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Evaluation of Antibacterial Efficacy of *Clerodendrum serratum* Linn. and *Clerodendrum viscosum* Vent. Leaves against some Human Pathogens causing UT and GIT Infection.

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ABSTRACT

The plant *Clerodendrum serratum* Linn. and *Clerodendrum viscosum* Vent. commonly known as Bharangi and Bhant respectively belonging to the family Verbenaceae was investigated for its antibacterial activity against some selected urinary tract and gastrointestinal tract infection causing pathogens such as *Salmonella paratyphi* MTCC-3220, *Salmonella enterica typhimurium* MTCC-98, *Salmonella enteric ser.typhi* MTCC-733, *Shigella flexeneri* MTCC-9543, *Shigella 5151, Escherichia coli MTCC-118, Escherichia coli* MTCC-614, *Streptococcus mitis 2798, Streptococcus salivarous subsp thermophiles 1938, Pseudomonas aeruginosa 1035, Bacillus circulans* MTCC-490, *Vibrio cholera* MTCC-3906, *Pectobacterium cartovorum* MTCC-1428*, Micrococci, Klebsiella pneumoniae and Bacillus subtilis.* The activities in terms of zone of inhibition were evaluated by Agar well diffusion method at 10 mg/ml of the test extracts. The petroleum ether extract of *C. serratum* and methanol extract of *C. viscosum* exhibited highest activities (in mm) against *Escherichia coli* MTCC-614 (25.14±0.38) and *Klebsiella pneumonia (*25.12±0.51*)* respectively. The minimum inhibitory concentration (MIC) value of the test extracts was determined by two fold dilution assay at range of 0.001-10 and 0.001-1.56 mg/mL for test extracts and reference antibiotics respectively. Methanol extract of *C. viscosum* broadly inhibited UT and GIT infection causing pathogens at a comparative lower concentration when compared to *C. serratum.*

Keywords: Antibacterial activity, *Clerodendrum serratum* Linn., *Clerodendrum viscosum* Vent.*,* Zone of inhibition, urinary and gastrointestinal tract pathogens.

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INTRODUCTION

Herbal drugs constitute a major part in all the traditional systems of medicine. This herbal medicine is a triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive. For these and other reasons, the use of plants for medicines around the world still vastly exceeds the use of modern synthetic drugs. Such activity is not completely dismissed in scientific society and plants are also appreciated in pharmaceutical research as the major resource for new medicines and a growing body of medical literature supports the clinical efficacy of herbal treatments [1].

Urinary tract infections (UTIs) are one of the mostly common infectious diseases caused by *E. coli, K. pneumoniae, P. auruginosa, Proteus sp., Providencia sp, Enterobactor and Serratia sp.* in most of the developed as well as under developed countries. The majority of UTIs that occur in the community are caused by uropathogenic *E. coli* and upto 25% of women who have a first UTI will have a second infection within 6 months. 50-60 % of women report having a UTI during their lifetime [2]. Gastro intestinal tract (GIT) infection caused by *E. coli, Salmonella enteritidis, Salmonella typhi, Salmonella paratyphi, Vibrio cholerae, and Shigella* invades the body from the GIT to cause systemic illness. In India GIT related infections are the major causes of health burden and registered prevalence of abdominal pain to be 57.3%, constipation 61% and chronic diarrhoea 39% [3].

Clerodendrum serratum Linn.(Family: Verbenaceae) (CS) is a slightly woody shrub with bluntly quadrangular stems and branches, leaves usually three at a node distributed in the deciduous forests of the western Ghats of India [4]. In Indian system of medicine the plant is well known as Bharangi (Hindi). It is commonly known as Blue glory (English), Brahmani (Sanskrit), Gantu Bharangi (Kannad). As per the traditional claims the leaves are applied in the form of poultice in skin suppurations [5]. It is recommended for inflammations of the eye [6]. The leaves can be used for external application in headache and also used in sinusitis. The extracts has been locally consumed to treat hypertension [7], inflammation [8] and cancer [9]. Priliminary phytochemical analysis of CS revealed the presence of flavonoids, tannins, terpenoids and saponins [10].

