Antimicrobial efficacy of a traditionally important medicinal plant - 
*Tiliacora acuminata* (Lam.) Hook. f.

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Received 6 August 2012; Accepted 15 July 2013

The present investigation was aimed to screen the antimicrobial potential of *Tiliacora acuminata* (Lam.) Hook. f. against various human bacterial and fungal pathogens. Antimicrobial efficacy was performed by disc diffusion method against the bacterial pathogens, viz. *Proteus mirabilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* incubated for 24 h at 37° C as well as the isolates of fungal pathogens, *Fusarium oxysporum, Aspergillus niger, Aspergillus terreus, Rhizopus oxysporum* and *Candida albicans*. All the extracts of the plant, viz. acetone, chloroform, petroleum ether and distilled water, studied in the present investigation exhibited varying degree of inhibitory effect against the selected human pathogens. Further it is revealed that the activity was significant in acetone and chloroform extracts. *Proteus mirabilis* (fungus) and *Aspergillus terreus* (bacterium) forms the most susceptible microbial strains. Hence, *T. acuminata* forms a potentially good source of antimicrobial agent and demonstrates the importance of such plant in medicinal systems for curing various ailments.

**Keywords:** Antibacterial, Antifungal, Disc diffusion, Plant extracts, *Tiliacora acuminata*.

**IPC code:** Int. cl. (2011.01)−A61K 36/00.

**Introduction**

Plants have been used in the treatment of various diseases from the time immemorial. The use of plant as source of medicine lies in the root history of mankind. Many of thousands of plant species growing throughout the world have medicinal properties and contain active constituents that have direct action on the body. Undoubtedly, the plant kingdom still holds many medicinal species, yet to be discovered, therefore, large number of plants are constantly screened for their possible antimicrobial activity. Among the estimated 2,50,000 - 5,00,000 plant species, only a small percentage has been investigated phyto-chemically and the fraction submitted to biological or pharmacological screening is even smaller. Antimicrobial drug resistance is a global problem today as the resistant microorganisms have emerged and spread throughout the world because of their genetic plasticity. Over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strain of several group of microorganism. For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents. The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed. Literature reports and ethnobotanical records suggest that plants are the sleeping giant of pharmaceutical industry. They may provide natural source of antimicrobial drug or lead compounds which may be employed in controlling some infection.

*Tiliacora acuminata* (Lam.) Hook. f. & Thom. (Menispermaceae), a wonder plant where all its parts are effectively utilized for various medicinal properties (Plate 1a). The ethnomedicinal uses of this plant include its use as an antidote for snake bite. The leaf or root paste is applied on the bitten area soon after bite. Juice from macerated leaves is applied to cuts in folks. Crude and solvent extract of *T. acuminata* flowers shown high activity against the larval form of *Culex quinquefasciatus*. A new lactone, two alkaloids, tilioresine and (+) N-methyl tiliamosine from leaves and an oil accuminatide from

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seeds have been isolated and characterized. But the antimicrobial and antifungal effect of this plant is not clearly known. In this study, the possible inhibitory effects of different extracts of T. acuminata on the growth of various selected human pathogens were tested by using agar disc diffusion method.

Materials and Methods

Collection of plant material

The aerial plant parts of T. acuminata (Lam.) Hook. f. & Thom. were collected from Thengapattinam coastal area (8°14′25″N & 77°10′20″E) of Kanyakumari District, Tamil Nadu. The plant was identified and voucher specimen was deposited to the Herbarium, Department of Botany, Nesamony Memorial Christian College, Marthandam (NMCCH-5029). The parts collected were washed thoroughly with normal tap water, followed by sterile distilled water. These plant samples were then shade dried separately in room condition, powdered and stored for further use.

Sample extraction

500 g of powdered plant samples were weighed and taken separately. These samples were extracted with acetone, chloroform, petroleum ether and distilled water individually using Soxhlet’s apparatus. The organic extracts obtained were evaporated to dryness by heating in a water bath to obtain a semisolid mass. Equal volume of DMSO was added to each extracts and was later subjected to microbial bioassays.

Microbial Bioassays

Bacterial Isolates and Bioassay

The extracts of acetone, chloroform, petroleum ether and distilled water extracted from the selected plant tissues were screened against five bacterial strains. The test organisms, Proteus mirabilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus were obtained from “Edu-tech Educational and Research Institute”, Melpuram, Kanyakumari.

Preparation of inoculum

Stock cultures were maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton broth (MHB) for bacteria and were incubated without agitation for 24 h at 37°C.

Antimicrobial Susceptibility Test

The disc diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 15 mL of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extracts (20 µL) were loaded on 4 mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimetre. These studies were performed in duplicates.

