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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of Various Solvent Extracts of the Indian Herbal Plant *Acalypha indica* against Human Pathogens Causing Nosocomial Infection

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ABSTRACT

Nosocomial infections occur worldwide, both in the developed and developing world. They are a significant burden to patients and public health. They are a major cause of death and increased morbidity in hospitalized patients. They may cause increased functional disability and emotional stress and may lead to conditions that reduce quality of life. In this present study, the herbal plant *Acalypha indica* was tested for its antibacterial activity against Nosocomial infection causing bacteria. The *Acalypha indica* was shade dried and the antimicrobial principles were extracted with Methanol, Acetone, Chloroform, Petroleum Ether and Hexane. The antibacterial activity of Acalypha indica was determined by Agar Well Diffusion Method. It was found that 50mg/ml of methanolic extract of the plant able to inhibit the growth of nosocomial infection causing bacteria when compared to other solvent extracts. From this it was concluded that the solvent methanol able to leach out antimicrobial principle very effectively from the plant than the other solvents. The phytochemicals present in the *Acalypha indica* was tested and it conferred that the possible antibacterial principle resided in tannins and alkaloids.

Key words: *Acalypha indica*, Organic solvents, Antibacterial activity, Nosocomial infection and phytochemical analysis.

INTRODUCTION

A nosocomial infection also called "hospital acquired infection" can be defined as: An infection acquired in hospital by a patient who was admitted for a reason other than that infection. An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility [1]. Nosocomial infections are most frequently infections of the urinary tract, surgical wounds, and the lower respiratory tract. A World Health Organization prevalence study and other studies have shown that these infections most commonly occur in intensive care units and in acute surgical and orthopedic wards. Infection rates are also higher in patients with increased susceptibility due to old age, underlying disease, or chemotherapy.

Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. They are a significant burden both for the patient and for public health. The organisms that cause nosocomial infections are often drug-resistant. The regular use of antimicrobials for treatment therapy or prophylaxis promotes the development of resistance. Through antimicrobial-driven selection and the exchange of genetic resistance elements, multi-drug resistant strains of bacteria emerge. Antimicrobial-sensitive microorganism that are part of the endogenous flora are suppressed, while the resistant strains survive. Many strains of pneumococci, staphylococci, enterococci, and tuberculosis are currently resistant to most or all antimicrobials which were once effective [2].

India has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicine. A country like India is very much suited for development of drugs from medicinal plant. Because of its vase and wide variations in soil and climate, the Indian sub – continent is suitable for cultivation of large

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number of medicinal and aromatic plant which can be used as raw materials for pharmaceutical, perfumery, cosmetics, flavour and food and agrochemical industries. A large number of these plants grow wild and exploited especially for use in indigenous pharmaceutical houses. Some of these plants produce valuable drugs which have high export potential [3].

Developing a medicinal plants sector, across the various states of India has become an important issue. Different stakeholders in the medicinal plants sector have projected Tamil Nadu, one of the southern states, as an "Herbal State". This nation has made medicinal plants as a commodity of high value across the state. At the same time, realizing the continuous depletion of this valuable resource, attempts are being made for its large-scale cultivation and multiplication in order to meet its escalating demand as well as long-term sustainability. There are many aspects of research associated with the medicinal plants sector. The significant contribution to the society, traditional medicine has experienced very little attention in modern research and development and less effort has been done to upgrade the practice [4].

The antimicrobial properties of plants have been investigated by number of researchers' world wide. Since past few decades antibiotics from microbial origin and other chemotherapeutic agents have been used for control of bacterial disease. However due to indiscriminate use of these drugs, various pathogenic bacteria have developed resistance to many of the currently available antibiotics [5,6,7]. Other drawbacks are their high cost and undesirable side effects [8]. *Acalypha indica* is an annual erect herb commonly called as "Kuppai meni". It belongs to the family Euphorbiaceae. It is a common shrub in Indian gardens, backyards of houses and waste places through the plains of India. The root, stem and leaf of *Acalypha indica* possess herbal activity. In the present study, different solvent extracts of the herbal plant *Acalypha indica* leaf was screened for its antibacterial activity against nosocomial infection causing bacterial pathogens. The extracts also were subjected to phytochemical analysis to find out the chemical principle behind the bioactivity.

MATERIALS AND METHODS

Collection and Drying of plant materials:

Mature leaves of *Acalypha indica* were collected from Dhesiga Perumal temple at Chengelpet, Kanchipuram District, Tamil Nadu. The leaves of *Acalypha indica* were washed thoroughly three times with water and once with distilled water. The plant materials were shade dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of plant extract

40 g of powdered leaves were extracted successively with 200 ml of Methanol (56-60°C), Acetone (60-62°C), Chloroform (60-62°C), Petroleum Ether (40-60°C) and Hexane (62-66°C) in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [9].

