Preliminary phytochemical screening and antibacterial activity of 
 Acalypha indica L.

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Received 03 December 2013; accepted 14 December 2013

Abstract
The antibacterial effect of leaves, stem, and root of Acalypha indica L. was evaluated on bacterial strains like Staphylococcus aureus and Salmonella typhi. The solvent used for the extraction of plants were water and methanol. The in vitro antibacterial activity was performed by agar cup method. The most susceptible bacteria was Staphylococcus aureus. The significant antibacterial activity of active extract was compared with the standard antibiotic streptomycin (100ppm). The results obtained in the present study suggest that Acalypha indica can be used in treating diseases caused by the test organisms.

Key words: Acalypha indica, antibacterial activity, aqueous extract and methanol extract.

1. Introduction
Medicinal plants represent a rich sources of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extracts as raw drugs and they possess varied medicinal properties. The plant part used include root, stem, flower, fruit twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value (Nostro et al., 2000). Some of them are also used for prophylactic purposes. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources it has been reported that between the year 1983 and 1994 (Cragg et al., 1999). The systematic screening of antibacterial plants extracts represents a continuous effort to find new compounds with the potential to act against multi resistant bacteria.

Acalypha indica is a common annual herb, found mostly in the backyards of houses and waste places throughout the plains of Tamilnadu. Plants are emetic, expectorant, laxative and diuretic; useful in bronchitis, pneumonia, asthma and pulmonary tuberculosis. Leaves are laxative antiparasiticide, ground with common salt or quicklime or lime juice applied externally in scabies. Leaf paste with lime juice prescribed for ringworm. Leaf juice is emetic for children. A decoction of the leaves is giving in earache. Powder of the dry leaves is giving to children to expell worms; also given in the form decoction with little garlic. In homeopathy, the plants are used in severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis.

The plant contains kaempferol, a cyanogenetic glycoside, a base triacetonamine and an alkaloid, acalyphine. It is also contains the amide, acalyphamide and some other amides, 2-methyl athraquinone, tri-O-methyl elegiac acid and γ-sistosterol, β- sistosterol, glucoside, stigmatoler, n-octacosanol, quinine, tannin, resin and essential oil (Ghani et al., 2003). The plant is traditionally used as an expectorant against asthma and pneumonia, and also as an emetic, emmenagogue and anthelmintic (Shivayogi et al., 1999). Acalypha indica contains acalyphine which is used in the treatment of sore gums (Bedon et al., 1982). The plant is reported to have a post-coital antifertility effect (Shivayogi et al., 1999), anti- venom properties (Annie et al., 2004), wound healing effects (Suresh Reddy et al., 2002), antioxidant activities (Ruchi et al., 2007), anti-
inflammatory effects (Mohana vamsi et al., 2008) and antibacterial activities (Govindarajan et al., 2008). In the present study evaluates to the potential of Acalypha indica extracts for their antibacterial activity against important human pathogens.

2. Materials and methods
Acalypha indica L. collected from in and around area of Annamalai nagar, Annamalai University, Chidambaram, Tamilnadu. The plant species were identified and authenticated by the Dept. of Botany, Annamalai University. The entire plant was washed thoroughly 2-3 times sterile distilled water following by the removal of dust and foreign particles. The parts like leaf, stem and root were shade dried and used for extraction.

2.1 Solvent Extraction
To prepare the methanol extracts, 150gm of each of the plant material was collected dried in the oven at 70°C for 4 hr and reduced to powder. It was separately macerated with methanol, allowed to stand for 72hr and then filtered, dried extracts were stored in labelled sterile screw capped at 5°C in the refrigerator, until when required for use.

2.2 Preparation of plant extract
10 g of air dried powder of plant samples were added to 10 times of solvent such as water and methanol. The sample was kept in dark for 48hr, the extract was filtered and was then supernatant was centrifuged at 8,000 rpm for 15 min. at room temperature. Supernatant was filtered through whatman No. 1 filter paper and heat sterilized at 120°C for 30 min. The extracts were preserved aseptically in a brown bottle stored at 40°C until further use.

2.3 Microorganisms
The species of bacterial organism, Staphylococcus aureus and Salmonella typhi. They were clinical isolate obtained from Microbiology Laboratories, Rajah Muthaiya Medical College & Hospital, Annamalai University, Annamalai nagar. The cultures of bacteria were maintained on nutrient agar slants at 4°C, re-identified by biochemical tests (Cheesbrough 1982, Cowan and Steel, 2004) and sub-cultured on to nutrient broth for 24h prior to testing.

