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Screening of Antibacterial and Phytochemical activity of *Acalypha indica Linn* against isolated respiratory pathogens

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Medicinal plants are extensively used to cure various infectious diseases in human. Our present study was undertaken to investigate antibacterial activity and phytochemical studies in leaf extraction of *Acalypha indica* against the Respiratory tract pathogens. The shade dried leaves powder was used to prepare extracts by using Aqueous and Alcohol under crude and soxhlet method. The antibacterial activity was studied by using agar well diffusion method. The result showed that crude aqueous extract is effective against all tested pathogen. Highest inhibition of zone was recorded in *Pseudomonas aeruginosa* (16mm). Aqueous and Alcoholic extracts reveals the presence of Alkaloids, Flavonoids, Steroids, Saponin, Tannins, Quinine, Coumarin and Phenol.

Key words: *Acalypha indica*, Antibacterial activity, phytochemical studies.

Respiratory tract infections are an important cause of morbidity and mortality for all age groups. Each year approximately seven million peoples are died as direct consequences of acute and chronic respiratory infection. Bronchitis and pneumonia are the most common infection. Respiratory pathogens like *Klebsiella pneumoniae, Pseudomonas aeroginosa* and *Staphylococcus aureus* are some of the causative agents responsible for bronchitis and pneumonia (Ponni *et al.*, 2000). In recent years multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use and commercial antibacterial drugs commonly used in treatment and injections diseases. This situation forced scientists for searching new antimicrobial substances from various sources like medicinal plants which are the good sources and novel antimicrobial chemotherapeutic agents (Karaman *et al.*, 2003). The toxicity produced by the antimicrobial agents can be cured or prevented or antagonize with herbs (Lin and song, 1989). Herbal molecules are safe, will overcome the resistance produced by the pathogens. Some herbs have antibacterial properties, which will be useful to clinical use (Kalemba and Kunica, 2003). Some *invitro* studies have been conducted that herbal oral liquids can be given to clinical drug resistant strains and

different serotype strains and infection (Lu *et al.*, 2002). World Health Organization (WHO) (1976) describes a medicinal plant as any plant in which one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and combacting diseases. Currently, people of Asia and India are utilizing plants as part of their routine health management (Perumal samy *et al*., 2008).

Acalypha indica Linn belongs to the family Euphorbiaceae. It is a common weed in many parts of Asia .It is an annual herb, about 80 cm high and commonly found in waste places or fields. It is locally known as "kucing galak" or "rumput lis-lis", "kuppaimeni" in India and "t'ie han tsai" in China (Kirtikar and Basu, 1975).This plant is used as diuretic, antihelmintic and for respiratory problems such as bronchitis, asthma and pneumonia (Varier, 1996). According to the Siddha text, 'Pathartha Guna Chinthamani' (page no: 179), *Acalypha* cures diseases of the teeth and gums, burns, toxins of Plant and mixed origin, stomach pain, diseases due to Pitha, bleeding piles, irritations, stabbing pain, wheezing, sinusitis and neutralizes predominance of the Kabha factor. According to Siddha Materia Medica the leaf powder when given in the dose of 950 mg to 1300 mgs, cures respiratory diseases.

In the present study, an attempt has been made to enrich the knowledge of anti bacterial activity of *Acalypha indica* leaves extracts against pathogenic bacteria like, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* which cause respiratory diseases.

Materials and Methods:

Collection and authentification Plant Material:

The plant material was collected from in and around Trichy Districts of Tamilnadu in India. The plant was authenticated by Prof. Dr. S. Ahmed John, and it has been deposited in the Herbarium, Department of Botany, Jamal Mohamed College, Tiruchirapalli, Tamil Nadu, India, for reference.

Preparation of Extracts:

Fresh *Acalypha indica* leaves were washed thoroughly in tap water and with distilled water and air dried in the shade at room temperature for five days. Shade dried leaves were powdered and the dried powder passed through sieve of 60 mesh (#)size and stores in airtight containers.The plant powders (100 g) were successively extracted by soxhlet and crude extraction methods with aqueous and alcohol. The extracts were dried in vaccum desicator and were stored in a sterile container for further use.

