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Biological Efficacy of Quassia Indica (Geratn) Nooteb and Centella Asiatica (L.) Urban Against Selected Strains of Bacteria

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Abstract: *Antimicrobial activity of different extracts of two selected plants, Quassia indica (Geartn) Nooteb and Centella asiatica (L.) Urban were tested against selected strains of bacteria viz; Escherichia coli, Staphylococcus aureus, Pseudomonas sps, Bacillus thuringiensis, Klebsiella sps and Serratia sps using agar disc diffusion technique. Petroleum ether, acetone, methanol and water extracts of seed and leaf of Q. indica and leaf of C. asiatica showed moderate to significant antimicrobial activity. Of the four extracts tested, petroleum ether and water fractions showed more activity against all the bacterial strains. The zone of inhibition of the active fractions for the petroleum ether ranged from 8.57 to 12.63 mm and 8.53 to 14.5mm for the water fraction of Q. indica seed and leaf and C. asiatica leaf; and exhibited comparable results with widely used commercial antimicrobial agents (16 to19 mm for Kanamycin and 14 to 17mm for Cefotaxime). The results prove the efficacy of the most active fractions of the selected plant extracts to be used for developing potent antimicrobial formulations.*

Keywords: *Antimicrobial activity, B. thuringiensis, Centella asiatica, E.coli, Klebsiella sp, Pseudomaona sp, Staphylococcus aureus, Serratia sp, Quassia indica.*

I. Introduction

Secondary metabolites of plants are well known for their various properties such as larvicidal, bactericidal and fungicidal activities (Ikram and Immanual 1984, Sousa *et al* 1991). Several components having antimicrobial properties were also isolated and identified from plants (Li and Xu, 2008, Verma *et al* 2008) which have great significance in treatment of various microbial infections. Many plants and their crude extracts are used in traditional medicine for the treatment of various diseases such as fever, cough, diarrhea, skin diseases, gout, heart diseases, cuts and wounds and burns etc from time immemorial (Kirtikar and Basu 1935, Arther 1992). The systematic screening and isolation of active components of ethno botanicals used in folk medicines as efficient antimicrobial agents may contribute to a greater demand for plant based therapeutics in the near future; due to their environment friendly, non- narcotic properties and easy availability for affordable prices. The objective of the present study is to evaluate the antimicrobial activities of the different extracts of the two selected plants viz; *Quassia indica* (Geratn) Nooteb and *Centella asiatica* (L.) Urban against the selected strains of bacteria in laboratory conditions.

II. Materials And Methods

1. ***Extraction of Plant materials :***

Fruits and leaves of *Quassia indica* (Geratn) Nooteb of the family Simaroubaceae and leaves of *Centella asiatica* (L.) Urban of the family Umbelliferae were selected for extraction. The fruits/leaves were collected, washed with water, dried under shade and powdered using a kitchen machine. The powder is then extracted with acetone, petroleum ether (PE), methanol (MeOH) and water separately using a soxhlet apparatus. The extracts were dried and made stock solutions in dimethyl sulfoxide (DMSO).

2. ***Bacterial strains used :***

Selected strains of gram-positive and gram-negative bacteria were collected from the pure culture maintained in the department of Life Sciences, University of Calicut. They are as follows;

a) Gram-positive strains: *Staphylococcus aureus* and *Bacillus thuringiensis*

b) Gram-negative strains: *Escherichia coli, Pseudomonas* sps. *Klebsiella* sps. and *Serratia* sps.

3. ***Media used and their composition :***

Nutrient agar media was used for maintaining pure cultures of bacteria for detecting antimicrobial activity. The plates were prepared with nutrient agar (0.5 gm peptone, 0.3 gm beef extract, 2 gm agar, 1 gm NaCl in 100 ml distilled water). The pH of the medium was adjusted to 7.0 before mixing agar to the media. The medium was autoclaved, cooled to 60ºC and poured on to sterile plates.

Nutrient broth was prepared with 0.5 gm peptone, 0.3 gm beef extract, 1 gm NaCl in 100 ml distilled water. The pH of the medium was adjusted to 7.0, autoclaved, cooled to room temperature and used for making suspension of bacteria.

