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Evaluation of Anti Microbial Activity of *Acalypha indica*

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ABSTRACT

To investigate the antimicrobial activity of Ethanol, Methanol, Acetone, Chloroform, Hexane and Petroleum ether extract of *Acalypha indica* was tested against infectious disease causing bacterial pathogens such as *E.Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella Pneumonia* fungus like *Aspergillus niger*, *Candida albicans*, *Candida kefyr* and *Candida tropicalis* using the Agar Well diffusion method. . It was observed that Ethanol, Methanol, Acetone, Chloroform, Hexane and Petroleum ether showed activity against Bacteria and Fungi. The Ethanol extract of *Acalypha indica* showed more activity against *Staphylococcus aureus* zone of diameter 13.83 ± 0.29 and *E.coli* zone of diameter 11.0 ± 0.20 and the ethanol extract of *Acalypha indica* showed more activity against *candida albicans* zone of diameter 13.93 ± 0.12 and *Aspergillus niger* of diameter 9.97 ± 0.96 , when compared to other solvent extracts. In the present study, both in bacteria and fungi ethanol extract showed a varying degree of inhibition to the growth of tested organism than Methanol, Acetone, Chloroform, Hexane and Petroleum ether. The results confirmed that presence of antibacterial and antifungal activity in the sundried extract of *Acalypha indica* against the human pathogenic organisms.

KEYWORDS: *Acalypha indica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Aspergillus niger*, *Candida albicans*

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INTRODUCTION

Antibiotics are powerful medicines that fight bacterial infections. Used properly, antibiotics can save lives. They either kill bacteria or keep them from reproducing. Antibiotics provide the main basis for the therapy of microbial and bacterial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents. With increased knowledge of the causative agents of various infectious diseases, antibiotics has come to denote a broader range of antimicrobial compounds, including antibacterial, antifungal and other compound¹. An antibacterial is a compound or substance that kills or slows down the growth of bacteria² With advances in medicinal chemistry most of today's antibacterial's chemically are semisynthetic modifications of various natural compounds³ These include, for example, the beta-lactum antibacterials, which include the penicillins (produced by fungi in the genus *Penicillium*), the cephalosporins and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterials for example, the sulfonamides, the quinolones, and the oxazolidinones are produced solely by chemical synthesis. In accordance with this, many antibacterial compounds are classified on the basis of chemical/biosynthetic origin into natural, semisynthetic and synthetic. Another classification system is based on biological activity, in this classification, antibacterials are divided into two broad groups according to their biological effect on microorganisms, bactericidal agents kill bacteria, and bacteriostatic agents slow down or stall bacterial growth.

In the recent years, the interest in medicinal plants has increased in a great deal. Apart from this people from the west have also taken this matter seriously by conducting various researches on plant based medicines. Traditional medicines are used by about 60 per cent of the world's population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While the traditional medicines are derived from medicinal plants, minerals, and organic matter, the herbal drugs are prepared from medicinal plants only. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes, hence this requires proper documentation and research. In western world also, the use of herbal medicines is steadily growing with approximately 40 per cent of population reporting use of herb to treat medical illnesses within the past year. Public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine, against microbial infections⁴. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by

pathogenic microbial infectious agent's has led to the screening of several medicinal plants for their potential antimicrobial activity^{5,6}. *Acalypha indica* Linn Is a member of Euphorbiaceae family. It is essentially a weed which grows in waste areas. It is an annual herb, about 80 cm high and commonly found in waste places or fields⁷. Previous studies on *A. indica* revealed that this plant has antibacterial activity against several gram positive bacteria^{8,9} the petroleum ether and ethanol extract were found to have anti-implantation activity when they were given to female albino rats. This effect was reversible upon withdrawal of the treatments with the extracts. This effect is due to some oestrogenic activity as evidenced by histological studies of the uterus¹⁰. Studies found that *Acalypha indica* does have wound healing ability, however it is inferior to *Heliotropium indicum* which has better activity and tensile strength¹¹. *Acalypha indica* is a common weed while *Viper russelli russelli* is amongst the deadliest snakes in the world. Ethanol leaf extract of *Acalypha indica* possess potent snake venom neutralizing properties¹². A drug having a marked action on the alimentary canal and respiratory organs. It is indicated in incipient phthisis, with hard, racking cough, bloody expectoration, arterial hemorrhage, but no febrile disturbance. In congestive headache a piece of cotton saturated with the expressed juice of the plant or leaves and inserted into each nostril is said to relieve it by causing hemorrhage from the nose¹³.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Acalypha indica were collected from in Maduravoyal region, Tamilnadu India and used for this study. All the lab works are done in microlabs, Institute of Research and tech. Arcot, Tamilnadu.