Clerodendrum viscosum Vent. (Family: Verbenaceae) (CV) is a flowering shrub or small tree and produce circular leaves with 6 inch diameter. Leaves are simple, opposite, both surfaces sparsely villous-pubescent, elliptic, ovate or elongate ovate [11]. The plant is commonly known as Hill glory bower (English), Bhant (Hindi), Barhibarha (Sanskrit), Ibbane (Kannada). The leaves are widely used as antidandruff, antipyretic, ascaricide, laxative, vermifuge [12] and in the treatment of convulsion [13], diabetes, malaria [14], skin diseases, spasm, snake bite and tumor [15]. In Thai medicine the leaves are known to be diuretic and used for the treatment of intestinal infections and kidney dysfunction. In many traditional practices the leaves are widely used as antihyperglycemic [16]. Preliminary phytochemical screening of CV revealed the presence of alkaloids, flavonoids, saponins, cleodendroside and β-sitoterol [17].

The present investigation was undertaken with an aim and objective to evaluate the comparative *in-vitro* antibacterial potential of the leaves of two species of *Clerodendrum* against human pathogens causing UT & GIT infection based on the folkloric claims.

MATERIALS AND METHODS

Collection and identification of plant materials

The leaves of CS and CV were collected from Utkal University campus, Vani Vihar, Bhubaneswar and Naharkanta village of Khordha district, Odisha, India respectively. Identification of voucher specimen was authentificated by Dr.K.B. Satpathy, P.G. Department of Botany, Utkal University, Bhubaneswar and voucher specimen (SVN-537,SVN-536) was deposited in the departmental herbarium.

Processing of plant material and preparation of extract

The collected leaves were shade dried and ground to a coarse power .The powdered leaves were successively extracted [18] with petroleum ether (PE), chloroform (CH) and methanol (ME) by soxhlation and

the solvent was evaporated under reduced pressure in a rotary evaporator. The extracts were kept in a desiccator for further use. The yield of petroleum ether, chloroform and methanol extracts of CS and CV leaves were 2.62%, 1.89%, 11.52% and 2.71%, 2.12%, 12.16% w/w respectively.

Evaluation of the extract for antibacterial activity

The *in-vitro* antibacterial screening was carried out against selected bacterial pathogens causing Urinary tract (UT) and Gastro intestinal tract (GIT) infections in human. The bacterial pathogens viz., *Salmonella paratyphi* MTCC-3220, *Salmonella enterica typhimurium* MTCC-98, *Salmonella enteric ser.typhi* MTCC-733, *Shigella flexeneri* MTCC-9543, *Shigella* MTCC-5151*, Escherichia coli MTCC-118, Escherichia coli* MTCC-614, *Streptococcus mitis 2798, Streptococcus salivarus subsp thermophiles 1938, Pseudomonas aeruginosa 1035, Bacillus circulans* MTCC-490, *Vibrio cholera* MTCC-3906, *Pectobacterium cartovorum* MTCC-1428*.* These species were procured from Microbial Type Culture Collection Centre (MTCC) & Gene Bank, Chandigarh, India. Other strains viz., *Micrococci, K. Pneumonia* and *B. subtilis* were obtained from Pharmaceutical Biotechnology Division, University Department of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India. These organisms were identified by standard microbiological methods [19]. The antibacterial screening of the extracts were carried out by determining the zone of inhibition using agar well diffusion method [20]. The minimum inhibitory concentration (MIC)[21] was studied by two fold serial dilution method .

Agar well Diffusion assay [20]

