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Evaluation of antimicrobial activity of aqueous extracts of Acalypha indica

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Abstract

Acalypha indica is a small herb which is distributed in most of Asian countries such as Sri Lanka, India, and Pakistan. It is commonly known as Indian Copper leaf and it is used in traditional medicines for bacterial and fungal diseases. Aqueous extraction of plant materials such as leaf and whole plant were investigated for anti-microbial property without changing concentration. Results of this study revealed that all extracts had good inhibitory activity against gram positive and gram negative bacteria. The clear zone for Staphylococcus aureus and Klebsiella were found with the diameter of 1.85cm and 1.9cm respectively, though the inhibitory diameters of these bacteria were smaller than the streptomycin control. Antifungal activity of whole plant and leaf extract of Acalypha indica against all fungal species showed the inhibitory growth less than 20%. The findings exhibit that aqueous extracts have broad spectrum activity and there is a possibility in treatment of infectious diseases.

Key words: Acalypha indica, leaf and whole plant extracts, antimicrobial activity

1. INTRODUCTION

Acalypha indica (family: Euphobiaceae) is a small annual herb grows up to 60cm along the road sides having medicinal properties (Burkill, 1985). It is distributed in Asian countries such as Sri Lanka, India, Pakistan, Africa and South America (Ramachandran, 2008; Parveen et al, 2007; Mohan et al, 2012). It is commonly known as Indian Copper leaf (T- Kuppaimeni, S- Kuppameniya) (Kirtikar et al, 1975). Leaves are little triangular and ovate. Leaf stalks are longer than the 3-5 cm long blades. Flowers are borne on erect of axillary spikes which are stalkless. Male flowers are minute where the female flowers are scattered along the inflorescence axis (Kirtikar and Basu, 1994; Prajapati et al, 2003; Nadkarni,1995).

Human have worthy remedy from the nature for health and life. Practice of indigenous treatment is the leading edge of medicinal science (Kaushik and Dhiman, 1999; Jain, 1996). The different parts of plant such as leaf, stem, root, flower and seed are used in a variety of ailment in Ayurvedic medicine. The leaves of the plant are used for the treatment of scabies (Gurib-Fakim et al, 1993), as purgative, diuretic, antihelmintic (Varier, 1996), syphilitic ulcer (Dhar et al, 1968), rheumatoid arthritis. The roots are used as a laxative (Panthong et al, 1991). It has the properties of wound healing (Reddy et al, 2002), as an anti-snake venome (Siddiqui and Husain, 1990; Shirwaikar et al, 2004; Mahishi et al, 2005; Samya et al, 2008), anti-inflammatory effect (MohanaVamsi et al, 2008), anti-oxidant activity (Ruche et al, 2007) and anti-estrogenic activity (Hiremath et al, 1999). Microbial infectious diseases are a global issue in human health (Hamer et al, 2010; Khan et al, 2013) and leads death (Avery, 2006; Tekwu et al, 2012). The plant extract is used for treating pneumonia, jaundice, piles, asthma, rheumatism, bedsores, wounds, skin infections and eczema. It has been stated to have wound healing activity, snake venom neutralizing properties, antibacterial activity and antiurolithiatic activity (Reddy et al, 2002; Suresh; Govindarajan et al, 2008; Sathya et al, 2011; Jain, 1987; Jain, 1996; Sumathi and Puspha, 2005). The whole plant is diuretic, expectorant, emetic, anthelmintic. (Oudhia, 2003; Valsara, 1994).

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The previous findings of Suresh et al, revealed that *A. indica* showed considerable antibacterial activity against *S. aureus and E. coli*. The aqueous extracts of *A. indica* showed the inhibitiaon against E.coli and alcoholic extact show inhibition *tow*ards *Staphylococcus aureus* and *Salmonella typhi* (Divya et al, 2014). The present study is to investigate the anti-microbial properties of the aqueous extracts of *A.indica* leaves and whole plant.



Figure (1): Acalypha indica leaf and whole plant

2. MATERIAL AND METHODS

2.1. Sample collection and extract preparation

Leaf and bark of *C. fistula* was collected from the premises of University of Jaffna, Sri Lanka. For aqueous extract preparation, 2.5g of plant material was weighed and washed well in tap water. Then they were sterilized by giving a quick dip in alcohol and washed with sterilized water again. The weighed plant material was crushed with 10mL of sterile water and it was filtrated using Whatman's filter paper No.1. The filtrate was collected in sterile beaker. Anti-microbial activity was carried out using disc diffusing method (Kirby et al, 1966).