Collection of Samples:

Hundred sputum samples were collected from clinically diagnosed patients from Government Hospital, Srirangam. Samples were collected in the sterile containers and transported to laboratory for further analysis.

Isolation and identification of pathogens in sputum samples:

For the isolation of causative agents the sputum samples were inoculated in Blood agar and MacConkey agar. Plates were incubated at 37^{o}C for 24-48 hrs. Colonies were analyzed by physiological and biochemical test and confirmed as *Escherchia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogens* (Koneman et al., 1998).

Testing of antibacterial activity of various extracts:

Agar well diffusion method was followed by using Muller-Hinton Agar (MHA).The plates were seeded with 24 hours old culture of the isolates. The organic fractions were dissolved in Dimethyl sulfoxide (DMSO) and sterilized by using sortorious syringe filter of pore size 0.22µm. various concentrations of the extracts (250µl, 500µl, 750µl and 1000µl) were added into the sterile 8mm diameter well. Incubation was made at 37^0C for 24hrs. Antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well using standard (Hi-Media) scale. The experiment was repeated thrice and the average values were calculated for antibacterial activity (Perez *et al.,*1990).

Phytochemical screening

The plant extracts were screened for the presence of biologically active compounds like giycosides, phenolic, alkaloids, tannins, flavonoids, saponin and steroids under qualitative analysis (Chitravadivu et al., 2009).

Test for alkaloids:

To 2ml of test solution, added HCL, aqueous layer formed was decanted and to that added few drops of Mayer's reagent. The test result was observed.

Test for Anthraquinones:

To 2ml of test solution, added magnesium and acetate solution. The result was observed.

Test for Catachol:

To 2ml of test solution in alcohol added Erlich's reagent and few drops of concentrated HCL. The result was observed.

Test for Flavonoids:

To 2ml of test solution, added alcohol and bit of magnesium then few drops of concentrated HCL was added and boiled. The result was observed.

Test for Phenol:

To 2ml of test solution, added alcohol and then few drops of neutral ferric chloride solution was added. The result was observed.

Test for Saponin:

2ml of test solution, added 2ml of water and shake well. The result was observed.

Test for steroids:

To 2ml of test solution, added minimum quantity of chloroform. Then 3-4 drops of acetic anhydride and 3 drops of concentrated sulphuric acid were added. The result was observed.

Test for Tri Terpenoids:

To 2ml of test solution, added pieces of tin and 2 drops of thionyl chloride. The test result was observed.

Test for Tannins:

To 2ml of test solution, added lead acetate solution. The result was observed.

Thin layer chromatography:

Preparation of TLC Plates:

25x10 cm glass plates were washed with distilled water followed by smearing with acetone. After drying the plates were placed on the template in row. The slurry of silica gel G prepared with glass distilled water in the ratio 1:2 (w/v) was poured in the applicator. The glass plates were immediately coated with a layer of silica gel in 500µm thickness. The coated plates were activated at 80 c for 3 hours. Then the plates were stored in a plate chamber for further study. In that study chloroform and methanol (solvent) was used in 96:4 ratios.

Loading of substances:

The concentrated plant extract of 2.5 mg was loaded on the TLC plates just above 2 cm from the bottom using a capillary tube. The plates were reserved in a developing jar containing the solvent mixture. After, the solvent front reached approximately 18cm height. The plates were removed and allowed at room temperature for 30 min. Then the plates were also observed under UV light (240 and 300 nm) and recorded the

Rf value of fluorescence substances (Anushia *et al*., 2009).

Result and discussion

Antibacterial activity:

Antibacterial activity of *Acalypha indica* was investigated against isolated pathogens. crude aqueous shows best activity in *Pseudomonas aeroginosa* (16mm) followed by *Staphylococcus aureus* (10mm), *Streptococcus pyogenes*(10mm), *Escherichia coli*(8mm), *Klebsiella pneumoniae* (6mm). *Klebsiella pneumoniae was not* sensitive at low concentration. In crude alcohol extracts *Streptococcus pyogenes* (14mm) high sensitivity followed by *Staphylococcus aureus*(12mm), *Pseudomonas aeroginosa* (11mm), *Escherichia coli* (8mm), *Klebsiella pneumoniae* (5mm).Soxhlet aqueous shows inhibition against *Streptococcus pyogenes, Pseudomonas aeroginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus as* 15mm, 14mm, 9mm, 9mm and 8mm respectively. Soxhlet alcohol extracts shows highest zone in *Klebsiella pneumoniae* (12mm) and minimum zone of inhibition in *Escherichia coli* (6mm). Among all the extracts crude aqueous extracts found to be best for the bacterial inhibition (Table-1).

