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Original Research Paper

SCREENING OF ANTIBACTERIAL ACTIVITY OF *CEPHALENDRA INDICA* PLANT EXTRACTS IN INVITRO SYSTEMS

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ABSTRACT

The present study was aimed to investigate the antibacterial activity of aqueous, methanolic and ethanolic extracts of root, fruit and whole plant of *Cephalendra indica* using disc diffusion method. The antibacterial activity was detected against Gram-positive and Gram-negative bacteria, *Bacillus subtilis, Bacillus ceresu, Staphylococus aureus, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* Different solvent extracts of root, fruit and whole plant of *Cephalendra indica* was taken in 50,100,150 and 200 µg as per CLSI standards. The zones of inhibitions obtained were recorded and analyzed against standard controls, Streptomycin and Ampicillin. The methanolic extracts of fruit and whole plant were showed highest antibacterial activity than ethanolic and aqueous extracts. The higher concentration of methanolic extract of whole plant, maximum antibacterial activity was observed against to Gram-negative bacteria than Gram-positive bacteria. When compared with fruit and root extracts of different solvents, methanolic extract of whole plant exhibit high antibacterial activity. The results indicate that *Cephalendra indica* whole plant also has potent antibacterial activity similar to fruit and leaves.

Keywords: *Cephalendra indica*, Gram-positive, Gram-negative bacteria, Antibacterial activity, Disc diffusion method Whole plant.

INTRODUCTION

The herbal medicine represents one of the most important fields of traditional medicine all over the world. Because increase in bacterial infections and their treatment due to indiscriminate use of commercial antibacterial drugs and development of multiple drug resistance in most of the microorganisms, the investigation of natural materials as sources of new antibacterial agents has been increased from the past 20 years. The source of the therapeutic effects to identify different medicinal plants have been tested, and to identify novel substances that are active towards pathogens with high resistance, as a result new antibacterial drugs have been approved some natural products (Cragg *et al*., 1997; Recio 1989**).** The natural products of higher plants may give possible novel mechanisms of action with new source of anti microbial agents (Barbour *et al*., 2004; Hamil *et al*., 2003; Motsei *et al*., 2003**).** Each and every part of the plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for various diseases anti-spasmodic, anti-periodic, stimulant, diaphoretic, bronchial catarrh, bronchitis, psoriasis, small pox, scabies and other ulcers and itchy skin eruptions (Perry, 1980., Behl *et al*., 1993). *Cephalendra indica* is a member of the family Cucurbitaceae, is distributed in tropical Asia, and is commonly found in India and commonly known as Ivy guard. The fruit of

Cephalendra indica is used as a vegetable when green and eaten fresh when ripened; it is a climbing shrub with white flowers (Sastri, 1950). The selection of the plant for evaluation was based on its traditional usage, every part of the plant exhibit pharmacological activities. *Cephalendra indica* roots, leaves and fruits are used to treat various anti-inflammatory, antioxidant, antimutagenic, antidiabetic, antibacterial, antiprotozoal, antiulcer, hepatoprotective, expactorants, analgesis, antiinflamatory are the reported pharmacological activities (Goldy Yadav *et al*., 2010**;** Tamilselvan *et al*., 2011). Mother tincture of *Cephalendra indica* showed anti-diabetic factor and it regenerates pancreatic beta cells (Rastogi *et al*., 1988). It is used to treat diabetic type II patients and results showed that decrease in blood glucose level in diabetic patients (*Zaubair Qureshi et al., 2002)*. Extracts of *Cephalendra indica* were eluted for the potential anti-therapeutic and antibacterial properties and the extracted juice from tuberous roots or leaves of this plant has been used by Ayurvedic Physicians in diabetes (Ajagoankar, 1968; Chopra, 1968). Antibacterial activity of different solvents of leaves and fruit extracts of *Cephalendra indica* was reported (Farrukh *et al*., 2008; Syed *et al*., 2009; Shaheen *et al*., 2009). In pharmaceutical industries, due to varied medicinal properties in all plant parts include root, stem, flower, fruit, whole plant and modified plant is used for extract as drugs. Due to the potent antibacterial properties of *Cephalandra indica* plant parts like stem, leaf, fruit etc. The present work was considered of interest to carry out antibacterial activity of root and whole plant extract with compared to fruit extract of medicinal plant *Cephalendra indica* against to an array of bacterial pathogens.