2.2. Anti-bacterial activity

The preliminary screening of antibacterial activity was done using well in agar method. *Bacillus sp, Staphyllococcus aureus, Klebsiella, E.coli* and Pseudomonas bacteria were selected for this study. These bacteria were streaked on pure nutrient agar plates separately and stored in refrigerator at 10^oC with labelling. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121^oC for 15min). Then the petri dishes were used for the experiment. Peptone broth and agar standard solution were prepared and 100ppm streptomycin standard solution was used as positive control. The inoculum was spread in nutrient agar plates with bacterial strain and incubated at 37^oC for 24 hours. Wells were prepared with UV sterile and wet heat sterile extracts, streptomycin solution and sterile water in agar plates for each bacterial species. The diameter of the clear inhibitory zone around the well was measured.

2.3. Anti - fungal activity

Candida sp, Aspergillus sp, Pencillium, Rhizopus fungus were selected for this study. Plant materials were sterilized using different methods such as UV sterilization (UV light at 336nm) and wet heat sterilization (autoclave at 121°C for 15min). The sterilized extracts and Potato dextrose agar (PDA) media were mixed

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well and poured in petridishes. They were incubated at room temperature for 2 days. After it had grown enough, disc with the diameter of 7mm were cut using the sterile cork borer. The disc of each fungus was placed on the middle of the plate, which contain herbal product with PDA by using sterile loop. Control plates were also maintained without plant extract. These plates were incubated at room temperature for 2 days. Then the diameter of the clear inhibitory zone around the well was measured.

3. RESULTS AND DISCUSSION

3.1.Anti-bacterial activity

The extract of *Cassia fistula* found to be effective against the pathogenic bacteria. The inhibition zone is shown in Table 1 has exposed the power against pathogenic bacteria.

Table (1): The diameter of the zone obtained from samples plant material under UV sterilization

Bacteria	Diameter of inhibitory zone (cm)			
	Leaf extract	Leaf extract Whole plant extract		
E.coli	1.5	1.5	3.0	
Bacillus sp 1	Reduced growth	Reduced growth	2.6	
Bacillus sp 2	Reduced growth	Reduced growth	1.5	
Pseudomonas	0	0	1.9	
Staphylococcus aureus	1.85	1.45	3.3	
Klebsiella	1.75	1.9	2.4	

Table (2): The diameter of the zone obtained from samples plant material under wet heat sterilization

Bacteria	Diameter of inhibitory zone (cm)			
	Leaf extract Whole plant extract		Streptomycin	
E.coli	2.0	2.0	3.0	
Bacillus sp 1	Reduced growth	Reduced growth	2.7	
Bacillus sp 2	Reduced growth	Reduced growth	1.5	
Pseudomanas	0	0	1.95	
Staphylococcus aureus	1.3	1.3	3.2	
Klebsiella	1.3	1.6	2.2	



Figure (2): Effect of UV sterilised extract of leaf (A) and whole plant (B) of *Acalypha indica* on the growth of *Staphylococcus aureus* and *Klebsiella* (C- Streptomycin, D-sterile water)

Figure (3): Effect of UV sterilised extract of leaf (A) and whole plant (B) of *Acalypha indica* on the growth of *Pseudomonas* and *Bacillus sp 2* (C- Streptomycin, D- sterile water)



Figure (4): Effect of UV sterilised extract of leaf (A) and whole plant (B) of *Acalypha indica* on the growth of *E.coli* and *Bacillus sp 1*(C- Streptomycin, D- sterile water)

Figure (5): Effect of wet heat sterilised extract of leaf (A) and whole plant (B) of *Acalypha indica* on the growth of *Klebsiella*, *E.coli*, *Pseudomonas*, *Staphylococcus aureus*. *Bacillus sp 1* and *Bacillus sp 2* (C-Streptomycin, D-sterile water)

Madhavi Adhav, 2016 stated that water extract show strong antibacterial activity against gram positive bacteria such as *S.aureus* and *B. subtilis* and gram negative bacteria such as *Shigella dysenteriae* and *E.coli*. Another study indicated that the aqueous extract showed 1.0 - 1.3cm zone of inhibition against *Pseudomonas* and 0.8 - 1.0cm zone of inhibition against *E.coli* (Ashwini and Asha, 2017). In the present study stated: among the herbs tested *Acalphya indica* showed a considerable antibacterial activity. The leaf extract produced clear zone on *E.coli*, *Staphylococcus aureus* and *klebsiella* as 1.5, 1.85, 1.75cm with 0.25g/mL of extract concentrationtively. The *Bacillus sp 1* and *Bacillus sp 2* showed reduced growth but had no clear zone for Pseudomonas showed resistance to the plant.

The whole plant extract also showed the similar result, it produced clear zone with *E.coli, Staphylococcus aureus* and *Klebsiella* with diameter of 2.0, 1.3 and 1.9cm respectively and it reduced the growth of *Bacillus sp* 1 and *Bacillus sp* 2. Pseudomonas showed resistance. The wet heat sterilised extracts of whole plant and leaf extracts of *Acalypha indica* showed a small difference in inhibition.