EXTRACTION OF PLANT MATERIAL

They were washed thoroughly with sterile distilled water in order to remove any dirt or filthy particles present on the surface and were dried in sunlight¹⁴ then made into fine powder, this powdered samples (100g/100ml) in ethanol, methanol, acetone, chloroform, hexane and petroleum ether for overnight at room temperature., soxhelt apparatus are used for this extraction^{15,16}. The extract from these solvents are soaked and evaporated under pressure.

TEST ORGANISMS

The bacterial species used for the test were *E.Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*. The fungus species used for the test were *Aspergillus niger*, *Candida albicans*,

Candida kefyr, *Candida tropicalis*. All the stock cultures were obtained from Microlabs, Institute of Research and technology, Vellore Tamilnadu, India.

CULTURE MEDIA AND INOCULUM PREPARATION

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

ANTIBACTERIAL ACTIVITY

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic ciprofloxacin (100mg/ml) in vitro by well diffusion method (17, 18). Lawn culture was used using the test organism on Muller Hinton Agar (MHA). The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. In each step of well cutting the well cutter was thoroughly wiped with alcohol. Using sterilized micropipettes 30ml of different solvents with selected *Acalypha indica* extract was added into the well. The plates were incubated at 37°C for overnight. 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 extracts were used.

ANTIFUNGAL ACTIVITY

The extracts were also screened for their antifungal activity in comparison with standard antibiotic Ketoconazole (10mg/ml) invitro by well diffusion method (17, 18). Lawn culture was prepared using the test organism on Sabouraud's Dextrose Agar (SDA). The inoculated plates were kept aside for few minutes using well cutter, four wells were made in those plates at required distance. Using sterilized micropipettes 30ml of different solvents with selected leaf extract was added into the well. The plates with fungi were incubated at room temperature for 48hrs. The activity of the root extract was determined by measuring the diameter of zone on inhibition. For each fungal strain controls were maintained where pure solvents were used.

RESULTS AND DISCUSSION:

The efficacy of different extracts of *Acalypha indica* is shown in the table1. The Ethanol, methanol and acetone extracts have shown better activity against these pathogenic organisms. Ethanol extract was more effective against *Staphylococcus aureus* and *Escherichia coli*. Methanol extract was more effective against *Staphylococcus aureus* and *Escherichia coli*. Acetone extract was more effective against *Escherichia coli* and *Staphylococcus aureus*. Chloroform extract was more effective against *Escherichia coli* and *Klebsiella pneumonia*. Hexane extract was more effective against *Klebsiella pneumonia* and *Staphylococcus aureus*. Petroleum ether extract was more effective against *Staphylococcus aureus* and *Klebsiella pneumonia*. Among these 6 extracts Ethanol, methanol, acetone shows better activity against Chloroform, hexane and petroleum ether against the standard drug Ciprofloxacin. The results of antibacterial activity are shown in the table 1 and figure1.

