Evaluation of Anti Microbial and Anti Fungal Activity of Acalypha indica L., Leaf Extract

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ABSTRACT
Anti-microbial and anti-fungal activity of different solvent extracts of Acalypha indica (Euphorbeace family) was tested against bacterial pathogens (Pseudomonas aeruginosa, E.Coli, Klebsiella Pneumonia and Staphylococcus aureus) and fungal strains (Candida albicans, Aspergillus niger, Candida tropicalis and Candida kefyr) using the Agar Well diffusion method. It was observed that all the extracts showed positive activity against bacteria and fungi. Ethanolic extract of Acalypha indica showed more potency against Staphylococcus aureus with an inhibition zone of 12.46 (mm) and Methanolic extract exhibited higher activity against E.coli with an inhibition zone of 11.26 (mm). Ethanolic extract of Acalypha indica showed prominent antifungal activity against candida albicans with an inhibition diameter of 12.53 (mm) and Aspergillus niger with a diameter of 9.21 (mm) when compared to other solvent extracts. Erythromycin and Ketoconazole were used as positive standards for antimicrobial and anti fungal experiments. In the present study, Ethanol extract showed a varying degree of inhibition to the growth of tested organisms compared to Methanol, Acetone and Chloroform against Bacteria and Fungi. The results confirmed the presence of antibacterial and antifungal compounds in shade dried extracts of Acalypha indica against human pathogenic organisms.

Keywords: Acalypha indica, Pseudomonas aeruginosa, E.Coli, Klebsiella Pneumonia, Staphylococcus aureus, Candida albicans, Candida tropicalis, Aspergillus niger, Candida tropicalis, Candida kefyr

INTRODUCTION
Herbal medicines have been playing a vital role in treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant derived compounds as potent drugs have been a part of human evolution and healthcare for thousands of years. Acalypha indica Linn. belongs to family Euphorbiaceae, and is a common weed found in Asia including India, Pakistan, Yemen, Sri Lanka and is well distributed in Tropical areas of Africa and South America. It is an annual herb, about 80 cm in height with its predominant habitant being waste places or fields. It has several common names: “kuceing galak” or “runpuit lis-lis”, “kuppaiemen” in India and “t’ie han tsai” in China. The root, stem and leaves of Acalypha indica possess potent medicinal value. The plant extracts are commonly used as an expectorant against asthma and pneumonia and as an emetic, emenagogue and anthelmintic. Acalypha indica has antibacterial activity against human pathogens causing nosocomial infection and several other microbes. The search for new antimicrobial and antifungal agents from natural sources has intensified in response to the limitations of currently available therapy and the emergence of drug-resistant strains. Several chemical and biological investigations have been carried out on the leaf extract of Acalypha indica. Its leaf juice is added to oil or lime to treat a variety of skin disorders and other ailments. Antibiotics have come to denote a broader range of antimicrobial compounds, including antibacterial and antifungal compound. Acalypha indica is a common weed while viper russelli russelli is amongst the deadliest snakes in the south Asia. Ethanol leaf extract of Acalypha indica possesses potent snake venom neutralizing properties. A drug having a marked action on the respiratory organs and alimentary canal is Acalypha indica L. (family: Euphorbiaceae) is a weed widely distributed throughout India. It has been reported to be useful in treating hemolysis, analgesis and anticancer and several other diseases. In the present study, an attempt has been made to enrich the knowledge of antibacterial and antifungal activity leaf extract of Acalypha indica against Gram-positive and Gram-negative bacteria and fungal cultures. In the recent years, interest in medicinal plants has increased in a great deal. Apart from this people from the West and East have also taken this matter seriously by conducting various researches on plant based medicinal compounds. Traditional medicines are used by about 80 per cent of the world’s population. These plant based medicinal compounds are not only used for primary health care in rural areas of developing countries, but also rural.

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areas of developed countries as well, where modern medicines are predominantly used\textsuperscript{13}.

**MATERIALS AND METHODS**

**Collection of plant material**

*Acalypha indica* were collected from in CIMAP (Central Institute of Medicinal and Aromatic Plants) Boduppal, Hyderabad and Authenticated at Department of Botany, Osmania University, Hyderabad.

**Extraction of plant material**

The plant leaves were washed twice thoroughly with sterile distilled water in order to remove traces of dust and soil particles present on the surface and were dried in shade\textsuperscript{14} then made into fine powder. This powder was subjected to solvent extraction using soxhelt apparatus. Powdered samples were extracted (100g/200ml) in Ethanol, Methanol, Acetone, and Chloroform, for 72 hours at room temperature. The extracts from these solvents were evaporated under rotar vapour to concentrate the extract.

**Test organisms**

The bacterial species used for the test were, *Pseudomonas aeruginosa*, *E.Coli*, Klebsiella pneumonia and *Staphylococcus aureus*. The fungus species used for the test were *Candida albicans*, *Aspergillus niger*, *Candida tropicalis* and *Candida kefy*. All the stock cultures were obtained from Department of Microbiology, Osmania University, Hyderabad, Telanaga, INDIA.

**Culture media and inoculum preparation**

Nutrient agar broth (NBA) (Himedia) was used as the media for culturing bacteria. Loops full of all the bacterial cultures were inoculated in nutrient broth and incubated at 37°C for 72 hrs. (Potato dextrose agar and potato dextrose broth (Himedia, India) were used to culture fungal strains. Loops full of the fungus were inoculated in the Potato dextrose broth (PDA) and incubated at room temperature for 72hrs.

