Research Article

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IN VITRO ANTIBACTERIAL ACTIVITY OF *ACALYPHA INDICA* LINN LEAVES EXTRACT AGAINST GRAM NEGATIVE BACTERIA

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ABSTRACT

The objective of this study was to determine antibacterial potentials of extracts of *Acalypha indica (A.indica)* against standard and drug resistant human microbial pathogens. Active antibacterial compounds were extracted using six different solvents (petroleum ether, chloroform, ethyl acetate, n-butanol, ethanol and aqueous). The antibacterial activities of six crude extracts of *Acalypha indica* leaves were determined using agar well diffusion assay. Chloramphenicol used as positive controls. Zone of inhibition of each crudeextracts were also determined against test microrganism. Crude extracts of *Acalypha indica* showed various degrees of antimicrobial activity towards test pathogenin a dose dependent manner. Of the different crude extracts, petroleum ether extract of *Acalypha indica* showed the highest zone of inhibition of 36mm and a minimum inhibitory zone of 6mm by aqueous extract of *Acalypha indica*. According to this study, crude extracts of the plants under imvestigation have potential inhibitory effects and thus manage the diseases caused by pathogenic microorganism.

KEY WORDS: *Acalypha indica*, Six extracts, Five pathogens, Well diffusion method, Zone of inhibition, Inhibitory activity.

INTRODUCTION

Acalypha indica Linn. (family :Euphorbiaceae) is a small annual shrub, generally occurs in gardens, roadsides, waste places or fields and throughout the plains of India[1]. It is found in many parts of Asia including India, Pakistan, Sri Lanka and throughout Tropical Africa and South America[2,3]. It is an annual herb commonly known as "Kuppaimeni" in India[4]. Traditionally, the leaves of *A. indica* is usedfor the treatment of scabies[5],as diuretic, purgative, antihelmintic[6], rheumatoid arthritis, and syphilitic ulcer[7]. The roots of *A. indica* is used as a laxative[8]. It is also found to have property of wound healing[9],as an anti-snake venom [10-13] antioxidant effect [14] and anti-inflammatory effects [15], anti-implantation and anti-estrogenic activity [16]. Infectious diseases caused by bacteria remains as a large global issue on public health [17,18] and causes death [19,20]. For many of these diseases treatments are difficult and have no specific effective therapy. Since no vaccines are available for most of the diseases[17]. In addition to this in recent years the emergence of rapid resistance against antibiotic has tremendously increased. Consequently, the discovery of natural antibacterial agents to treat the infectious diseases has developed into a new way to prevent the spread of diseases and thereby improve their treatment. Plants are still an important traditional source of medicine used for the treatment of various diseases[21]. Naturally, plants are rich in a wide variety of large number of organic compounds the so called secondary metabolites[22]with antimicrobial properties. The secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids [23-29] were found to have anti-microbial property. Thus, the present study was aimed at validating the antibacterial activities of petroleum ether, chloroform, ethylacetate, n-butanol, ethanol and water extracts of *A. indica* and to compare the anti-bacterial activity with standard antibiotics drug.

MATERIALS AND METHODS

Collection of plant materials

The *A.indica* (family: Euphorbiaceae) leaves were collected in and around Velapadi, Vellore district, India. The collected plant materials were identified and a vocher specimen was deposited and kept for further reference (herbarium voucher specimen 1316).

Preparation of plant extract

The collected leaves were washed completely to remove sand particles and shade dried at environmental temperature until completely dry. Then the materials were powdered into fine materials in a electrical stainless steel blender. The powdered materials were stored in a air tight container until use.

Extraction method

A weighted amount of *A.indica* leaf powder (30gm) was placed in a thimble of soxhlet apparatus and extracted with solvents like petroleum ether, chloroform, ethyl acetate, n-Butanol and ethanol. Same quantity of powder was macerated with 300ml of distilled water for 24 h at room temperature. All the extracts were concentrated in a vaccum evaporator under reduced pressure at 40°C. The so obtained semi solid crude extracts of all solvents were stored and used for antibacterial screening.

Antibacterial activity

Stock solution of each extract *A.indica* viz petroleum ether, chloroform, n-butanol, ethyl acetate, ethanol and aqueous was dissolved with 100ml of 10% DMSO solution at a concentration of 1gm/10ml (w/v).

Bacterial strains

The selected test organisms were supplied by the Department of microbiology, D.K.M College, Tamil Nadu, India, for antibacterial activity. Five gram positive (*E.coli, Salmonella, Vibrio, Proteus* and *Pseudomonas*) bacteria strain were used in study.

Inoculum preparation

The inoculums were prepared using nutrient broth medium and the 24h old cultures were used for the antimicrobial studies using agar well diffusion method. Loopfull of test organisms include *E.coli, Samonella, Vibrio, Proteus* and *Pseudomonas* culture were inoculated into the nutrient broth medium. The bacterial strains were subcultured in the nutrient broth medium, incubated at 37ºC for 24h and maintained on nutrient agar slant at 4ºC as stock culture.