Test microorganisms

Eight pathogenic nosocomial infection causing bacterial isolates, *viz*., *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Pseudomonas aeruginosa* were used during the present study and were obtained from SGS India laboratories – Thoraipakkam, Chennai – 96. The cultures were sub-cultured and maintained on Nutrient agar slants and stored at 4°C.

Determination of Antibacterial Activity:

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards.

Determination of antibacterial activity (Agar well Diffusion Method or Cup Plate Method)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculum on the surface of the media. Wells (8 mm in diameter) were punched in the agar. Ethanol and ethyl acetate extracts with different concentrations (25 mg/ml, 50mg/ml, 75mg/ml and 100 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. Well containing DMSO alone act as a Negative control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

Phytochemical Analysis:

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

Test for Alkaloids (Meyer's Test)

The extract of *Acalypha indica* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent [10]. The samples were then observed for the presence of turbidity or yellow precipitation [11].

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer [10].

Test for Tripenoid and Steroid

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids [10].

Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones [10].

Test for reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Triterpenes

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

Test for Phenolic Compounds (Ferric chloride test)

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins [12].

RESULTS AND DISCUSSION

Nosocomial infections in the developing countries pose greater threats to patient safety than in Western countries. In West, the crude mortality rate for patients with device-associated infections ranged from 35.2% (for CVC-associated bloodstream infection) to 44.9% (for VAP) [13]. In India, *Pseudomonas aeruginosa* was the commonest species isolated from VAP patients in ICUs (55%) and from wound infections (59%) with high mortality rates ranging from 16% to 46% [14]. Emergence of MDR in *Pseudomonas aeruginosa* in many hospitals across the country is of great concern [15]. Although the present study had limitations in number of isolates tested and short duration of screening, multiple resistance among isolates was clearly evident in the study.

In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [16].

In this present study, the antibacterial activity of the various solvent extract (leaf) of the herbal plant *Acalypha indica* was investigated against nosocomial infection causing bacterial isolates *viz*., *Staphylococcus aureus, Serratia marcescens, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The findings of the present investigation were showed in (**Table 1,2,3,4&5**). The antibacterial activity was determined by Agar Well Diffusion Method. Among the various solvents tested, the methanol extract showed more inhibitory activity when compared to other solvent extracts. Next to methanol extract, acetone extract showed good inhibitory activity followed by chloroform extract and petroleum ether extract. The inhibitory activity of hexane extract was relatively low when compared to the other tested solvent extracts.

Sumathi and Pushpa, (2007)[17], evaluated the antibacterial activity of some Indian medicinal plants. The aqueous extract of *Acalypha indica* was tested against different bacterial pathogens. The aqueous extract of *Acalypha indica* showed 9 mm inhibition zone to *Escherichia coli* and no zone was showed against *Staphylococcus aureus*,

Salmonella typhi and *Shigella flexneri*. Alcoholic extract of *Acalypha indica* showed 10 mm inhibition zone towards *Staphylococcus aureus* and *Salmonella typhi*.

Siva Sakthi *et al*., (2011)[18], evaluated the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Datura metel* against nine pathogenic bacteria isolates *viz., Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumoniae, Vibrio cholerae* and *Pseudomonas aeruginosa*. The ethanol extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (26 mm) against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (8 mm). The ethyl acetate extract of *Datura metel* (100 mg/ml) showed maximum zone of inhibition (19 mm) against *Escherichia coli*.

John De Britto and Herin Sheeba Gracelin, (2011)[19], investigated the phytochemicals present in leaves, stem, flowers and fruits of *Datura metel* which have some medicinal applications. Phytochemical analysis gave positive results for steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins. The stem and fruits extracts did not show marked antibacterial activity. The phytochemical compounds present in the *Acalypha indica* extract was analyzed in the present study and the results were showed in (**Table 6**). The *Acalypha indica* showed the presence of alkaloids and tannins. Saranraj *et al*., (2011)[20], tested the phytochemical characteristics of *Datura metel* and *Acalypha indica*. The *Datura metel* showed the presence of alkaloids, tripenoid, steroids, flavonoid, triterpenes, phenolic compounds and tannins. The presence of alkaloids and tannins was seen in *Acalypha indica*. They concluded that the antifungal activity of *Datura metel* and *Acalypha indica* was due to the presence of phytochemical compounds.

Some studies concerning the effectiveness of extraction methods highlight that methanol extract yields higher antibacterial activity than n-hexane and ethyl acetate (Sastry and Rao, 1994)[21]. Whereas other report that chloroform is better than methanol and benzene (Febles *et al*.,1995)[22]. It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method (Lima-Filo *et al* .,2002)[23].