Fungal Isolates and Bioassay

Isolates of fungal pathogens Fusarium oxysporum, Aspergillus niger, A. terreus, Rhizopus oxysporum and Candida albicans were obtained from “Edu-tech Educational and Research Institute”, Melpuram, Kanyakumari. The cultures were maintained on potato dextrose agar until further study. Aliquotes of potato dextrose agar medium was poured in sterile petridishes. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was
swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extract (20 µL) was loaded on 4 mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were incubated for 72 hours at 28 ± 2º C. Observations were made on the growth of fungal mycelium as influenced by the plant extracts. Based on the growth rate of fungi in response to plant extract, the rate of inhibition was measured in millimeter.

**Results and Discussion**

In the present investigation, *in vitro* antibacterial and antifungal efficacy of the crude extracts of *T. acuminata* was quantitatively assessed on the basis of zone of inhibition. The stock crude extracts prepared from the aerial parts of *T. acuminata* by using distilled water, acetone, chloroform and petroleum ether were subjected to antimicrobial activity against *Proteus mirabilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* and the results were recorded (Table 1 and Fig. 1). The results obtained indicate that all the extracts tested were efficient against *Proteus mirabilis* as shown in Plate 1 b. The zone of inhibition formed were 7, 7, 14 and 8 mm, respectively. The acetone, chloroform and petroleum ether extracts were found to be more effective against *Enterobacter aerogenes* with 13, 13 and 6 mm zone formation as shown in Plate 1c. While distilled water extract was found ineffective. Same condition was shown against the pathogen *E. coli* with 6, 5 and 8 mm zone formation. In the case of *Staphylococcus aureus*, all the extracts showed antibacterial effect with 7, 6, 7 and 7 mm zone formation, respectively. The extract doesn’t exhibit any activity against the pathogen *Pseudomonas aeruginosa*.

The results of antifungal assay tested against *Fusarium oxysporum, Aspergillus niger, A. terreus, Rhizopus oxysporum* and *Candida albicans* using the crude extracts obtained from the aerial parts of *T. acuminata* by using distilled water, acetone, chloroform and petroleum ether are shown in Table 2 and Fig. 2. The results reveal that, the distilled water, acetone and chloroform extracts were found to be effective against *A. terreus* based on the formation of inhibition zones area of around 10, 6 and 5, respectively. Similarly, acetone and chloroform extracts were efficient against *A. niger* with 8 and 10 mm zone formation, (Plate 1d). In *Candida albicans*, distilled water, chloroform and petroleum ether extracts showed activity with 7, 6 and 5, respectively. The extracts tested were found to be ineffective against fungal pathogens *Fusarium oxysporum* and *Rhizopus oxysporum*.

The results obtained indicate that the plant exhibits a varying degree of inhibitory effect against the selected human pathogens. The presence of antimicrobial and antifungal activity in a particular part of a selected species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins, etc. Recently, a number of plants have been reported for antimicrobial properties across the world.

### Table 1—Antibacterial assay of *Tiliacora acuminata* (Lam.) Hook. f. & Thom. using different solvents

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Microorganisms</th>
<th>Distilled Water (mm)</th>
<th>Acetone (mm)</th>
<th>Chloroform (mm)</th>
<th>Petroleum ether (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Proteus mirabilis</em></td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td><em>Enterobacter aerogenes</em></td>
<td>-</td>
<td>13</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>
Based on the previous literature, there exists some studies on biochemistry and ethnobotany of *Tiliacora acuminata*, but there is no report on its antimicrobial activity so far. Thus, the present study shows the presence of antibacterial activity in *Tiliacora acuminata* for the first time.

Further it is authenticated that *Tiliacora acuminata* possess a multipotent efficacy to act against several groups of pathogens. This result was strengthened by a literature report on larvicidal activity of *Tiliacora acuminata* which was due to a spectrum of six secondary phytochemicals present in its flowers along with steroids and cardiac glycosides. Some secondary metabolites in combination may be responsible for better effect of larvicidal activity. Further this plant was reported in 19 sacred forests of Vilavancode Taluk and 23 sacred forests of Kalkulam Taluk of Kanyakumari District. The common occurrence of this plant was also reported in Sendirakillai sacred grove of Cuddalore district of Tamil Nadu and also in Nanmangalam reserve forest of Chennai, Tamil Nadu. It was also found to possess more medicinal importance in folkloric usage such as phyto-antidote for snake bite, curing skin diseases, etc. Hence plenty of this plant resource availability and its distribution pave the way for its utility as an effective antibiotic against various ailments caused by these selected human pathogens.

### Conclusion
The current study reveals that the activity exhibited by the plant extract against human bacterial and fungal pathogens was significant in acetone and chloroform extracts when compared with other solvents used. Based on these observations the most susceptible bacterium is *Proteus mirabilis* and in case of fungus, *Aspergillus terreus* was more susceptible. Also the results of the present study supplement the folkloric usage of the studied plant which possesses several known and unknown bioactive compounds with antibacterial and antifungal properties. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various infectious diseases. A further phytochemical and pharmacological study on this lesser known ethnomedicinally important plant is absolutely necessary for its successful utility.

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