MATERIALS AND MATHODS

Collection of Plant Material

Cephalendra indica roots, fruits and whole plant were collected in Tirupati, Andhra Pradesh, India. The parts of the plant were separately shade dried and were homogenized to fine powder and further subjected to extraction.

Crude Extracts

The crude methanol extract was obtained by extracting 5 g of dried root powder, 5 g of dried fruit powder and 5 g of whole plant separately in 50 ml methanol and kept on a rotary shaker for 24 hrs. The extract was filtered, centrifuged at 5000 g for 15 mins, and was dried under reduced pressure. The extract was stored at 4ºC in airtight bottles. The crude ethanol extracts of root, fruit and whole plant was obtained by extracting 5 g of dried root powder, 5 g of dried fruit powder and 5 g of dried whole plant powder separately in 50ml ethanol and kept on rotary shaker for 24 hrs. The extract was filtered, centrifuged at 5000 g for 15mins, and was dried under reduced pressure. The extract was stored at 4°C in airtight bottles. The aqueous extract was obtained by extracting with same weight of dried root, fruit andwhole plant powder separately in 50 ml of distilled water and boiled for 20 mins. The extract was cooled and filtered, centrifuged at 5000 g for 15 mins and stored at 4ºC in airtight bottles.

Microorganisms Used

To assess the antibacterial properties of crude extracts of *Cephalendra indica,* Gram-positive bacteria *Bacillus subtilis, Bacillus cereus* and *Staphylococus aureus,* and Gram-negative strains of *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aureginosa* were used. The organisms were maintained on nutrient agar slants at 4°C and sub-cultured into nutrient broth for 24 hrs before use.

Determination of Antibacterial Assay

In vitro antibacterial activity of different extracts was studied against for bacterial strains by disc diffusion method. Nutrient agar media (Hi Media) was used as bacteriological medium. The extracts were made to different concentrations 50 mg, 100 mg, 150 mg and 200 mg respectively and the activity was eluted. The nutrient agar plates were prepared and standardized inoculums were added ascetically to the agar plates and spreaded over the medium. The antibiotic discs prepared with different concentrations were placed on the agar plates and incubated at 37ºC. The antibacterial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each disc. The diameters of zone of inhibition

produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin and ampicillin. For each bacterial strain controls were maintained where pure solvents were used instead of the extract.

RESULTS

The antibacterial activity of crude extracts of *Cephalendra* root, fruit and whole plant were determined against for bacterial strains. Antibacterial activity at different concentrations 50, 100 and 150 and 200 mg/ml of aqueous, methanol and ethanol root, fruit and whole plant extracts were presented in Table 1. The antibacterial activity of root, fruit and whole plant extracts were observed in dose dependent manner, 200 mg/ml showed more activity in tested microorganisms. The results were also compared with the standard antibiotic and all these six micro organisms are sensitivity to standard antibiotic (Table 1). Gram-positive bacteria *B. subtilis* was most resistant in contrast to this Gram-negative bacteria *K. pneumonia* and *P. auregnosa* showed antibacterial activity at different concentrations of aqueous, methanol and ethanol fruit and whole plant extracts. In the higher concentration of methanolic fruit and whole plant extracts, maximum antibacterial activity was observed against to Gram-negative bacteria than Gram-positive bacteria. The minimum concentration (50 mg/ml) of methanolic extract of fruit extract and whole plant extracts also showed antibacterial activity against to Gram +ve and Gram –ve bacteria. But no zone of inhibition was observed in 50mg/ml of aqueous root extract. Whereas high concentration of methanolic extract of root showed minimum zone of inhibition but no zone of inhibition was observed in less concentration. Higher concentrations of aqueous and ethanolic extracts of fruit and whole plant were showed minimum antimicrobial activity against to microorganisms but no zone of inhibition was observed in less concentration (Table1).

