A study on antimicrobial activity of Acalypha indica crude extraction of methanolic compound

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Abstract

Dried leaves of Acalypha indica was extracted with methanol by cold percolation. From the observed data, crude extraction concentration 500 and 250 µg completely arrest the bacterial growth except Enterococcus faecalis and Shigella boydii. 125 µg concentration crude extract also completely inhibit the bacterial cell growth in Escherichia coli, Pseudomonas aeruginosa, and P. mirabilis and Staphylococcus aureus. In Vibrio cholera 62.5 and 32.25 µg concentration of the crude extraction also shows inhibitory activity. The concentration which is completely arrest the bacterial growth, that is determined as minimum bactericidal (500, 250, 125, 62.5, and 31.25 µg concentration) concentration and which concentration is inhibit the bacterial growth comparing with the control culture that is determined as minimum inhibitory concentration (62.5 and 31.25 µg concentrations). From the above investigation the experimental plant may solve the multidrug resistant bacteria problem and further higher studies is need for qualitative study for the present investigation.

Keywords: antimicrobial activity, methanolic extraction, Acalypha indica

Introduction

There is evidence of the medicinal use of various Plants since pre-history. (Cowan, 1999). In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. In recent years pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population (Farnsworth, 1994). Medicinal plants constitute an effective source of traditional (Ayurvedic, Unani and Homeopathy) and modern medicine. About 80% of rural population depends on medicinal herbs as their primary health care (WHO, 1978). Siddha medicine is mainly based on the use of plants as medicine to cure many diseases. Similarly, Homeopathy, Ayurvedic and folk medicines utilize plants for therapeutic use. WHO (1993) in its special report on traditional medicine has emphasized the need to scientifically evaluate the therapeutic value of these plants.

Today, nearly 88% of the global population turns to plant derived medicines as their first line of defense for maintaining health and combating diseases. 119 secondary plant metabolites derived from plants are used globally as drugs; 15% of angiosperms have been investigated chemically and of which 74% are pharmacologically active plant derived components.

Biologically active compounds from natural sources have always been of great interest to scientists working on various infectious diseases. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against Cancer, as well as viral and microbial infections (Galal et al., 1991; Hoffmann et al., 1993). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko et al., 2002). In recent years, drug resistance of human pathogenic bacteria has been commonly reported from all over the world. Many
synthetic drugs may cause the side effects such as allergy, drug resistance etc; forcing scientists to seek for alternative drugs (Purma and Babu, 1998). Contrary to the synthetic drug, antimicrobials of plant origin do not cause side effects and also lodge an enormous therapeutic potential to heal many infectious diseases (Iwu et al., 1999). The characteristics of the plants that inhibit microorganisms are important for human health have been researched in laboratories since 1926 (Vondrban, 1949, Erdogru, 1999, Erdogru et al., 2001, Erdogru et al., 2002). Plants are also known to contain numerable biological active compounds (Alade and Irobi, 1993), which possess antibacterial properties (Brander and Grein, 1994 and Perumal Samy and Ignacimuthu, 1998). Apart from being antibacterial few medicinal plants also exhibit antiparasitic activity (Tshbangu et al., 2002).

Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues (Hirasa and Takemasa, 1998). Many medicinal plants contain large amount of antioxidants other than vitamin C, vitamin E and carotenoids (Velioglu et al., 1998). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the search for new infection fighting strategies (Sieradski et al., 1999). Due to the increasing development of drug resistance in human pathogens as well as the appearance of undesirable effect of certain antimicrobial agents, there is a need to search for new agents. A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Malcom and Sofowora, 1969; Blakuni et al., 1974; Taniguchi et al., 1978; Moskuleko, 1986; Brander and Grein, 1994; Grosnenor et al., 1995; Perumal Samy and Ignacimuthu, 1997). The objectives of the present investigation are: extraction of crude from dried leaves of Acalypha indica and screening of antibacterial activity using crude extractions.