Table1: Inhibition zone diameter different extracts of *Acalypha indica* against different organisms (Mean±SEM) (mm)

Organisms (Bacteria)	zone of inhibition (mm)						
	Ethanol	Methanol	Acetone	Chloroform	Hexane	Petroleum ether	Ciprofloxacin
<i>Escherichia coli</i>	11.0±0.20	12.9±0.12	11.10±0.15	8.17±0.15	Nil	Nil	10.7±0.11
<i>Pseudomonasa eruginosa</i>	9.97±0.6	11.0±0.20	10.7±0.58	7.03±0.58	Nil	Nil	14.7±0.29
<i>Staphylococcus aureus</i>	13.83±0.29	12.9±0.12	10.0±0.11	7.20±0.10	6.00±0.00	8.10±0.10	18.17±0.15
<i>Klebsiella pneumoniae</i>	7.90±0.10	12.0±0.00	10.0±0.15	8.07±0.12	7.03±0.58	6.03±0.58	11.17±0.15

The results of antifungal activity are given in the table2. Which clearly show that all the extracts have shown antifungal activity against the tested organisms. Ethanol, methanol and acetone have shown better activity against against these pathogenic organisms. Ethanol extract was more effective against *Candida albicans* and *Aspergillus niger*. Methanol extract was more effective against *Candida tropicalis* and *Candida albicans*. Acetone extract was more effective against *Candida tropicalis*, *Candida albicans* and *Candida kefyrr*. Chloroform extract was more effective against *Candida kefyrr*.

Hexane and Petroleum ether extract was less effective only for *Candida kefy* and others show no active.

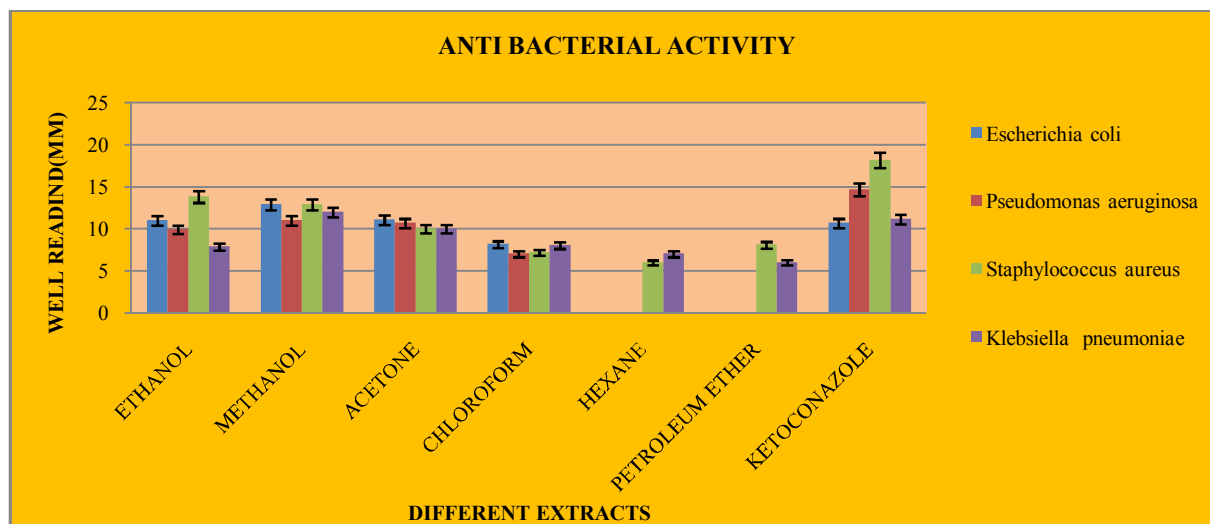


Figure 1: Antibacterial activity of different extracts of *Acalypha indica* (*Euphorbiaceae*) against different organisms

Table 2: Inhibition zone Diameter of different extracts of *Acalypha indica* of Against different organisms (Mean± SEM) (mm)