Table 1: Antibacterial of Methanol extract of *Acalypha indica.*

S.No	Organisms	Concentration of extract (Zone in mm)							
		25mg		50mg		75mg		100mg	
		Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
1	*Staphylococcus aureus*	-	R	17	S	22	S	26	S
2	*Serratia marcescens*	15	S	30	S	33	S	36	S
3	*Escherichia coli*	20	S	30	S	32	S	35	S
4	*Salmonella typhi*	15	S	25	S	30	S	32	S
5	*Shigella flexneri*	-	R	25	S	28	S	31	S
6	*Klebsiella pneumoniae*	-	R	25	S	29	S	33	S
7	*Vibrio cholerae*	-	R	25	S	27	S	30	S
8	*Pseudomonas aeruginosa*	-	R	30	S	32	S	35	S

R – Resistant; S - Sensitive

Table 2: Antibacterial of Acetone extract of *Acalypha indica.*

S.No	Organisms	Concentration of extract (Zone in mm)							
		25mg		50mg		75mg		100mg	
		Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
1	*Staphylococcus aureus*	-	R	15	S	20	S	23	S
2	*Serratia marcescens*	12	S	27	S	30	S	32	S
3	*Escherichia coli*	17	S	28	S	32	S	35	S
4	*Salmonella typhi*	10	S	21	S	26	S	30	S
5	*Shigella flexneri*	-	R	23	S	25	S	30	S
6	*Klebsiella pneumoniae*	-	R	20	S	24	S	28	S
7	*Vibrio cholerae*	-	R	22	S	25	S	29	S
8	*Pseudomonas aeruginosa*	-	R	29	S	32	S	35	S

R – Resistant; S – Sensitive

Table 3: Antibacterial of Chloroform extract of *Acalypha indica.*

S.No	Organisms	Concentration of extract (Zone in mm)							
		25mg		50mg		75mg		100mg	
		Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
1	*Staphylococcus aureus*	-	R	12	S	15	S	20	S
2	*Serratia marcescens*	9	S	25	S	27	S	30	S

3	*Escherichia coli*	12	S	27	S	30	S	30	S
4	*Salmonella typhi*	6	S	20	S	24	S	28	S
5	*Shigella flexneri*	-	R	22	S	25	S	26	S
6	*Klebsiella pneumoniae*	-	R	17	S	20	S	25	S
7	*Vibrio cholerae*	-	R	20	S	23	S	27	S
8	*Pseudomonas aeruginosa*	-	R	25	S	28	S	33	S

R – Resistant; S – Sensitive

Table 4: Antibacterial of Petroleum ether extract of *Acalypha indica.*

S.No	Organisms	Concentration of extract (Zone in mm)							
		25mg		50mg		75mg		100mg	
		Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
1	*Staphylococcus aureus*	-	R	-	R	-	R	14	S
2	*Serratia marcescens*	-	R	20	S	23	S	25	S
3	*Escherichia coli*	-	R	22	S	23	S	25	S
4	*Salmonella typhi*	-	R	-	R	15	S	22	S
5	*Shigella flexneri*	-	R	15	S	20	S	24	S
6	*Klebsiella pneumoniae*	-	R	-	R	15	S	20	S
7	*Vibrio cholerae*	-	R	-	R	17	S	22	S
8	*Pseudomonas aeruginosa*	-	R	18	S	20	S	25	S

R – Resistant; S - Sensitive

Table 5: Antibacterial of Hexane extract of *Acalypha indica.*

S.No	Organisms	Concentration of extract (Zone in mm)							
		25mg		50mg		75mg		100mg	
		Zone	Rating	Zone	Rating	Zone	Rating	Zone	Rating
1.	*Staphylococcus aureus*	-	R	-	R	-	R	9	S
2.	*Serratia marcescens*	-	R	-	R	18	S	20	S
3.	*Escherichia coli*	-	R	-	R	20	S	20	S
4.	*Salmonella typhi*	-	R	-	R	-	R	18	S
5.	*Shigella flexneri*	-	R	-	R	15	S	20	S
6.	*Klebsiella pneumoniae*	-	R	-	R	-	R	10	S
7.	*Vibrio cholerae*	-	R	-	R	10	S	15	S
8.	*Pseudomonas aeruginosa*	-	R	-	R	15	S	20	S

R – Resistant; S - Sensitive

Table 6: Phytochemical analysis of *Acalypha indica* extracts

S. No	Test	Result
1	Alkaloids	+
2	Glycosides	-
3	Tripenoid and steroid	-
4	Flavonoid	-
5	Reducing sugars	-
6	Triterpenes	-
7	Phenolic compounds	-
8	Tannins	+

CONCLUSION

From this present study, it was concluded that the methanol extract of *Acalypha indica* have more inhibitory activity against nosocomial infection causing bacterial isolates when compared to other solvent extracts and the antibacterial potentiality was due to the presence of phytochemical compounds like alkaloids and tannins. The findings of the present investigation suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they supported by many investigation. The present study justifies the claimed uses of leaves in the traditional system of medicine to treat various infectious diseases caused by the microbes. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators in the country. The obtained results may provide a support to use of the plant in traditional medicine. Based on this further chemical and pharmacological investigations can be done to isolate and identify minor chemical constituents in the seeds and to screen other potential bioactivities may be recommended.

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