4. ***Antimicrobial activity test :***

Antimicrobial activity of the selected extracts was carried out by the disc diffusion method by following the guidelines of National Committee for Clinical Laboratory Standards (1993). The tests were conducted in agar plates inoculated with bacteria from broth culture. The discs with desired concentrations (30 ug and 40 ug) and standard antibiotics were placed on the plates and incubated with a temperature of about 28ºC for 24 hrs. The clear zones formed around the disc were measured as zone of inhibition. A control disc was also placed on the plate with highest amount of DMSO used for the corresponding test. All experiments were conducted in triplicates.

III. Results

The different extracts of *Q. indica* fruits and leaves and *C. asiatica* leaves were tested against different strains of bacteria and measured antimicrobial activity by disc diffusion method. The zone of inhibition for each plant extracts to the selected strains of bacteria is provided in Table 1. The zone of inhibition for different extracts of *Q. indica* seed ranged from 5.6 mm (*Staphylococcus)* for acetone extract to 12.63 mm (*Serratia*) for PE at a concentration of 40 ug/disc.Of the four extracts of *Q indica* leaf, the zone of inhibition exhibited a range from 7.27 mm (at 30 ug/disc for *Serratia*) to 14.5 mm (at 40 ug /disc of water extract for *E. coli*). Selected extracts of *C. asiatica* showed zone of inhibition between 8.27 mm and 12.73 mm.

IV. Discussion

The present study demonstrates the antimicrobial property of the fruit and leaf extracts of *Q. indica* and leaf extract of *C. asiatica*. All the three materials in four different solvents showed moderate to significant antimicrobial effects. The activity of the materials measured by disc diffusion method indicates concentration depended antimicrobial property. Comparing the four extracts of the selected plant materials, PE and water extracts of the materials exhibited higher activity than the acetone and methanol extracts irrespective of the materials. The activities of the extracts for the different materials are also varied for the different strains of bacteria. However the water extract of all the tested materials showed high activity against the selected strains of bacteria except for *Serratia* sps. which showed highest zone of inhibition at 40 ug/disc concentrations for PE extract of *Q. indica* fruit. Of all the tested fractions, a concentration of 40 ug/disc of water extract of the leaf of *Q. indica* exhibited the largest zone of inhibition (14.5 mm) against *E coli* (figure 1) which is comparable with that of the commercially available antibiotic Kanamycin (16 mm) tested as positive control thus emphasizing the importance of selection of suitable solvents to get maximum antimicrobial activity. The result also supports the susceptibility of gram negative bacteria to inhibition. The crude ethanolic extract of *Gymnema sylvestre* leaves and the saponins isolated from *G. sylvestre* were reported to possess antimicrobial activity against *B. pumilis*, *B. subtilis* and *S. aureus* (Satdive et al; 2003) and *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae* (Khanna and Kannabiran; 2008) respectively. The isolation of the active components from the water and PE extracts of the selected plants may render much higher activity than their crude extracts. Hence the present study explore the scope for developing better cost effective and indigenous alternatives which can substitute conventional antibiotics or antimicrobial agents which suffer reduced efficiency due to the development of single or multidrug resistance by most of the pathogenic microorganisms.

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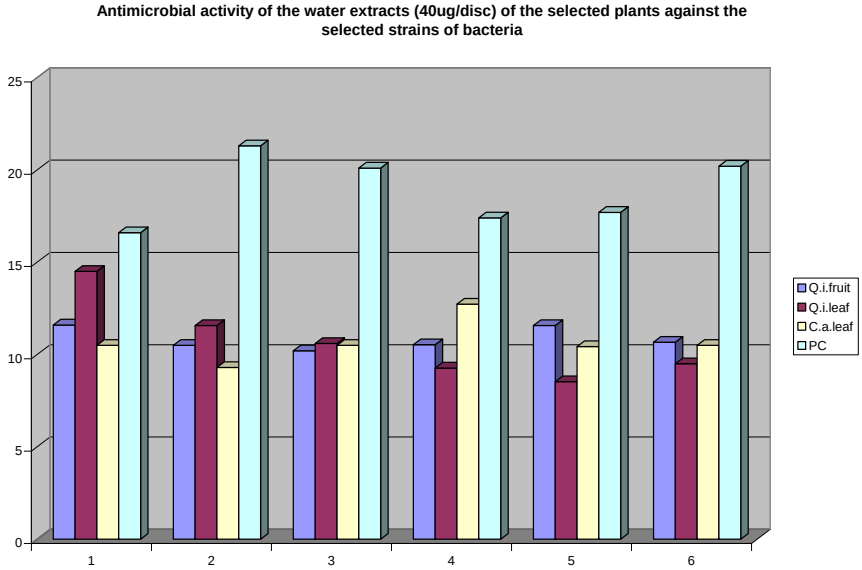

