al*., 2008; Shete and Bodhankar, 2010). 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 (Ahmad and Beg, 2001). Recently, Chitravadivu *et al*. (2009),

Jazani *et al*. (2009) 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 (Sieradzki *et al*., 1999).

*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 (Anoop, 2008). Roots are woody and aromatic. Highly valuable medicinal plant, *H. indicus* roots are used as antipyretic, anti-diarrhoeal, astringent and tonic (Gayathri and Kannabiran, 2009). Roots are also useful in blood diseases (Verma *et al*., 2005) biliousness (Balasubramanian and Prasad, 1996; Kavitha *et al*., 2006). Furthermore, Das and Devaraj (2006) reported the antibacterial activity of the chloroform and methanol extracts of *H. indicus* root was demonstrated using a variety of methods and different enterobacterial strains. Furthermore (Aqil and Ahmad, 2007) has been identified the 105 Indian plant species ant its antibacterial, antifungal activites against the pathogenic and non-pathogenic microorganisms. Since, there is an ample of works has been done, hence this research 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 during the year of January, 2009. Healthy rapidly growing plant roots were collected from the tropical areas of Kanyakumari District (South India) also in and around the College campus Malankara Catholic College in Kaliakkavilai (Kanyakumari District). Then using the Herbarium of TBGRI (Tropical Botanical Garden Research Institute), Trivandrum and the flora of the presidency of Madras identified this specimen.

**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 25 g powdered root and soaked separately in 100 mL of petroleum ether, chloroform, methanol, ethanol and aqueous by increasing order of their polarity (Kokate, 1999). Each solvents were kept 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 *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 1688), *Salmonella paratyphi* (MTCC 735), *Salmonella typhii* (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 5000 rpm 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 (8 mm 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°C for 18 h and measuring the diameter of the zone of inhibition assessed the antibacterial activity. 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. Clear zones of inhibition indicated the antibacterial activity of Hemidesmus indicus root extracts. 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 stagnated 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 that the mosquito larvae were introduced in to the beaker to this 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. Counting the number of dead larvae each day up to three days monitored the larvicidal effects of the extracts. 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.

## RESULTS

**Antimicrobial activity:** *H. indicus* extracts (1 mg $mL^{-1}$) inhibited the growth of *S. aureus* and *K. pneumonia* (13.70 mm) and *P. aeruginosa* (15.54 mm). 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 10.65 mg against tested bacterial pathogens (Table 1). Standard antibiotics amphicillin, tetracycline and chloromphenicol exhibited marked inhibition with the MIC values ranging from 9.5-21.6 mg $mL^{-1}$.

Table 1: Antibacterial activity of five different extracts of *H. indicus* (Linn.) root on human pathogenic organisms

| | Zone of inhibition in mm (Mean±SD) | | | | | |
|---|---|---|---|---|---|---|
| Name of the bacteria | M | E | C | PE | AQ | CHL |
| *Escherichia coli* | 16.00±0.18s | 15.23±0.19 | 08.23±0.20 | - | 08.50±0.18 | 13.3±0.50 |
| *K. pneumoniae* | 13.70±0.00s | 13.21±0.00 | 12.50±0.34 | - | 11.70±0.25 | 14.5±0.40 |
| *Proteus mirabilis* | 10.65±0.19s | 09.50±0.19s | 06.20±0.25 | - | 10.50±0.19 | 09.5±0.40 |
| *P. aeruginosa* | 15.54±0.16s | 16.30±0.19 | 07.00±0.60 | - | 11.24±0.19s | 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. typhii* | 17.69±0.40s | 14.26±0.00s | 10.54±0.50 | - | 12.30±0.27s | 20.5±0.20 |
| *S. typhimurium* | 16.30±0.20 | 13.62±0.19 | 09.31±0.18 | - | 11.50±0.20 | 15.5±0.20 |
| *Shigella boydii* | 13.02±0.30s | 12.35±0.00 | 07.24±0.40s | - | 09.25±0.23 | 19.1±0.60 |
| *S. sonnnei* | 16.26±0.20 | 15.23±0.44is | 09.35±0.27is | - | 15.50±0.19s | 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±0.23s | 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 |

M: Methanol, E: Ethanol, C: Chloroform, PE: Petroleum ether, AQ: Aquoeus CHL: Chloromphenicol (10 μg $disc^{-1}$ as a positive reference standard) Values are mean inhibition zone (mm)±S.D of six replicates.- : No activity observed, s: 5% level of significant, is: Insignificant

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 100 μL 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. Eventhough, 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.