**Materials and methods**

*Collection of plant samples*

The fresh leaves of Acalypha indica was collected in between Kancheepuram and Chennai. Healthy leaves were separated and shade dried for 10 days at room temperature. Dried leaves were powdered using mixer grinder. They were tightly packed in sterile polythene covers and stored at room temperature.

*Preparation of plant extract*

The leaf powder was extracted with 70% Methanol by cold percolation method. Three hundred grams leaf powder was soaked in 900 ml of Methanol and incubated for 10 days. After 10 days the filtrate was collected and concentrated by air drying method. The concentrated leaf extracts were stored at 4ºC in sterile vials for further studies.

*Antibacterial activity*

The antibacterial activity was screened by double dilution method for crude extraction of Acalypha indica leaf extraction. 2 ml of sterile nutrient broth medium is containing 0.1 ml of corresponding bacterial culture with 500mg of plant crude extract and followed by 1ml is transferred to subsequent test tubes containing nutrient broth with corresponding culture. The last test tube containing 0.97 mg of plant crude extraction with bacterial culture. The discs were impregnated with 100 µl (20 mg) plant extract.

*Bacterial strains*

A loop full of overnight grown culture was inoculated in test tubes containing sterile nutrient broth medium and kept for incubation at 37°C for 6 hrs and 0.1 ml of corresponding culture is transferred into new sterile nutrient broth with already plant crude extract doubly diluted test tubes ant the test tubes is kept incubation for 24 hrs at room temperature. Then the culture was taken in inoculation needle and single streaked in the sterile nutrient agar plates. After 24 hrs the bacterial strain streaked plates were examined and recorded the observations. Bacterial strains used in the present study were Escherichia coli,
Enterococcus faecalis, Pseudomonas aeruginosa, Pseudomonas mirabilis, Shigella boydii, Staphylococcus aureus, Staphylococcus epidermis, Salmonella typhimuri and Vibrio cholera. The strains were subcultured and maintained on nutrient agar slants.

**Results and discussion**

In the present investigation, the experimental plant Acalypha indica leaves methanol crude extraction and column chromatography fractions were screened against human pathogenic bacteria for antibacterial activity and the data’s was presented following.

**Antibacterial activity of crude extraction**

The antibacterial activity of crude extraction was determined for Acalypha indica leaves by double dilution method as described in materials method and the observed data’s were presented.

*Escherichia coli*

In *Escherichia coli*, 500 µg to 125 µg concentration of crude extraction added test tube culture does not show any growth throughout the experiment in streaking on nutrient agar plate and 62.5 µg to 0.97 µg concentration of crude extraction additive culture streaking showed the well bacterial growth (Table 1).

*Pseudomonas aeruginosa*

The bacterial culture of *Pseudomonas aeruginosa* shows susceptibility against the concentration of 500 µg to 62.5 µg of crude extract and rest of the concentration of crude extract concentration does not showed any inhibitory activity throughout the experiment (Table 1).

*Proteus mirabilis*

*Proteus mirabilis* showed growth inhibition against 500 µg to 62.5 µg but rest of the concentration of crude extract shows growth of the bacterial cells (Table 1).

*Staphylococcus aureus*

Among ten different concentration of crude extraction 500, 250, and 125 µg concentration showed susceptibility activity for the bacteria *Staphylococcus aureus* and remaining seven concentration does not showed any activity (Table1).

*Staphylococcus epidermis*

In *Staphylococcus epidermis*, 500 µg and 250 µg of crude extract concentration showed susceptibility and 62.5 µg showed slightly inhibitory activity and form the fourth concentration to tenth concentration does not show any inhibitory activity for the bacterial culture (Table 1).

*Salmonella paratyphi*

In *Salmonella paratyphi*, among ten different concentrations the crude extraction only first three concentration showed complete bacterial growth arresting activity and remaining seven concentrations does not inhibit the bacterial growth except fourth concentration (Table 1).

*Vibrio cholera*

In *Vibrio cholera*, there was no bacterial growth observed between first to fifth concentration of crude extract and sixth and seventh concentration crude extract showed inhibitory activity. Remaining three concentration does not showing any susceptibility activity (Table 1).