The microorganisms were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37^0C for 24 h and were referred to as seeded broth. On the surface of sterile agar plates, an inoculum of 100 µl was aseptically placed and sterilized glass spreader was used for even distribution of the inoculum. In the agar plates, wells were prepared by using a sterile cork borer of 6.0 mm diameter, loaded with 50 µl of each test extracts and reference antibiotics (RA). The PE, CH and ME extracts were dissolved in dimethyl formamide (5% v/v) (DMF) which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. The extracts were made solution at a concentration of 10 mg/mL. Amoxicillin + clavulinic acid (AC) and Ciprofloxacin (CF) were used as reference standards at a concentration of 1.56 mg/mL which were finally sterilized by filtration using 0.45 µm Millipore filters. The reference antibiotics were appropriately diluted in DMF (5% v/v) to give a stock solution having concentration of 1.56 mg/mL. Then 50 µl of each of the test extracts (500 µg/well) and reference antibiotics (78 µg/well) from the above stock solutions were introduced into the wells for comparative evaluation of antibacterial efficacy. The *in-vitro* antibacterial activities of the plant extracts were determined by using selected pathogens by agar well diffusion method [20]. The plates were then refrigerated at 4^0C for 1 h that allows the test extracts and RA to diffuse and then incubated at 37^0C for 24 h. The diameter of the zone of inhibition exhibited by each of the test extracts and reference antibiotics were measured and compared using the Hi-Antibiotic zone Scale (Hi-Media). The test extracts exhibiting inhibitory zones less than 10 mm were considered to be inactive against the test pathogens and evidenced the resistant nature of the organism. The PE, CH and ME extracts of CS and CV leaves that exhibited highest inhibitory zones against the bacterial pathogens were further subjected to determination of MIC. The density of the bacterial suspension was standardized by standard McFarland[22] method. The results of agar well diffusion method is shown in Table-1.

Determination of MIC [21]

The minimum inhibitory concentration (MIC) value of the tested extracts was determined by two fold dilution assay at range of 10 - 0.001 and 1.56 –0.001 mg/mL for RA respectively. The density of the bacterial suspension was standardized by standard McFarland [22] method. The MICs of the extracts were determined by using two fold serial dilution assay for the microorganisms which were determined sensitive to various plant extracts. The inoculums were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts were dissolved in dimethyl formamide (5% v/v) and then diluted by two folds. MIC values of the extracts against UT and GIT pathogens were determined with some modifications. The dilutions were performed by dispensing into each tube 1 mL of nutrient broth and 1ml of each extract and then serially diluted to achieve desired concentration of 10-0.001 mg/mL. 50 µl of freshly prepared inoculum was then added to all the tubes. Control was chosen using 1 ml of broth, 1 ml of solvent DMF and then adding 50 µl inoculum without the extract. Contents of each tube containing UT and GIT pathogens under test were

treated with different concentrations of each extract for 24 h. The contents of the tube were then subcultured on nutrient agar plates by adding 10 µl of the above inoculums (treated with test extracts) and incubated at 37^0C for determination of MIC. The solvent control tubes were also observed for any inhibitory action and found to have no zone of inhibition.

RESULTS AND DISCUSSION

The leaf extracts of two plants viz., CS and CV were subjected to antibacterial screening against four gram positive and twelve gram negative bacteria causing UT and GIT infection.

The results indicated that PE extract of CS exhibited highest zone of inhibition (in mm) against *E. coli* 614 (25.14±0.38), least against *S. mitis* 3906 (13.85±0.59) and resistant to *S. salivarous subsp thermophilus 1938* (9.91±0.21). CS exhibited moderate activity against *P. cartovorum* 1428 (16.01±0.91) followed by *E. coli* 118 (16.79±0.510), *V. cholera* 3906 (14.90±0.51), *S. enterica typhimurium* 98 (14.89±0.45), *S. enteric ser.typhi* 733 (15.15±0.37). The CH extract of CS showed highest zone of inhibition against *S. enterica typhimurium* 98 (16.98±0.63), least against *B. circulans* 490 (10.79±0.53) and ineffective against *S. flexneri* 9543. CS exhibited moderate activity against *E. coli* 614 (15.91±0.56) followed by *P. aeruginosa* 1035 (15.89±0.61), *Shigella* 5151 (15.06±0.56), *P.cartovorum* 1428 (15.08±0.37) and *Micrococci (*15.98±0.36). The MT extract of CS showed highest zone of inhibition against *K. pneumoniae* (25.12±0.51), least effective against *S.mitis* 2798 (8.81±0.86) and was resistant to *S. salivarous subsp thermophiles* 1938 (8.97±0.56). CS exhibited moderate activity against *Micrococci* (15.87±0.76) and *E. coli* 614 (16.02±0.61). Among the three extracts of CS, the PE extract registered broad spectrum antibacterial activity.

