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### *In vitro* antimicrobial activity of *Clerodendrum viscosum* (Vent)

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#### ABSTRACT

*Clerodendrum viscosum* a common plant belonging to the family verbenaceae, grows as a weed on roadside and waste lands. The various extracts of the leaves of *C. viscosum* were prepared by successive solvent extraction with increasing polarity order using Soxhlet apparatus. The various extracts prepared were subjected to antimicrobial screening for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella* and *Vibrio cholerae* at a concentration of 20, 60, 100mg/ml in DMSO. Punch well method is used to assess the activity, the results obtained showed that the petroleum ether fraction exhibited a significant activity against *Staphylococcus aureus*, chloroform extract showed activity against *Staphylococcus aureus*. *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella*, *Vibrio cholerae*, acetone extract showed significant activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella*, *Vibrio cholerae* and alcoholic extract exhibited the activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella*, *Vibrio cholerae*. Benzene and aqueous extract did not show any activity against any of the pathogens used in the test.

**Key words:** *Clerodendrum viscosum* (Vent), Mueller Hinton Agar, Successive extraction, Clerodin, Antimicrobial activity.

#### INTRODUCTION

*Clerodendrum viscosum* vent verbenaceae known as Bhandirah in Sanskrit is an important plant in the Indian system of medicine. It is a large gregarious shrub found throughout India up to 1800m and weed along the roadside and waste lands. The plant has been used as an antiseptic, anti-inflammatory, antipyretic, vermifuge, treatment of tumors, leprosy and skin diseases [1].

Areal part of the plant contains sterols, a diterpene clerodin, the root contains lupeol,  $\beta$ -sitosterol, the flower contains glycoside acetoside, fumaric acid, esters of caffeic acid, lupeol,  $\beta$ -sitosterol, cleridine [2]. Essential oil analysis of the root, leaf of the plant revealed the presence of monoterpene, limonene, pinene, cymene, myrcene and sesquiterpenes [3]. Phytochemical study of the leaves revealed the presence of carbohydrates, phytosterols, fixed oil, saponins and phenolic compounds [6]. The present study was undertaken to screen for the antimicrobial activity of various fractions of the leaf extract of *C.viscosum* against various pathogens

## MATERIALS AND METHODS

### Plant material

The leaves of *C.viscosum* were collected from Udupi district, Karnataka, India in the month of April, the plant was authenticated by Dr Gopalakrishna Bhat, Taxonomist, Professor of botany, Poorna Prajna College Udupi. A voucher specimen pp 515 has been deposited in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India

### Preparation of the Extract:

The collected leaves were shade dried, were ground to coarse powder, the powder was successively extracted with different solvents like petroleum ether, benzene, chloroform, acetone, ethanol and water with their increasing order of polarity by soxhlation for 6 hours.[4,5] Extracts were concentrated in vacuo and stored in a desiccator. The above extracts were subjected for antimicrobial screening

### Pathogens screened for antimicrobial property

*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysenteriae*, *Vibrio cholera*.

### Preparation of the concentration of the extracts

Each of the above extracts were dissolved separately in DMSO, the final concentration of the extracts screened for antimicrobial activity of *c.viscosum* were 20, 60, 100 mg/ml

### Preparation of media

The dehydrated Mueller hinton agar medium was obtained from Hi-media laboratory Pvt Ltd, Mumbai, India. It contained the following ingredients:

38g of the MHA was suspended in 1000ml of distilled water, boiled and dissolved completely. It was sterilized by autoclaving at 15 lbs. pressure (121<sup>0</sup>C) for 15 min and poured into sterile Petri dish and allowed to set. Sterility check was done after the agar was solidified [7].

### Inoculation method

The plates were inoculated by dipping a sterile swab into the microbial growth. Excess of the inoculate was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid. The medium was inoculated by evenly streaking the swab over the entire surface of the plate in three directions.

### Punch well method

Sterile MHA (at 45<sup>0</sup>C) was poured into sterile Petri dishes, which was inoculated with the test organisms. Wells (6mm diameter) were made with the aid of flamed cork borer on the surface of the agar plates. About 10µl of the each concentration were delivered into each of the wells. These were incubated at 37<sup>0</sup>C for 24h. The presence of zone of inhibition was regarded as the antimicrobial action. From the inhibition zones seen, antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured. DMSO was used as a control [8, 9].

**Table 1: Antimicrobial activity of *C.viscosum* vents against various pathogens**

Extract used	<i>Staphylococcus aureus</i>			<i>Pseudomonas aeruginosa</i>			<i>Escherichia coli</i>			<i>Klebsiella pneumoniae</i>			<i>Shigella dysenteriae</i>			<i>Vibrio cholerae</i>			
	mg/ml	20	60	100	20	60	100	20	60	100	20	60	100	20	60	100	20	60	100
	Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)			
Pet ether	12	14	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
benzene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
chloform	10	13	14	9	15	18	-	8	11	-	-	-	8	13	15	7	10	17	
acetone	9	12	14	15	17	20	-	-	-	-	-	12	18	20	22	12	15	18	
alcohol	-	-	-	15	17	20	-	-	-	-	-	12	16	18	20	9	14	18	
Aqueous	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

(-) indicates no activity

## RESULTS AND DISCUSSION

From the results obtained it was showed that the petroleum ether fraction exhibited a significant activity against *Staphylococcus aureus*, chloroform extract showed activity against *Staphylococcus aureus*. *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella*, *Vibrio cholerae* acetone extract showed significant activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella*, *Vibrio cholerae* and alcoholic extract exhibited the activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Shigella*, *Vibrio cholere*. *Benzene* and aqueous extract did not showed any activity against any of the pathogens used in the test .moreover from the result it is clear that the actone fraction of the extract of *C,viscosum* leaf exhibited a significant activity against all the pathogens used in the text except *E.coli*, it was also clear from the result that chloroform , acetone and alcoholic extract showed a significant activity against two enteric bacteria *Shigella dysenteriae type II* and *Vibrio choleraei*.

## CONCLUSION

From the results, it could be concluded that the *C.viscosum* leaves can be used as antimicrobial agents as a natural source, moreover the results showed that the chloroform ,acetone and alcoholic extracts possess powerfull activity agaist two enteric bacteria *Shigella dysenteriae type II* and *Vibrio cholerae* the two life threatening pathogens affecting the rural population with reduced level of sanitation. Further ,the more advanced study on this plant and the isolation of the active constituents in the pure form and establishing its antimicrobial property will be more beneficial.

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