DISCUSSION

The present study shows that extracts of fruit and whole plant were effective inhibitors of bacteria growth than root extracts. The methanolic extracts of fruit and whole plant were more effective against to Gram- negative bacteria than Gram-positive bacteria but not in the aqueous and ethanol extracts. This may be due to the ability of methanol to extract a wide range of chemical constituents of plant material (Cowan, 1999). The present result confirm the previous studies, the methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants as compared to other solvents (Ahmad *et al*., 1998; Eloff, 1998; Lin *et al*., 1999, Karaman *et al*., 2003; Parameswari *et al*., 2012). In the present study whole plant extract of *Cephalendra indica* showed a potent antibacterial activity. Similar type of results reported in different whole plant extracts of different plants, *Camellia sinensis* (Yam *et al*., 1997), *Tridax procumbens*, (Aniel Kumar and Mutyala Naidu 2010), *Andrographis paniculata* (Aniel Kumar *et al*., 2010), *Andrographis serpyllifolia* (Revathi *et al*., 2012), *Sida spinosa* Linn. (Selvadurai *et al.*, 2011), *Solanum nigrum* etc.,(Prameswari *et al*., 2012). The presence of phytochemicals alkaloids, phenolic compounds, steroids, proteins, carbohydrates and tannins were investigated in leaves and fruit of *Cephalendra indica* (Umamaheswari and Chatterjee, 2008; Syed *et al*., 2009). Presence of alkaloids and phenolic compounds may be attributed to antibacterial activity in *Cephalendra indica*.

CONCLUSION

The present results showed that *Cephalendra indica* plant extract possess certain constituents with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. There is a further studies need to isolation of the therapeutic antimicrobials and carry out further pharmacological evaluation.

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Table 1: Antibacterial activity of crude aqueous, methanol and ethanol extract of *Cephalendra indica* root, fruit and whole plant against bacterial strains