*Enterococcus faecalis* and *Shigella boydii*

In *Enterococcus faecalis* and *Shigella boydii* does not shows any inhibitory activity throughout the experiment against the crude extract. From the above collected data, crude extraction concentration 500 µg and 250 µg completely arrest the bacterial growth except *Enterococcus faecalis* and *Shigella boydii*. 125 µg concentration crude extract also completely inhibit the bacterial cell growth in *Escherichia coli, Pseudomonas aeruginosa, P. mirabilis* and *Staphylococcus aureus*. In *Vibrio cholerae* 62.5 µg and 32.25 µg concentration of the crude extraction also shows inhibitory activity. The concentration which is completely arrest the bacterial growth, that is determined as minimum bactericidal (500, 250, 125, 62.5 and 31.25 µg concentration) concentration and which concentration is inhibit the bacterial growth comparing with the control culture that is determined as minimum inhibitory concentration (62.5 and 31.25 µg concentration).

In the plant kingdom, most of the plants are used in traditional medicine, but their entire phytochemical components and active principles are unexplored. In present
study, leaves of *Acalypha indica* were taken for partial purification of antibacterial components against human pathogens. Past few decades many of the species of *Acalypha indica* parts was studied for antimicrobial and antibacterial components. Govindarajan et al. (2008) studied the effect of methanol extract of *Acalypha indica* against gram positive microorganisms. In present investigation, leaves methanol extraction showed higher inhibition in vitro studies against ten human pathogens. Among ten human pathogens, *Salmonella paratyphi* and *Vibrio cholerae* showed maximum inhibition on the rest of the bacteria studied. A similar work was done by Krishnaraj (1998), in *Acalypha indica* and

**Table 1.** Screening of antibacterial activity of *Acalypha indica* leaves methanol extraction

<table>
<thead>
<tr>
<th>Strains used</th>
<th>Control</th>
<th>Crude extract (in µg) inhibit the bacterial growth or not</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500</td>
<td>250</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
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</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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</tr>
<tr>
<td><em>Pseudomonas mirabilis</em></td>
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<td>+</td>
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<tr>
<td><em>Shigella boydii</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
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</tr>
<tr>
<td><em>Staphylococcus epidermis</em></td>
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</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
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<tr>
<td><em>Vibrio cholerae</em></td>
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</table>

they investigated antibacterial activity of silver nano particles using *Acalypha indica* leaf extracts against *Staphylococcus* species *Bacillus* species, *Escherichia coli* and *Vibrio cholera* species. The acetone and ethanol extracts showed higher activity against nearly all test microorganisms. In present investigation, also sequential extract of acetone showed the highest inhibition zone against *Salmonella paratyphi* and less inhibitory zone against *E. coli*. In their experiment methanol extracts showed stronger growth inhibition against both bacteria and fungi. However, in present investigation sequential acetone extraction showed stronger activity than the remaining organic solvent extraction. On contrary to the above reviews, bacterial species were apparently susceptible to the column fractions and also crude extract of *Acalypha indica* methanol extract. The present study revealed the experimental plant posses bactericidal and as well as inhibitory activity against human pathogens and further studies need for specific scientific reason.

**Conclusions**

In the present investigation, dried leaves of *Acalypha indica* was extracted with methanol by cold percolation method. From the observed data, crude extraction concentration 500µg and 250µg completely arrest the bacterial growth except *Enterococcus faecalis* and *Shigella boydii*. 125 µg concentration crude extract also completely inhibit the bacterial cell growth in *Escherichia coli*, *Pseudomonas aeruginosa*, and *P. mirabilis* and *Staphylococcus aureus*. In *Vibrio cholera*, 62.5 and 32.25µg concentration of the crude extraction also shows inhibitory activity. The concentration which is completely arrest the bacterial growth, that is determined as minimum bactericidal (500, 250, 125, 62.5, and 31.25 µg concentration) concentration and which concentration is inhibit the bacterial growth comparing with the control culture that is determined as minimum inhibitory concentration (62.5 and 31.25 µg concentrations). From the above investigation the experimental plant may solve the
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References