E.coli Staphyloco B. ti Pseudo Klebsella Serraatia

Table 1: Antimicrobial activity of the selected plant extracts against selected strains of bacteria

Sl. No.	Plant (Material)	Extract	Conc. Tested µg/disc	Zone of inhibition (mm)					
				E.coli	*Staphy-lococcus*	*B.ti*	*Pseudo-monas*	*Kleb-siella*	*Serr-atia*
1.	*Q.indica* (Fruit)	Acetone	30	7.37±0.2	7.37±0.3	6.57±0.2	8.3±0.1	8.43±0.2	8.5±0.1
			40	6.2±0.1	5.6±0.2	7.6±0.2	8.5±0.3	8.7±0.2	8.3±0.1
		Pet.ether	30	10.2±0.1	10.4±0.3	10.3±0.1	9.57±0.3	8.57±0.1	10.5±0.3
			40	10.6±0.2	10.3±0.3	11.47±0.3	10.2±0.0	10.43±0.2	12.63±0.1
		MeOH	30	7.3±0.1	5.53±0.3	6.53±0.3	8.8±0.1	7.5±0.3	9.73±0.07
			40	7.33±0.1	8.47±0.3	7.5±0.1	8.7±0.1	8.47±0.3	8.2±0.16
		Water	30	11.4±0.2	10.4±0.1	11.2±0.1	9.53±0.3	8.43±0.3	9.2±0.08
			40	11.6±0.4	10.5±0.2	10.2±0.1	10.5±0.1	11.57±0.3	10.67±0.3
2.	*Q.indica* (Leaf)	Acetone	30	9.43±0.2	9.3±0.1	10.7±0.2	8.57±0.3	7.53±0.1	10.33±0.3
			40	9.73±0.2	10.3±0.2	9.4±0.4	9.6±0.1	8.67±0.3	8.53±0.05
		Pet.ether	30	12.5±0.3	10.6±0.1	10.4±0.1	9.37±0.3	7.57±0.3	9.5±0.16
			40	12.5±0.3	11.3±0.3	10.6±0.1	10.4±0.4	8.5±0.3	9.5±0.22
		MeOH	30	10.5±0.2	9.2±0.1	10.4±0.2	8.6±0.1	7.2±0.1	7.27±0.17
			40	10.4±0.2	9.57±0.3	10.53±0.3	9.27±0.2	8.27±0.2	8.5±0.33
		Water	30	13.2±0.1	10.4±0.2	10.4±0.1	9.2±0.1	7.2±0.1	9.4±0.36
			40	14.5±0.1	11.6±0.3	10.6±0.1	9.27±0.2	8.53±0.3	9.5±0.16
3.	*C.asiatica*	Acetone	30	8.47±0.4	8.7±0.1	9.43±0.3	9.23±0.1	10.26±0.2	9.4±0.36
			40	10.3±0.1	9.57±0.3	10.4±0.1	9.73±0.1	9.2±0.1	9.73±0.07
		Pet.ether	30	9.43±0.3	10.2±0.1	10.26±0.2	9.27±0.2	10.2±0.1	10.5±0.08
			40	11.6±0.3	10.6±0.1	10.53±0.3	10.7±0.3	11.3±0.1	10.53±0.1
		MeOH	30	8.3±0.1	9.53±0.3	8.27±0.2	10.5±0.2	10.2±0.1	10.33±0.3
			40	8.26±0.2	8.2±0.0	9.6±0.1	9.5±0.1	9.4±0.4	9.2±0.08
		Water	30	9.3±0.1	10.6±0.1	8.3±0.1	10.5±0.2	10.2±0.2	11.3±0. 7
			40	10.5±0.3	9.3±0.1	10.5±0.3	12.7±0.2	10.43±0.2	10.5±0.08
4.	Standard Antibiotics		30	K 23.4±0.22	K 24.43±0.25	K 20.2±0.18	C 17.43±0.24	K 17.73±0.07	K 20.2±0.08
			40	K 16.6±0.36	G 21.26±0.17	G 20.1±0.08	C 19.57±0.34	K 24.2±0.08	K 22.1±0.08
5.	DMSO control		40	0	0	0	0	0	0

(Values of mean ± standard deviation shown is the average of three replicates)
(K = Kanamycin, C = Cefotaxime, G = Gentamycin, DMSO = Dimethyl sulfoxide)