The PE extract of CV showed highest zone of inhibition against *S. enterica typhimurium* 98 (18.95±0.81), least effective against *P.cartovorum* 1428 (10.01±0.51) and was sensitive against all pathogens. PE extract of CV exhibited moderate activity against *S. paratyphi* 3220 (13.91±0.72) followed by *S. flexeneri* 9543 (12.90±0.32), *E. coli* 614 (14.93±0.51). The CH extract of CV showed highest zone of inhibition against *E.coli* 614 (18.91±0.71), least against *S. enteric ser.typhi* 733 (10.11±0.46) and was resistant to *B.circulans* 490 (9.83±0.58). The CH extract of CV exhibited moderate activity against *P. cartovorum* 1428 (12.93±0.74) and *S. mitis* (11.95±0.43). The MT extract of CV showed highest zone of inhibition against *K. pneumoniae* (25.12±0.51) *followed by E. coli* 614 (17.95±0.17), *Shigella* 5151 (20.12±0.39) ,*V. cholera* 3906 (16.89±0.45), *S. enterica typhimurium* 98 (20.12±0.62) and *Micrococci,* least zone of inhibition against *P. aerugenosa (12.23±0.39)* and was not resistant to any pathogen. The MT extract of CV exhibited moderate activity against *E. coli* 118 (15.11±0.36). Among the three extracts MT extract of CV exhibited highest efficacy against the test pathogens.

The results of Agar well diffusion method revealed broad spectrum activity of PE extract of CS and MT extract of CV against the test pathogens causing UT and GIT infection.

The MT extract of CV was found to be more potent against *Micrococci, S. enterica typhimurium* 98, *S. flexeneri* 9543, *Shigella* 5151, *B. subtilis, B. circulans* 490, *P. cartovorum* 1428, *S. mitis, P. Aerugenosa* when compared to CS. However, the findings of our investigation revealed inhibitory potential of CS against *S. enteric ser. typhi* 733, *E. coli 118, E. coli* 614 , *S. salivarous subsp thermophilus 1938 and V. cholera* 3906 as evidenced from the results of MIC shown in Table-2 .Thus MT extract of CV broadly inhibited microorganism at a comparative lower concentration registered higher potential against the test pathogens when compared to CS. Our findings are in accordance with the findings of Oly *et.al* and his coworkers [23]. Future investigation is aimed at isolation of phytoconstituents responsible for antibacterial potential.

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Table 1: In-vitro antibacterial activity of leaf extracts of *C. Serratum* and *C. Viscosum* by Agar well diffusion method.

Zone Of Inhibition (in mm)*							
Extract 500 µg/well							
Organisms	*C.SERRATUM*			*C.VISCOSUM*			
	PE	CH	MT	PE	CH	MT	RA
1	19.11±0.36	15.98±0.36	15.87±0.78	12.93±0.65	17.12±0.65	18.12±0.12	14.01±0.21(CF)
2	18.88±0.21	12.92±0.46	13.91±0.61	13.91±0.72	12.89±0.35	16.98±0.29	16.99±0.52(CF)
3	14.89±0.45	16.98±0.63	13.78±0.56	18.95±0.81	12.89±0.37	20.12±0.62	21.56±0.82(CF)
4	15.15±0.37	10.98±0.51	13.61±0.51	10.92±0.21	10.11±0.46	17.91±0.51	22.34±0.91(CF)
5	13.83±0.51	--	11.91±0.91	12.90±0.32	11.89±0.51	13.93±0.57	20.12±0.11(CF)
6	20.95±0.58	15.06±0.56	13.76±0.56	12.12±0.59	16.08±0.58	20.12±0.39	17.32±0.24(CF)
7	16.79±0.51	11.89±0.65	13.91±0.23	13.12±0.43	12.92±0.31	15.11±0.36	24.56±0.77(CF)
8	25.14±0.38	15.91±0.56	16.02±0.61	14.93±0.51	18.91±0.71	17.95±0.17	26.12±0.48(CF)
9	16.11±0.12	12.86±0.86	12.56±0.51	11.72±0.43	12.83±0.39	13.71±0.63	17.34±0.22(AC)
10	24.01±0.11	10.79±0.53	12.78±0.89	13.83±0.29	9.83±0.58	11.85±0.61	15.23±0.28(AC)
11	16.01±0.91	15.08±0.37	11.92±0.71	10.01±0.51	15.11±0.91	12.93±0.74	19.02±0.53(CF)
12	13.85±0.59	13.96±0.49	8.81±0.86	16.83±0.34	14.92±0.42	14.95±0.43	18.34±0.33(AC)
13	23.02±0.41	15.89±0.61	11.10±0.76	10.56±0.54	16.91±0.59	12.23±0.39	25.21±0.55(CF)
14	9.91±0.21	13.23±0.45	8.97±0.56	10.09±0.89	11.10±0.85	12.53±0.31	13.03±0.22(AC)
15	19.2±0.35	11.01±0.69	17.76±0.11	24.12±0.39	16.11±0.49	25.12±0.51	24.11±0.17(CF)
16	14.90±0.51	12.82±0.65	12.56±0.21	12.11±0.48	13.86±0.12	16.89±0.48	19.11±0.66(CF)