Infectious diseases are caused by pathogenic bacteria. The issue raised is to be developed of multi drug resistant microorganisms. So, the studies focused on new therapeutic alternatives using medicinal plants against especially *Staphylococcus aureus* and *Pseudomonas sp.* (Lakshmanan and Sankaranarayanan, 1990; Reynolds , 2009; Anuradha et al, 2010). Cholapandian, (2013) stated water extract of *A. indica* showed the inhibitory zone against *Staphylococcus aureus* (0.8cm) and *Pseudomonas* sp. (0.75cm). No zone was observed to water extract against *Klebsiella* sp. The mean range of antibacterial activity *Pseudomonas* sp were estimated for leaf extracts of *A.calypha indica in* methanol , ethanol and water 1.0 ± 0.022 , 1.2 ± 0.015 and 0.7 ± 0.035 respectively. As like of Pseudomonas, the antibacterial activity of plant extract for *Klebsiella* sp were estimated in methanol extract methanol , ethanol and water 0.50 ± 0.67 , 0.50 ± 0.076 and 0.50 respectively (Arulraj , 2017).

3.2.Anti-fungal activity

Table 3: the diameter of the Mycelial disc in cm and % of growth reduction of samples obtained from *A*. *indica* under UV sterilization

Fungus	Diameter of inhibitory zone (cm)			% of growth	% of growth
	Leaf extract	Bark extract	Control (without	reduction in leaf	reduction in bark
			plant extract)	extract	extract
Yeast	2.65	1.2	3.3	19.69	19.69
Rhizopus	7.5	7.5	9.0	16.60	16.60
Aspergillus	2.5	2.5	3.0	16.60	16.60
Pencillium	1.2	1.2	1.2	0	0

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Table 4: the diameter of the Mycelial disc in cm and % of growth reduction of samples obtained from *A*. *indica* under wet heat sterilization

Fungus	Diameter of inhibitory zone (cm)			% of growth	% of growth
	Leaf extract	Bark extract	Control (without	reduction in leaf	reduction in bark
			plant extract)	extract	extract
Yeast	2.8	2.8	3.3	15.15	15.15
Rhizopus	7.5	7.5	9.0	16.60	16.60
Aspergillus	2.5	2.5	3.0	16	16.00
Pencillium	1.2	1.2	1.2	0	0



Figure (6): Effect of UV sterilised leaf extract of *Acalypha indica* on the mycelial growth of Yeast, *Rhizopus, Aspergillus* and *Pencillium* (A- Control, B- extract added)

Figure (7): Effect of UV sterilised whole plant extract of *Acalypha indica* on the mycelial growth of Yeast, *Rhizopus, Aspergillus* and *Pencillium* (A- Control, B- extract added)



Figure (8): Effect of UV sterilised whole plant extract of *Acalypha indica* on the mycelial growth of Yeast, *Rhizopus*, *Aspergillus* and *Pencillium* (A- Control, Bextract added)

Figure (9): Effect of UV sterilised whole plant extract of *Acalypha indica* on the mycelial growth of Yeast, *Rhizopus, Aspergillus* and *Pencillium* (A- Control, B- extract added)

Considering the *Acalypha indica* there is no distinct difference in the reduction by whole plant and leaf extract. Yeast was reduced by 21.2% on its growth while *Rhizopus* and *Aspergillus* showed 16.60% and 16% respectively. *Pencillium* showed resistant to extrcts of *A. indica*. The results showed a similar inhibition zones in wet heat and UV sterilization.

Sudhakar et al, 2018 stated that the methanol extract was more effective against *Candidatropicalis* and *Candida albicans* and ethanol extract was more effective against *Candida albicans* and *Aspergillus niger* (Aushi Nag1a et al, 2018). The antifungal activity of *Acalypha Indica* L. is similar to antifungal drug ketaconazole. This is able to use for curing of of transmittable disease cause through veteran strain as well as latent anti-microbial agent can exist prepared. (Mahesh et al, 1984).

Leaves of Acalypha indica contain Alkaloids, Tannins, Saponins and Proteins. The qualitative analysis of A.indica showed the presence of biomolecules such as alkaloids, catechols, flavonoids, phenolic compounds,

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saponins and *steroids* respectively (Komathy et al, 2013). *Family* Euphorbiaceae showed the elevated concentration of phenols, alkaloids and flavonoids (Mahesh Vk et al., 1984). Phenolic, Flavonoids (quercetin, kaempferol, isorhamnetin, isoquercetin) derivatives have strong anti-fungal activity.

4. CONCLUSION

The present study revealed that the extracts obtained from leaf and bark of *Acalypha indica* figure out strong activities against the gram negative and gram positive bacteria. And it has a little range of anti-fungal activities. This study leads to the possibility for the treatment of infectious diseases, however further studies need to be conducted to find isolation and antimicrobial active constituents from the plant.

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