Antibacterial assay

In vitro antibacterial activity was carried out using agar well diffusion method[30-32]. Previously auto claved sterile petriplates were poured with 25ml of sterile Muller Hinton agar and allowed to solidification. Sterilized cotton swabs were dipped in the nutrient broth containing the test cultures. After solidification the respective test organisms were swabbed uniformly on the top of the individual solidified agar plates. Allow all the plates to dry for 10minutes. In each plate well of equal size (6mm diameter) were cut using a sterile borer, with proper gaps in the medium and the plant extract were added into it. About 80µl, 90µl, 100µl concentrations of the test extracts were added into the wells by sterile micropipette and allowed to diffuse at room temperature for 10minutes. Chloramphenical (30mg/ml) was used as positive control for comparing the bioassay. The plates were incubated for 24hr at 37ºC for bacterial growth, after which zone of inhibition was recorded in millimeters using a transparent scale.

RESULT AND DISCUSSION

In our present study we utilized petroleum ether, Chloroform, n-Butanol, ethyl acetate, ethanol and aqueous solution for successive extraction of leaf material of *A.indica*. Five selected strains *E.Coli, Salmonella, Vibrio, Proteus* and *Pseudomonas*, were treated against different leaf extracts of *A.indica* for antibacterial activity. The results of antibacterial activity of *A.indica* leaf extracts are presented in Table 1.

From the table it can be noted that *Vibrio* 100µl (36mm), 90µl (32mm), 80µl (30mm), *Proteus* 100µl (31mm), 90 µl (28mm), 80µl (24mm) , *Pseudomonas*100µl (27m), 90 µl (24*mm*), 80µl (21mm) was most sustainable among the five organisms and *E.coli* 100µl (19mm), 90 µl (15mm), 80 µl (10mm), and *Samonella* 100µl (22mm), 90 µl (15mm), 80 µl (10mm) are the least against petroleum ether extract.

The ethyl acetate extract at different concentrations 100µl, 90µl, 80µl showed highest zone of inhibition against *Pseudomonas* (25mm, 20mm, 12mm, respectively) *E.coli* (17mm, 13mm and 10mm, respectively) and *vibrio* (16mm, 12mm,10mm, respectively). While the ethyl acetate extract had lowest zone inhibition against *Proteus* (20mm, 17mm and 9mm, respectively) and *Salmonella* (12mm, 10mm and 8mm, respectively).

From the result, it was observed that zone of inhibition varied from one organism to another against the tested extract (n-Butanol). The n-Butanol showed broad spectrum activity against the five tested organism in the following order *Pseudomonas* > *Salmonella* > *Proteus* > *Vibrio* > *E.coli*. The zone of inhibition of *Pseudomonas* at 100 µl, 90 µl and 80 µl was found to 14mm, 15mm, and 20mm respectively. The zone of inhibition of *Salmonella* at 100µl,90µl and 80µl was found to 15mm, 13.6mm, 12.5mm respectively. The zone of inhibition of *Proteus* at 100µl, 90µl and 80µl was found to 16mm, 12mm and 11mm reapectively. The zone of inhibition of *vibrio* at 100µl, 90µl and 80µl was found to 13.8mm,11.6mm and 10.6mm respectively. The zone of inhibition of *E.coli* at 100µl, 90µl and 80µl was found to 11mm, 10mm and 07mm respectively.

When chloroform extract of *Acalypha indica* treated against five bacteria, the highest value zone of inhibition of 19mm, 18mm, 17mm, 16mm and 13mm (100µl respectively) against *E.coli, Samonella, Proteus* and *Pseudomonas* and *Vibrio* and the lowest values of 14mm, 12mm, 10mm and 8mm (80 µl respectively) against *E.coli, Proteus, Psedomonas, Samonella* and Vibrio was recorded. But the inhibition zone value due to 90µl concentration were recorded to be 14mm (*E.Coli*), 13mm (*Samonella* and *Proteus*), 11mm (*Pseudomonas*) and10mm (*Vibrio*).

The aqueous extract showed 11mm (80µl), 13mm (90µl), 14mm (*100*µl) zone of inhibition against *Proteus* and 10mm (80ml), 13mm (90ml), 15mm (100ml) zone of inhibition against *Vibrio* and 10mm (80µl), 11mm (90µl), 13 mm (100µl) zone of inhibition against *Pseudomonas* and 9mm (80µl), 11mm (90µl), 13mm (100µl) zone of inhibition against *Salmonella* and 8mm (80µl), 10mm (90µl), 10mm (100µl) zone of inhibition against *E.coli.*

This antibacterial screening experiment had a concertable effect to suppresss *Samonella, Proteus, Vibrio, E.Coli* and *Pseudomonas* growth and this inhibition was increased parallel to increasing concentration. The highest activity (about 16mm, 15mm, 15mm, 14mm, 9mm inhibition zone) was showed against organism *Samonella, Proteus, Vibrio, E.coli* and *Pseudomonas* at 100µl and followed by 90µl (13mm, 12mm, 11mm, 11mm and 8mm) against organism *Samonella, Proteus, Vibrio, E.coli* and *Psedomonas.* On the other hand Samonella (12mm), and *Proteus* (10mm) were nearly resistance to ethanolic extract except *E.coli* (9mm), *Proteus* (9mm) and *Pseudomonas* (7mm).