2.4 Screening for Antibacterial activity
The Antibacterial activity was carried out by employing 24hr cultures of Staphylococcus aureus and Salmonella typhi. Activity of the above mentioned extracts was tested separately using agar cup method (Bauer et al., 1966) for both solvent extracts, The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1x10⁸ cfu/ml) and poured into the Petri plate (Hi-media). For agar cup method, a well was prepared in the plates with the help of a cork-borer (10 mm), 200µl of the test compound was introduced into the well. The plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter.

3. Phytochemical study
3.1 Test for Alkaloids (Mayer’s test)
The extract of Acalypha indica was evaporated to dryness and residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Mayer’s reagent (Siddiqui and Ali, 1997). The samples were then observed or the presence of turbidity yellow precipitate (Evans et al., 2002).

3.2 Test for Tannins
To 0.5ml of extract solution, 1ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed or gallic tannins and green black catecholic tannins (Iyengar M A, 1995).

3.3 Test for Terpenoid and Steroid
4mg of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids (Siddiqui and Ali, 1997).

3.4 Test for Reducing Sugars
To 0.5ml of extract solution, 1ml of water and 5-8 drops of Fehling’s solution was added at hot and observed for brick precipitate.

3.5 Test for Glycoside
To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and concentrated Sulphuric acid are added, and observed for reddish coloration at the junction of two layers and the bluish green colour in the upper layer (Siddiqui and Ali, 1997).

3.6 Test for Saponins
The extract (50mg) was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of form showed the presence of saponins (Gothandam et al., 2010).

3.7 Test for Flavonoids
To 1 ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow color was produced in the plant extract, which become colorless on addition of a few drops of dilute acid indicates the presence of flavonoids (Gothandam et al., 2010).

4. Results and discussion
Preliminary phytochemical analysis revealed the presence of secondary metabolites like tannins, sterols, glycosides, etc. were present in trace amounts in the root, stem and leaves (Table S1 & S2). It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999).

Table 1: Phytochemical composition of Acalypha indica (Aqueous extracts)

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaves</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+)= indicates presence; (-) = indicates absence
Table 2: Phytochemical composition of Acalypha indica (Methanol extracts)

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Leaves</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

(+ = indicates presence; (-) = indicates absence)

The antibacterial activity of the leaves, stem and root of A. indica extracts was assayed by in vitro by agar cup method against two bacterial species. Table S3 & S4 summarizes the microbial growth inhibition of both aqueous and methanol extracts of the screened plants species. The methanol extracts of the investigated plants showed maximum antibacterial activity than aqueous extract. It was found that the metabolic extract of the leaves was effective against Staphylococcus aureus when compare to the stem and root. The minimum inhibitory concentration was found to be 6000 ppm. The stem and root a gradual bacterial activity occurred. While 8000 ppm for Staphylococcus aureus and Salmonella typhi. The comparative antibacterial activity between aqueous and methanolic extract of Acalypha indica and standard antibiotic streptomycin was studied. The methanolic extract showed significant antibacterial efficacy as compared to the standard antibiotic, streptomycin, (Table S3 & S4). The beneficial effects of treatment can be achieved with the treatment with the leaf, stem and root of Acalypha indica L. in various bacterial infectious diseases like bronchitis, pneumonia, & even some skin diseases.

Table 3: Minimum inhibitory concentration of Acalypha indica L. (Aqueous extract)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organisms</th>
<th>Aqueous Extract zone of Inhibition (mm)*</th>
<th>Control (Streptomycin) (100ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves 6000 ppm</td>
<td>7000 ppm</td>
</tr>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella typhi</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Values are the mean of three assays

Table 4: Minimum inhibitory concentration of Acalypha indica L. (Methanol extract)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organisms</th>
<th>Methanol Extract zone of Inhibition (mm)*</th>
<th>Control (Streptomycin) (100ppm)</th>
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<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>Salmonella typhi</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

* Values are the mean of three assays

5. Conclusion

Acalypha indica L. leaf, stem and root extract possess a broad spectrum of activity against a panel of bacterial responsible for the most common bacterial diseases. These promising extracts open the possibility of finding new clinically effective antibiotic compounds.

References

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Source of support: Nil; Conflict of interest: None declared