Bacterial strains	Conc (µg)	Zone of inhibition in mm									Standad drugs	
		Root			Friut			Whole plant				
		A	M	E	A	M	E	A	M	E	Strp	Amp
B. subtilus	50	-	-	-	1.0 ± 0.01	2.0 ± 0.01	1.9 ± 0.60	1.3 ± 0.14	2.5 ± 0.15	1.0 ± 0.01	10	9
	100	2.3 ± 0.11	3.3 ± 0.57	2.2 ± 0.25	1.0 ± 0.45	3.1 ± 0.15	3.9 ± 0.01	1.0 ± 0.45	3.3 ± 0.15	3.9 ± 0.01		
	150	3.2 ± 0.15	3.3 ± 0.55	3.5 ± 0.03	2.5 ± 0.45	5.5 ± 0.54	5.5 ± 0.98	2.5 ± 0.45	5.5 ± 0.54	5.5 ± 0.98		
	200	3.6 ± 0.18	4.0 ± 0.20	3.9 ± 0.27	3.5 ± 0.32	7.1 ± 0.25	8.3 ± 0.02	3.5 ± 0.32	8.1 ± 0.25	6.3 ± 0.02		
B. cereus	50	-	-	-	1.7 ± 0.50	2.3 ± 0.11	3.7 ± 0.00	1.4 ± 0.35	5.0 ± 0.11	2.4 ± 0.52	14	11
	100	1.4 ± 0.35	2.7 ± 0.21	2.6 ± 0.11	3.0 ± 0.2	8.0 ± 0.12	6.1 ± 0.20	2.2 ± 0.12	7.8 ± 0.22	2.7 ± 0.13		
	150	2.5 ± 0.00	3.1 ± 0.34	3.0 ± 0.64	3.1 ± 0.43	9.4 ± 0.25	5.7 ± 0.56	3.1 ± 0.43	9.7 ± 0.25	4.7 ± 0.56		
	200	3.0 ± 0.19	3.7 ± 0.31	3.9 ± 0.15	4.9 ± 0.15	11 ± 0.52	6.0 ± 0.0	4.9 ± 0.15	11 ± 0.52	8.0 ± 0.00		
S. aureus	50	-	-	-	1.3 ± 0.11	6.1 ± 0.55	4.3 ± 0.11	1.3 ± 0.11	7.6 ± 0.55	3.0 ± 0.11	16.2	10
	100	1.7 ± 0.50	1.9 ± 0.00	1.7 ± 0.22	5.1 ± 0.05	8.1 ± 0.15	7.0 ± 0.51	2.9 ± 0.95	10 ± 0.15	7.0 ± 0.51		
	150	2.3 ± 0.11	2.4 ± 0.57	3.0 ± 0.78	7.8 ± 0.15	9.4 ± 0.52	9.0 ± 0.00	6.0 ± 0.05	13 ± 0.52	10 ± 0.00		
	200	3.7 ± 0.00	3.7 ± 0.15	3.7 ± 0.66	9.6 ± 0.05	14 ± 0.44	12.0 ± 0.15	9.0 ± 0.15	16 ± 0.44	13 ± 0.15		
E. coli	50	-	-	-	2.3 ± 0.11	2.0 ± 0.04	1.3 ± 0.51	1.3 ± 0.11	2.0 ± 0.04	2.3 ± 0.11	11	10
	100	2.6 ± 0.15	3.0 ± 0.40	3.1 ± 0.10	3.0 ± 0.07	6.4 ± 0.05	4.9 ± 0.03	3.0 ± 0.07	6.4 ± 0.05	4.9 ± 0.03		
	150	3.2 ± 0.01	4.0 ± 0.00	3.5 ± 0.54	4.0 ± 0.40	10 ± 0.70	7.7 ± 0.20	4.0 ± 0.40	10 ± 0.90	7.7 ± 0.20		
	200	4.0 ± 0.11	4.4 ± 0.08	4.3 ± 0.11	7.0 ± 0.04	15 ± 0.99	9.0 ± 0.00	6.0 ± 0.04	11 ± 0.99	9.0 ± 0.00		
K. pneumonia	50	-	-	-	1.3 ± 0.11	4.0 ± 0.55	2.1 ± 0.13	1.3 ± 0.11	4.0 ± 0.55	3.2 ± 0.11	13	10
	100	2.4± 0.65	4.1 ± 0.05	3.0 ± 0.15	3.0 ± 0.03	8.0 ± 0.05	7.0 ± 0.20	3.3 ± 0.03	7.0 ± 0.05	6.0 ± 0.20		
	150	3.5 ± 0.15	4.4 ± 0.10	3.4 ± 0.45	3.4 ± 0.45	10 ± 0.51	7.0 ± 0.15	3.4 ± 0.45	10 ± 0.51	9.0 ± 0.15		
	200	3.0 ± 0.03	5.3 ± 0.30	3.7 ± 0.20	4.2 ± 0.26	16 ± 0.11	9.7 ± 0.32	4.2 ± 0.26	16 ± 0.11	12.7 ± 0.32		
P. aeruginosa	50	-	-	-	1.3 ± 0.11	4.5 ± 0.01	4.3 ± 0.11	2.3 ± 0.11	4.5 ± 0.01	3.5 ± 0.11	15	12
	100	2.3 ± 0.14	4.1 ± 0.08	3.4 ± 0.21	2.4 ± 0.21	10 ± 0.05	5.8 ± 0.45	3.4 ± 0.21	8.2 ± 0.05	5.4 ± 0.45		
	150	3.1 ± 0.45	4.4 ± 0.12	4.4 ± 0.44	4.8 ± 0.44	11 ± 0.09	6.8 ± 0.13	4.8 ± 0.44	10 ± 0.09	6.3 ± 0.13		
	200	4.0 ± 0.41	4.7 ± 0.33	4.6 ± 0.53	6.0 ± 0.53	18 ± 0.48	8.4 ± 0.55	6.0 ± 0.53	19 ± 0.48	7.0 ± 0.45		

Strp: Streptomcycin; Amp:Ampicilin, A: Aqueous, M: Methanol, E: Ethanol

Values are mean inhibition zone (mm) ± S.D of three replicates

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