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Evaluation of Antimicrobial Activity of Indian Nettle, Acalypha Indica

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Abstract: *Acalypha indica is a species of plant having catkin type of inflorescence. It occurs throughout tropical Africa and South Africa, in India and Srilanka, as well as in Yemen and Pakistan. This plant is held in high esteem in traditional tamil siddha medicine as it is believed to rejuvenate the body. Pharmacological investigation has shown that the plant has potent antibacterial, antifungal, anti – inflammatory, anti – osteoporotic, antioxidant, neuro protective, wound healing, post – coital antifertility activities. The present work is aimed to evaluate the antimicrobial activity of Acalypha indica leaf extract. Antibacterial activity is studied against strains Staphylococcus aureus, Escherichia coli, Salmonella typhi, Bacillus and Pseudomonas aeruginosa. We got maximum zone of inhibition of 13mm for Staphylococcus aureus and minimum zone of inhibition of 7mm for Escherichia coli. Antifungal activity is studied against species Rhizopus microsporus, Candida albicans, Aspergillus niger and Trichoderma viride We got maximum zone of inhibition of 10mm for Rhizopus microsporus and minimum zone of inhibition of 8mm for Aspergillus niger.*

***Keywords*:** *Acalypha indica*, antibiotics, antimicrobial.

INTRODUCTION

Acalypha indica (Kuppameni) is a plant found commonly in India, Sri Lanka, and Africa, as well as in Southeast Asia (Sanseera et al. 2012). Commonly known as the Indian nettle or three-seeded mercury, this herb has different names in various countries; in Indonesia it is known as lelatang, in China tie xian, and in India kuppikokli in Hindi and haritamanjari in Sanskrit (Globeinmed 2012). In Malaysia, as in India, this herb is found amidst fields and waste places throughout the country, hence its name kuppameni (pearl in the waste) in India. It is also known as galak kuching (Azmahani *et al*. 2002) in Malaysia. This herb plant is considered a troublesome perennial weed which can grow to a height of 1metre (Sanseera et al. 2012). Research suggested that this traditional herb is used to treat scabies (Gurib-Fakim *et al*. 1993), arthritis and relief of ulcer pains (Dhar et al. 1968), healing of wounds (Reddy *et al*. 2002), as a purgative (Panthong et al. 1991), snake bite anti-venom (for example, Siddiqui and Husain 1990; Samya et al. 2008), and as poultice, with lime or oil (Govindarajan *et al*. 2008). In India this plant is highly regarded in traditional Tamil siddha (traditional) medicine, as it is said to rejuvenate the body because of its anti-oxidant properties (Govindarajan *et al*. 2008). The traditional siddhas prepare a concoction for medicinal use. This herb is also extensively used from Asia to the Polynesia in traditional medicine (Burkill 1985). A recent study by Sanseera *et al*. (2012) suggested that this troublesome weed contains a rich source of antioxidant that is helpful in rejuvenating the body. Benefits of antioxidants first surfaced in the 1990s, when researchers found evidence on the role of free radicals in aging, vision loss and cancer (Valko *et al*. 2006; Sifferlin 2013) Free radicals are a

natural byproduct of the body's metabolism because body cells need oxygen to breakdown food for energy, which in turn can cause cell damage. The availability of antioxidants helps minimize cell damage (Sifferlin 2013).

Antimicrobial activity

Antibiotics provide the main basis for the therapy of microbial infection. Since the discovery of these antibiotics and their uses as chemotherapeutic agents, there was beleif in medical fraternity that this would lead to eventual eradication of infectious diseases(Rosina *et al* 2009). However, overuse of antibiotics has become the major factor for the emergence of multidrug resistant strains of several groups of microorganisms(Gardiner 2006). In the light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agent is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents indicates that even new families of antimicrobial agents will have short life expectancy(Contes *et al* 2002). A wide variety of antibiotics are commonly used for the treatment of serious infections caused by aerobic gram negative bacteria (Tamah, 2005). The increased use of antibiotics has resulted in the development of resistant bacteria (Derrida,2003). In recent years, misuse of antibiotics resulting in multidrug resistance among bacteria has accelerated the serch for drugs and dietary supplements effective against such multidrug resistant bacteria. It has been reported that in 1996, sales of botanical medicines increased by 37% over 1995 (Thongson *et al* 2004). In this connection, different parts of plants, herbs and spices have been used for prevention of infections. These are easily available and can be used in domestic setting for self medication.