Among the two 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 16 and 14 mm 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 10 mm and above was observed in *S. typhii, S. faecalis* and *Citrobacter* sp. Antibacterial activity of aqueous extract varied greatly among the different test pathogenic bacteria. Moreover, maximum antibacterial activity was observed against *S. sonnei* followed by *S. typhii*, even though antibacterial activity was observed against other pathogenic bacteria also it was not found significant. Inhibition zone more than 10 mm noted against *Klebsiella pneumoniae, Pseudomonas aeruginosa, 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.

Table 2: Mortality rate of *Culex quinquefasciatus* mosquito larvae at different concentrations of aqueous extracts of *Hemidesmus indicus* root

| Root extract concentration (mg mL$^{-1}$) | Mortality rate (%) 24 h | 48 h | 72 h |
|---|---|---|---|
| Control | 0.0 | 0.0 | 0.0 |
| 1 | 24.5 | 29.5 | 30.1 |
| 2 | 28.6 | 43.2 | 55.2 |
| 3 | 36.2 | 54.3 | 69.7 |
| 4 | 58.3 | 85.0 | 96.3 |
| 5 | 82.1 | 100.0 | 100.0 |

**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 12.50 mm 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 (Das *et al*., 2003). It is interesting to note that antibacterial activity was highly pronounced in solvent extract compared to aqueous extract of *H. indicus* (Gayathri and Kannabiran, 2009). It is also important to note that susceptibility of the pathogens was varied to solvent extracts and aqueous extract (Wiesman and Chapagain, 2006) 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*. (2006), Joseph and Sujatha (2011). Again, Khanna and Kannabiran (2008) demonstrated the time kill assay with most promising fractions of this plant extracts concentration-dependent killing of MRSA within 9-12 h of incubation also accompanying with Pure saponin extract exhibited remarkable antimicrobial activity against *Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and fungus like *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger*. Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of ethanolic extracts of four medicinal plants namely *Acorus calamus* (Rhizome) *H. 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$^{-1}$.

It was reviewed that the methanolic extracts of *H. indicus* possessed inhibitory activity against *S. typhimurium, E. coli* and *S. flexneri* in vitro cultures by agar well diffusion method (Das *et al.*, 2003; Das and Devaraj, 2006). For instance, Anoop *et al.* (2003), Anoop and Jegadeesan (2003) have been established the helicobactericidal activity of various extracts against *Helicobacter pylori*, which is comparable to standard antibacterial. 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 (Pelah *et al.,* 2002). Aluminium chloride, known for its phenolic complexing activity, obtained from alder leaf also reported to have the larvicidal activity against *A. aegypti* (David *et al.*, 2000). Monoterpene hydrocarbons showed a marked mosquito larvicidal activity against *C. pipiens* which is obtained from the fresh leaves of *Anthemis melampodina* and *Pluchea dioscoridis* (Massoud *et al.,* 2001). A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *C. pipiens* (Lee, 2000). Similarly an alkaloid derived from the tropical vine *Triphyophyllum peltatum* (Dioncophyllaceae), was found to have larvicidal activity against the malaria vector *Anopheles stephensi* (Francois *et al.*, 1996). 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 (Gopiesh and Kannabiran, 2007). Here, this result showed that the plant *H. indicus* having higher larvicidal activity, so it is suggested that the aqueous extract of this medicinal plant can be used as eco-friendly and sustainable insecticide to control mosquito. Several reports are available in support of antibacterial activity of several phytochemicals present in plant extracts (Slobodnikov *et al.*, 2004; Chacha *et al.,* 2005; Divysree and Cherupally, 2010). Antibacterial activity of tannins and saponins isolated from plant species are well documented (Mandal *et al.*, 2005). 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 phytochemicals could be responsible for the antibacterial activity (Chakradhar *et al.*, 2005). However, further studies are needed to establish that these plant extracts could form effective antimicrobial therapy against common bacterial diseases.

When the mosquito larvae were treated with various concentration and different treatment periods as a result of this minimum mortality rate has been observed on lowest concentration (1 mg $mL^{-1}$) 24.5% followed by medium level of mortality was observed on optimum level of concentration 3 mg $mL^{-1}$ (Table 2). When the concentration increased accompanied with prolonged exposure periods all the larvae were died, since this Table 2 clearly showed peak level concentration was 5 mg $mL^{-1}$ because this concentration was most probably suitable for control the mosquito larva (*Culex quinquefasciatus*) in our environment.

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