Table 1 Antibacterial activity of *Acalypha indica Linn* against Respiratory pathogens.

Organisms	Concentration of Extracts /Zone of inhibition in mm															
	Crude aqueous (µl)				Crude alcohol (µl)				Soxhlet aqueous (µl)				Soxhlet alcohol			
	250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Escherchia coli	-	4	6	8	-	-	6	8	-	-	7	9	-	-	-	6
Staphylococcus aureus	-	5	7	10	4	7	9	12	-	-	-	8	-	5	7	9
Pseudomonas aeruginosa	7	9	12	16	-	6	8	11	5	8	10	14	-	-	-	8
Klebsiella pneumoniae	-	-	-	6	-	-	-	5	-	-	6	9	-	-	8	12
Streptococcus pyogens	-	5	7	10	4	7	10	14	8	10	13	15	-	5	7	8

Table 2 Phytochemical Screening of *Acalypha indica Linn* Extracts

S.No	Phytochemical	Crude water	Crude alcohol	Soxhlet water	Soxhlet alcohol
1	Alkaloids	+	-	+	+
2	Flavonoids	+	+	+	+
3	Steroids	-	-	+	-
4	Anthraquinones	-	-	-	-
5	Tri Terpanoids	-	-	-	-
6	Tannins	+	-	-	-
7	Quinine	+	+	+	+
8	Coumarin	+	+	+	+
9	Saponins	+	+	+	+
10	Phenols	+	+	+	-
11	Catechols	+	+	-	+

Phytochemical activity:

The phytochemical screening reveals the presence of flavonoids, Quinine, coumarin, saponin present in all the four extracts. Alkaloids present in all the extracts except crude alcohol. Steroid present only in soxhlet aqueous extract. Anthraquinones and Triterpenoids were completely absent in all the four extracts. Tannin found to be present in crude aqueous where as phenol in crude alcohol, aqueous and soxhlet extracts. Catechols absent in soxhlet aqueous extract.

Thin layer chromatography (TLC):

Various spots were observed in all the four extracts. Pale yellow green spots were identified in crude extract. Yellow and pale yellow colour spots were observed in alcohol extracts of both crude and soxhlet methods. Green spot were identified in soxhlet aqueous extract. Ganga devi *et.al.*, (2008) also reported the similar observation for phytochemicals study (Table 3).

Table 3 TLC analysis of *Acalypha indica Linn* plant leaves extracts

S.No	Various extracts of *Acalypha indica* leaves extracts	Spot observed	Rf values
1	Crude aqueous	Pale yellow green	0.85
2	Crude alcohol	yellow	0.50
3	Soxhlet aqueous	Green	0.65
4	Soxhlet alcohol	Pale yellow	0.53

Discussion:

Plants are important sources of potentially useful structures for the development of new chemotherapeutic agents. Many reports are available regarding anti-viral, anti-bacterial, anti-fungal, anti-helminthic and anti-inflammatory properties of plants. These findings help to identify the active components responsible for the development of drugs for therapeutic uses. Our study was focused to find out the antibacterial activity and phytochemicals in *Acalypa indica* leaves. *Pseudomonas aeroginosa* was highly sensitive under low concentration some organisms shows absence of zone. *Klebsiella pneumoniae* were weakly sensitive when compare with other isolates. Crude extracts shows best activity than soxhlet.

Preliminary phytochemical analysis of plant extract of *Acalypa indica* shows the presence of Alkaloids, Flavonoids, Quinine, coumarin, Saponin, phenol and Catechol. The presence of these phytochemicals is a factor for antibacterial activity. The result of our study reveals that *Acalypha indica* is a good medicinal source to treat Respiratory Pathogens.

Conclusion

Various extracts of *Acalypha indica* leaves showed significant antibacterial activity and further purification is required to isolate active components which can be used as a lead compound for developing an antibacterial agent.

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