Organisms (fungus)	Zone of inhibition (mm)						
	Ethanol	Methanol	Acetone	Chloroform	Hexane	Petroleum ether	Ketoconazole
<i>Aspergillus niger</i>	9.97±0.10	7.03±0.06	8.0±0.00	7.10±0.10	Nil	Nil	10.93±0.12
<i>Candida albicans</i>	13.93±0.12	9.07±0.12	9.10±0.10	6.0±0.00	Nil	Nil	13.17±0.29
<i>Candida kefy</i>	8.07±0.12	8.03±0.06	9.07±0.12	8.17±0.15	7.03±0.06	7.92±0.02	10.90±0.10
<i>Candida tropicalis</i>	9.07±0.12	10.27±0.05	10.17±0.15	7.01±0.06	6.07±0.12	6.20±0.06	10.07±0.12

Antibacterial activity all 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 anti-microbial activity (19, 20, and 21). several studies have been conducted in the past three decades that focused on the antimicrobial properties of herbs, spices and their derivates such as essential oils, extracts and decoctions (22, 23, 24, 25).

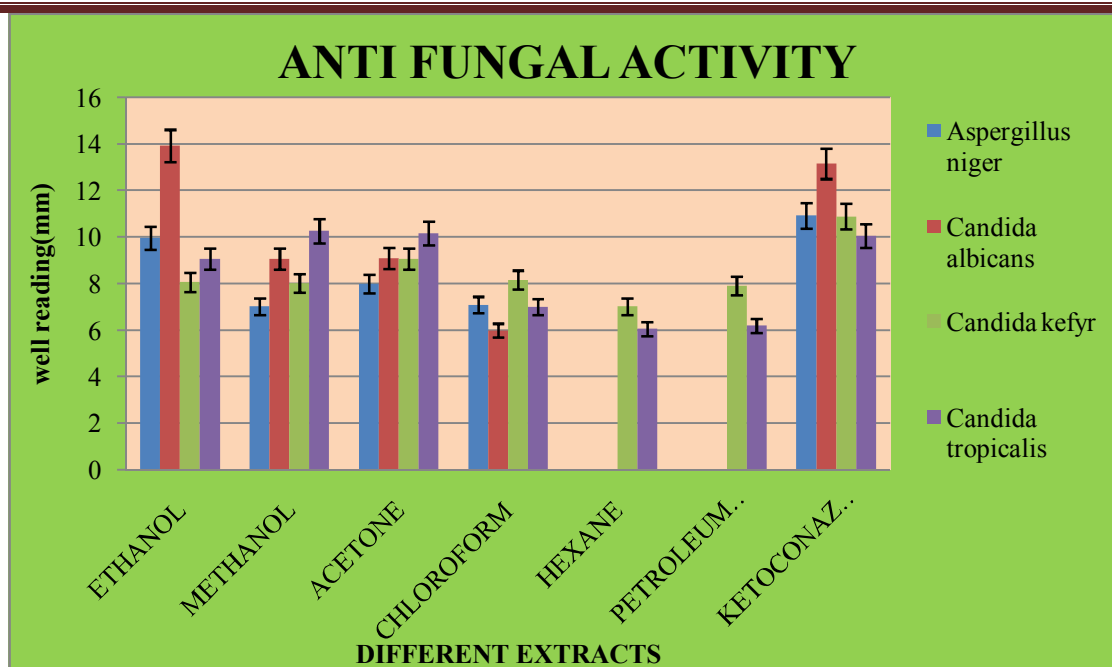


Figure 2: Antifungal activity of different extracts of *Acalypha indica* (*Euphorbiaceae*) against different organisms

From this study it can be said that, Ethanol, methanol and acetone sun dried extract of *Acalypha indica* showed wide range of Antibacterial and Antifungal activity and can be used and administered in the ethno medical practice. The present study has shown a spectrum of antibacterial activities which provides a support to some tradition uses of these few medicinal plants. But the effective biomolecules which act as antibacterial have to be identified isolated and subjected to extensive scientific and pharmacological screening that can be used as sources for new drugs.

CONCLUSION:

The result of this work suggests that the whole plant extract of *Acalypha indica* has number of medicinal properties. From this work it can be said that the sun dried *Acalypha indica* extract of Ethanol, Methanol and Acetone has more effective against these pathogenic organisms and can be used for the future references for various other diseases.

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