Standard antibiotic chloramphenical produced an inhibition zone of 36mm, 35mm, 30mm (each) and 23mm against *Vibrio, Proteus, Pseudomonas, Samonella* and *E.coli.*

The antibacterial result for different extracts of *A.indica* indicates that the petroleum ether extract were found to be more effective followed by ethyl acetate, n-Butanol and Chloroform when compare to ethanol and aqueous extracts.

Senthilkumar *et al*., (2011)[33] reported that ethanolic extract had a strong activity while the chloroform extracts possess moderate activity *Salmonella typi* and *Proteus vulgaris*. Whereas ethylacetate extract found to a moderate activity against *staphylococcus aureus*, *E.coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa.*

Table 1: Antimicrobial activity of *Acalypha indica*

Extracts	Concentration	Organisms				
		E.Coli	***Samonella***	***Vibrio***	***Proteus***	***Pseudomonas***
Petroleum ether	80µl	11mm	10mm	30mm	24mm	21mm
	90µl	15mm	15mm	32mm	28mm	24mm
	100µl	19mm	22mm	36mm	31mm	27mm
Ethyl acetate	80µl	10mm	8mm	10mm	9mm	12mm
	90µl	15mm	10mm	12mm	17mm	20mm
	100µl	17mm	12mm	16mm	20mm	25mm
n-Butanol	80µl	7mm	12.5mm	10.6mm	11mm	14mm
	90µl	10mm	13.6mm	11.6mm	12mm	15mm
	100µl	11mm	15.8mm	13.8mm	16mm	20mm
Chloroform	80µl	14mm	10mm	08mm	12mm	10mm
	90µl	14mm	13mm	10mm	13mm	11mm
	100µl	19mm	15mm	13mm	16mm	16mm
Ethanol	80µl	09mm	12mm	09mm	10mm	07mm
	90µl	11mm	13mm	11mm	12mm	08mm
	100µl	15mm	15mm	14mm	16mm	09mm
Aqueous	80µl	08mm	09mm	10mm	11mm	06mm
	90µl	10mm	11mm	13mm	13mm	11mm
	100µl	10mm	13mm	15mm	14mm	13mm
Control	80µl	35mm	47mm	25mm	33mm	37mm

µl-Microliter, mm-millimeter

There results are consistent with reports in the present study, where petroleum ether showed a strong inhibition activity while the ethylacetate and n-butanol showed a better activity against all the tested pathogens. The chloroform extract showed a moderate activity, whereas, ethanol and aqueous extract showed a less inhibitory activity against the human pathogens, tested in the present study.

Alzoreky and Nakshara (2003)[34] investigated that, both methanol and acetone extracts were proved to have an inhibitory activity on medicinal plants due to the presence of some inhibitory substances present in the extract. Along with solvent system, plant species as well as the test microorganisms should be considered during the study, which were found to play an important role in antimicrobial activity.

In general, among the Gram-negative bacteria and Gram-positive bacterias, Gram negative bacteria shows lesser sensitivity to plant extracts compared to gram- positive bacteria because of the presence of extra lipopolysaccharides in their outer membrane and protein cell wall [35]. This was reported in several previous findings[36-38]. These findings are in controversial with the results obtained since all of the six different extracts used in this study produced prominent activity against five tested gram negative bacteria's.

A similar finding were reported by Sunil Megi *et al*., (2010)[39] *A.catechu* methanol extract had exerted some degree of inhibition against gram negative organisms. Previous studies have noted that the polarity of solvents plays an important role in the extraction of natural products from the plant sources. Furthermore some other researchers also have shown that plant extracts contains some antimicrobial substance, responsible for the inhibition of microorganism by *in vitro* and *in vivo*[40].

In the current study, all the tested organisms were inhibited by six different solvent extracts used in this study. Thus, the efficacy of plant extracts evaluated as antimicrobial agents, found dependent on the solvent of extraction. Sunilson *et al*., (2009)[41] observed the antimicrobial activity of *C.longa* and *Z.officinalis* in four different solvents (Petroleum ether, chloroform, methanol and water) against food borne pathogens. The solvent extracts displayed antibacterial and anti yeast activity.

In many reports the water extract is not accepted more to extract compounds of antimicrobial activity or the extract shows weak antimicrobial effect as according to Parekh *et al*., (2005) and Afolayan *et al*., (2002)[42,43]. Unexpectedly, water extracts results in the present study showed prominent activity against gram negative bacteria. This is similar to the previous study reported by Obeidat *et al*., (2012)[44] whose findings reported that the water extracts of *A.discoridin* exhibited the highest inhibitory effects against gram negative bacteria and thus water extract could be used in drug industry. In support to these findings, in this study water extract of *A.indica* inhibited all the five tested microorganisms in a good manner.

CONCLUSION

From the overall study, it can be concluded that the results obtained confirms the therapeutic potential of *A.indica* leaf extract against various diseases causing pathogen might be due to the presence of certain compounds that have antibacterial activities and also supports the traditional usage of this plant as an alternative medicine in local community.

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