MATERIALS AND METHODS- COLLECTION OF PLANT PARTS

Leaves of mature *Acalypha indica* plants (1kg wet weight) were collected from Chennai, Tamilnadu, India and identified.

Extraction:

25g of each sample was weighed and extracted with 300ml of methanol by continuous hot percolation with the help of soxhlet apparatus for 10hrs of time. On completion the extracts were filtered and concentrated using rotary evaporator under reduced pressure and controlled temperature of 50^0C – 60^0 C. The concentrates were stored in the refrigerator for further use.

PHYTOCHEMICAL ANALYSIS: (Raman, 2006)

1. TEST FOR TANNINS:

1ml of sample was taken, to that few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black coloration.

2. TEST FOR SAPONINS:

1 ml of sample was taken, to that 2 ml of water was added .The suspension was shaken in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins.

3. TEST FOR FLAVONOIDS:

1 ml of sample was taken, to that concentrated hydrochloric acid and magnesium chloride was added and observed for tomato red colour.

4. TEST FOR ALKALOIDS:

1 ml of sample was taken, to that few drops of drag and off reagent was added. A prominent yellow precipitate indicates the test as positive.

5. TEST FOR PROTEIN:

1 ml of sample was taken, to that few drops of Millon's reagent was added. A white precipitate indicates the presence of Protein.

6. TEST FOR STEROIDS:

1 ml of sample was taken, to that two drops of 10% concentrated sulphuric acid was added and observed for brown colour.

7. TEST FOF ANTHRAQUINONES:

1 ml of sample was taken, to that aqueous ammonia was added and observed for change in colour. Pink, red, or violet colour in aqueous layer indicates the presence of anthraquinoness.

8. TEST FOR PHENOL:

1 ml of sample was taken, to that 3 ml of 10% lead acetate solution is added a bulky white precipitate indicates the presence of phenolic compounds.

ANTIBACTERIAL ACTIVITY ASSAY:

Number of samples: 01
Number of Microorganisms: 5
Staphylococcus aureus
Escherichia coli
Pseudomonas aeroginosa
Salmonella thphi
Bacillus
Standard: Ampicillin (20µl/disc)

PREPARATION OF INOCULUM:

Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth , that were incubated at 24hrs at 37ºC. The Assay was performed by agar disc diffusion method.

AGAR DISC DIFFUSION METHOD:

Antibacterial of extracts was determined by disc diffusion method on Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .The plates were incubated at 37ºC for 24 hrs. Then the antimicrobial activity was determined by measuring the diameter of zone of inhibition.

ANTIFUNGAL ACTIVITY ASSAY:

No of samples: 01
No of extracts: 01 (Methanol)
Number of Microorganisms: 4
1.Candida albicans
2.Aspergillus Niger
3.Trichoderma viride
4.Rhizopus microsporus
Standard: Amphotericin-B (20µl/disc)

PREPARATION OF INOCULUM:

Stock cultures were maintained at 4°C on Sabouraud Dextrose agar Slant. Active cultures for experiments were prepared by transferring the stock cultures into the test tubes containing Sabouraud Dextrose broth that were incubated at 48 hrs at room temperature. The assay was performed by agar disc diffusion method.

AGAR DISC DIFFUSION METHOD:

Antifungal activity of the extracts was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Amphotericin-B is taken as positive control. Samples and positive control of 20 µl each were added in sterile discs and placed in SDA plates. The plates were incubated at 37ºC for 24 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibition.