All the values are mean ± standard deviation of three determinations.
(-) Indicates no zone of inhibition.

1. Micrococci, 2.Salmonella paratyphi (MTCC-3220), 3.*Salmonella enterica typhimurium* (MTCC-98), 4.*Salmonella enteric ser. typhi* (MTCC-733), *5.Shigella flexeneri* (MTCC-9543), 6. *Shigella* (MTCC-5151), *7. Escherichia coli (MTCC-118), 8. Escherichia coli* (MTCC-614), 9. *Bacillus sabtilis,* 10. *Bacillus circulans* (MTCC-490), 11. *Pectobacterium cartovorum* (MTCC-1428), 12.*Streptococcus mitis, 13.Pseudomonas aerugenosa,* 14. *Streptococcus salivarous subsp hermophilus* (MTCC-1938), *Klebsiella pneumoniae,* 16.*Vibrio cholera* (MTCC-3906).
PE, CH, MT stands for petroleum ether, chloroform and methanol respectively.

Table 2: The MIC values of leaf extracts of *C. serratum and C. Viscosum* against the test pathogens tested by two fold serial dilution assay.

		MIC (mg/mL)			
Organism	**Solvent extract**	***C. serratum***	**Solvent extract**	***C. viscosum***	**Reference Antibiotic**
1	PE	0.312	MT	0.078	0.024
2	PE	0.156	PT	0.156	0.012
3	CH	0.078	MT	0.019	0.006
4	PE	0.039	MT	0.078	0.003
5	PE	0.078	MT	0.039	0.006
6	PE	0.156	MT	0.078	0.012
7	PE	0.078	MT	0.156	0.003
8	PE	0.019	CH	0.039	0.006
9	ME	0.312	MT	1.25	0.012
10	PE	0.625	PE	0.625	0.012
11	PE	0.312	CH	0.078	0.097
12	PE	1.25	MT	0.625	0.024
13	PE	0.039	CH	0.019	0.006
14	CH	1.25	MT	2.5	0.024
15	PE	0.078	PE	0.009	0.006
16	PE	0.039	MT	0.156	0.012

1. Micrococci, 2.Salmonella paratyphi (MTCC-3220), 3.*Salmonella enterica typhimurium* (MTCC-98), 4.*Salmonella enteric ser. typhi* (MTCC-733), *5.Shigella flexeneri* (MTCC-9543), 6. *Shigella* (MTCC-5151), *7. Escherichia coli (MTCC-118), 8. Escherichia coli* (MTCC-614), 9. *Bacillus sabtilis,* 10. *Bacillus circulans* (MTCC-490), 11. *Pectobacterium cartovorum* (MTCC-1428), 12.*Streptococcus mitis, 13.Pseudomonas aerugenosa,* 14. *Streptococcus salivarous subsp hermophilus* (MTCC-1938), *Klebsiella pneumoniae,* 16.*Vibrio cholera* (MTCC-3906).
PE, CH, MT stands for petroleum ether, chloroform and methanol respectively.

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CONCLUSION

The leaves of *Clerodendrum serratum* and *Clerodendrum viscosum* exhibited potent antibacterial activity against the selected UT and GIT infection causing pathogens. To summarize the methanol extract of *Clerodendrum viscosum* broadly inhibited pathogens causing infection of UT and GIT at a comparative lower concentration when compared to *Clerodendrum serratum*.

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