**Antibacterial activity**

The four extracts obtained were screened for antibacterial activity in comparison with standard antibiotic erythromycin (100mg/ml) in vitro by well diffusion method\textsuperscript{15,16} Lawn culture was used the test organism on Muller Hinton Agar (MHA). The inoculated plates were kept aside for 5 minutes, using a well cutter four wells were made in the plates at prescribed distance. After each step of cutting, the well cutter was thoroughly wiped with 70% ethanol. Using sterilized micropipettes 25ml of different solvents with selected *Acalypha indica* extract was added into the well. The plates were incubated at 37°C for 48 hours. The activity of the extract was determined by measuring the diameters of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents without leaf extracts were used.

**Antifungal activity**

The four extracts were also screened for antifungal activity in comparison with standard antibiotic Ketoconazole (10mg/ml) invitro by well diffusion method\textsuperscript{15,16} Lawn culture was prepared using the test organism on Sabouraud’s Dextrose Agar (SDA). The inoculated plates were kept aside for 5 to 10 minutes, using well cutter, four wells were made in those plates at prescribed distance. Using sterilized micropipettes 25ml of different solvents with selected leaf extract was added into the well.

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**Table 1: Inhibition zone diameter different extracts of *Acalypha indica* leaf extract against different microbial organisms (Mean) (Mm)**

<table>
<thead>
<tr>
<th>Bacterial cultures</th>
<th>+ve/-ve</th>
<th>Methanol extract</th>
<th>Ethanol extract</th>
<th>Acetone extract</th>
<th>Chloroform extract</th>
<th>Erythromycin (Antibiotic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.Coli</em></td>
<td>-ve</td>
<td>11.26</td>
<td>10.33</td>
<td>10.50</td>
<td>7.36</td>
<td>12.50</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>-ve</td>
<td>10.66</td>
<td>6.93</td>
<td>9.48</td>
<td>7.22</td>
<td>13.90</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+ve</td>
<td>11.55</td>
<td>12.46</td>
<td>9.23</td>
<td>6.28</td>
<td>15.20</td>
</tr>
</tbody>
</table>

**Graph:** Antimicrobial activity of different crude extracts against different microbial cultures.
Ketoconazole, acetone. Chloroform extract was more effective, whereas chloroform suggests that acetone, chloroform.

Antibacterial and antifungal activity against Candida kefyr effective against Candida albicans, Candida tropicalis effective against pathogenic organisms. Ethanol, methanol extracts have shown activity against the tested organisms. Ethanol, methanol, acetone have shown antibacterial and antifungal activity against all microorganisms tested. There are many reports of plants in the family Euphorbiaceae possessing antimicrobial activity and antifungal activity. Several studies have been conducted in the past three decades that demonstrate antimicrobial properties in herbs, and their derivatives such as essential oils and leaf extracts. From this study it can be said that, methanol, ethanol and acetone shade dried extract of Acalypha indica leaf extract showed wide range of Antibacterial and Antifungal activity and can be used and administered in medical practice. The present study has shown a spectrum of antibacterial activities which provides a support to some traditional uses of these medicinal plants. But the effective bio molecules which act as antibacterial are yet to be identified, separated and subjected to extensive scientific and pharmacological analysis that can be used as sources for new drugs.

RESULTS AND DISCUSSION
The efficacy of different extracts of Acalypha indica is shown in the table1. Methanol, Ethanol, and Acetone extracts have shown better activity against these pathogenic organisms. Methanol, ethanol and acetone extract were more effective against Staphylococcus aureus and Escherichia coli, whereas Chloroform extract was more effective against Escherichia coli and Klebsiella pneumonia. Among these four extracts Chloroform showed least efficacy on bacterial strains tested, in comparison with standard drug erythromycin. Extracts of different solvents have shown antifungal activity against the tested organisms. Ethanol, methanol and acetone have shown better activity against these pathogenic organisms. Methanol extract was more effective against Candida tropicalis and Candida albicans. Ethanol extract was more effective against Candida albicans and Aspergillus niger. Acetone extract was more effective against Candida tropicalis, Candida albicans and Candida kefyr. Chloroform extract was more effective against Candida kefyr. Among these four extracts Chloroform showed least potency towards the strains, in comparison with the standard drug, ketoconazole. The results of antifungal activity are shown in the table 2. Antibacterial activity leaf extracts of A. indica showed varying degrees of antibacterial and antifungal activity against all microorganisms tested. There are many reports of plants in the family Euphorbiaceae possessing antimicrobial activity and antifungal activity. Several studies have been conducted in the past three decades that demonstrate antimicrobial properties in herbs, and their derivatives such as essential oils and leaf extracts. From this study it can be said that, methanol, ethanol and acetone shade dried extract of Acalypha indica leaf extract showed wide range of Antibacterial and Antifungal activity and can be used and administered in medical practice. The present study has shown a spectrum of antibacterial activities which provides a support to some traditional uses of these medicinal plants. But the effective bio molecules which act as antibacterial are yet to be identified, separated and subjected to extensive scientific and pharmacological analysis that can be used as sources for new drugs.

CONCLUSION
The result of this work suggests that the leaf extract of Acalypha indica has various medicinal properties, which may be attributed to the compounds present in it. From this study it can be said that the shade dried Acalypha indica leaf extract of Methanol, Ethanol and Acetone is more effective against pathogenic strains (bacterial and fungal) and can be used for the future references for various other diseases.

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