Table 1: The Phytochemical studies of the sample

TEST	**kuppaimeni**
	Methanol
TANNINS	-
SAPONINS	+
FLAVONOIDS	-
ALKALOIDS	+
PROTEINS	-
STEROIDS	+
ANTHROQUINONES	-
PHENOL	+

(+) = Positive
(-) = Negative
The present study aimed at testing the antimicrobial, antioxidant and anticancer activity of *Acalypha indica*

Antibacterial activity

Antibacterial activity of *Acalypha indica* was studied against five bacterial strains *Bacillus, Staphylococcus aureus, Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa*. The extract of Acalypha indica showed the maximum zone of inhibition against Staphylococcus aureus and minimum inhibition against Escherichia coli.

Antifungal activity

Antifungal activity of Acalypha indica was studied against four fungal species Rhizopus, Candida, Aspergillus and Trichoderma. The extract of Acalypha indica showed maximum zone of inhibition against Rhizopus and minimum zone of inhibition against Aspergillus.

DISCUSSION

Acalypha indica commonly referred to as Kuppaimeni. The plant has wide uses in traditional medicines of various countries. *A.indica* leaves contain acalyphine which is used in the treatment of sore gums and have post coital and fertility effect, anti inflammatory effect, diuretic effects etc (Sumathi and Puspa 2007) was reported. The aqueous extract of *A.indica* shows 9mm inhibition zone to *Escherichia coli* and no zone were shown against *Staphylococcus aureus, Salmonella typhi* and *Shigella flexneri.* Alcoholic extract of *A.indica* shows 10mm inhibition zone towards *Staphylococcus aureus* and *Salmonella typhi.*

The leaf extract of *Acalypha indica* like chemical antibiotics exhibit antimicrobial properties by inhibiting the growth of the microorganism. This property is due to the presence of phytochemicals in the extract. If further studied they can be proven as even better antibiotics than chemical antibiotics. These antibiotics unlike chemical antibiotics do not have major side effects and are cheaper and easily available ones. In our study, we assayed for the antimicrobial, antioxidant and anticancer activity of extract of *Acalypha indica.* The antibacterial activity assay done against five bacterial strains *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus.* We got maximum zone of inhibition of 13mm for *Staphylococcus aureus* and minimum zone of inhibition of 7mm for *Escherichia coli.* The antifungal assay done against four fungal species *R. microsporus, C.albicans, A. niger* and *T. viride.* We got maximum zone of inhibition of 10mm for *R. microsporus* and minimum zone of inhibition of 8mm for *A. niger.* The acetone extract of *A. indica* was found to be more effective against majority of the bacterial strains (Shoba, 2008). (Khaleel Basha and Sudarshanam, 2011) reported the antimicrobial activity of *A. indica* leaves was due to the presence of phytochemical compounds like tannins, alkaloids, saponins, phenolics and flavanoids. In our present study we identified that the leaves of *A. indica* contains phytochemicals such as saponins, alkaloids, steroid and phenol.

CONCLUSION AND SCOPE FOR FURTHER STUDY

The presence of antimicrobial activity in *Acalypha indica* was confirmed in this study. If further investigated it could serve as a novel therapeutic agent for various problems.India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for ayurvedic, unani, siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value. One such plant is *Acalypha indica*. The study of antibacterial activity of herbal plant extract of *A.indica* shows maximum antibacterial activity against *Staphylococcus aureu*s and minimum antibacterial activity against *Escherichia coli.* The study of antifungal activity of the herbal extract of *Acalypha indica* shows maximum antifungal activity against *Rhizopu*s and minimum antifungal activity against *Aspergillus*. Phytochemical analysis showed that antimicrobial activity of *Acalypha indica* was due the presence of phytochemical compounds such as saponins, alkaloids, steroids and phenols.

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