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IN VITRO ANTIBACTERIAL ACTIVITY OF *CLEOME VISCOSA* LINN

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ABSTRACT

The present investigation is aimed to carryout *in vitro* antibacterial activities of the methanol and aqueous extracts of *Cleome viscosa* against seven bacterial species. To evaluate antibacterial activity, the agar well diffusion assay was performed. Methanol extract was showed more antibacterial activity than aqueous extract. Methanol extract has exhibited highest and significant antibacterial activity against all seven bacteria, while the aqueous extract showed activity against *Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*. The lowest MIC values were <3.125µg/ml and 6.25 µg/ml for the crude extracts of methanol of *Cleome viscose* against *Pseudomonas aeruginosa* and *Proteus vulgaris*, respectively. Reference antibiotic, Ampicillin was tested against all bacteria and the results were compared with the activity of both methanol and aqueous extracts of *Cleome viscosa*. The results were expressed as Mean±SD and SEM.

Keywords: *Cleome viscosa*, Antibacterial activity, Minimum Inhibitory Concentration, Ampicillin.

INTRODUCTION

The use of plants for healing is as ancient and universal as medicine itself. Plants act generally to stimulate and supplement the body's forces; they are the natural food for human beings [1, 2]. Many infectious diseases are known to be treated with herbal remedies throughout the history of man kind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [3, 4, 5].

Cleome viscosa Linn. (Capparidaceae) is also known as Tickweed, or Spider plant. It occurs in woodland and grassland, and is a weed of fallow land, fields, roadsides and wasteland, often occurring on sandy soils, but sometimes on calcareous and rocky soils. In Asia and Africa the leaves and seeds used as a rubefacient and vesicant and to treat infections, fever, rheumatism and headache. The whole herb is used in treatment of

inflammation of the middle ear and applied on wounds and ulcers. A decoction is used as an expectorant and digestive stimulant and the vapour from a steaming decoction of the whole plant is inhaled to treat headache [6]. The seeds and its oil have antihelminthic properties but they are ineffective in treating roundworm infections [7]. The roots are a remedy for scurvy and rheumatism [8]. An aqueous seed extract displayed significant analgesic activity in mice and local anaesthetic activity in guinea pigs [9, 10]. In tests with rats the anti-diarrhoeal [11] and antipyretic [12] activities of the extracts have been confirmed. The aim of the present study was conducted to investigate antibacterial properties of *Cleome viscosa* by preliminary bioassay screening.

MATERIALS AND METHODS

Plant material

Fresh aerial parts of *Cleome viscosa* was collected in and around Visakhapatnam district ($17^0 12^1$ to $18^0 33^1$ N latitudes and $82^0 18^1$ to $83^0 22^1$ E longitudes) at 159m above sea level. The specimen was authenticated in the herbarium unit of the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India and voucher specimen was deposited at the same herbarium.

Preparation of crude extracts

Hundred grams of the air dried and coarsely powdered plant material was exhaustively extracted for 2 hrs with Petroleum ether (60-80^0C) in Soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rotavapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). The extracted plant material was then air-dried, repacked in the Soxhlet apparatus and exhaustively extracted with methanol (98.8%) until solvent turned pure and colorless [13]. The methanol extract was filtered and evaporated under reduced pressure using Rotavapor.

Simultaneously, aqueous extract was prepared by adding boiled distilled water to 100g of coarsely plant powder on water bath with occasionally stirring for 4 hrs. The extract was then filtered using Whatmman No.1 filter paper and the filtrate was evaporated *in vacuo* and dried using Rotavapor [14]. The final dried extracts of both methanol and aqueous were dissolved in dimethyl-sulphoxide (DMSO) to make final concentrations of 500µg/ml and 750µg/ml, which kept in refrigerator till used.

Sources and maintenance of bacteria

The bacteria used in this study included *Escherichia coli* MTCC-B2401, *Klebsiella pneumoniae* MTCC-2405, *Proteus vulgaris* MTCC-B1771, *Pseudomonas aeruginosa* MTCC-B4996, *Bacillus subtilis* MTCC-B2274, *Staphylococcus aureus* MTCC-B1144 and *Streptomyces pneumoniae* MTCC-B4734. They were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The viability of the organisms were maintained by regular transfer into freshly prepared nutrient agar (Himedia) and stored at 4^0 C until used.

Antibacterial assay

Antibacterial activity was carried out by Agar well diffusion [15]. Mueller- Hinton broth was applied for growing and diluting the bacterial suspensions. Bacterial strains were grown to exponential phase in Mueller-Hinton at 37^o C for 18 hrs and adjusted to a final density of 10^8 CFU/ml by diluting fresh cultures and comparison with Mc Farland density. For susceptibility testing, 50µl of Mueller- Hinton broth was inoculated into 100ml of nutrient agar and care was taken in ensure proper homogenization and poured into petridishes and allowed them to cool under strict aseptic conditions. After the medium was solidified a well was made in petridishes with the help of a sterile metal borer (6mm). 50µl of each extracts were filled in each well by using adjustable volume digital Finn pipette. After that the plates were incubated at 37^0 C for 24 hrs. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well by using Hiantibiotic zone scale-c and the activity was compared with standard antibiotic, Ampicillin (30µg/ml). Simultaneously, control (DMSO) was also maintained without extract. Three replicates were carried out for each extract against each of the test organism. The results were expressed as Mean± SD and SEM.

The minimum inhibitory concentration (MIC) was determined by a modification of agar diffusion [16]. A two-fold serial dilution of each extract was prepared in DMSO to achieve a decreasing range of extract concentrations from 100µg/ml to approximately 3.125µg/ml. A 0.1 ml sample with bacterial cells as described above, incubation was at 37^0C for 24hrs. The lowest concentration of extract showing a zone of inhibition was taken as the MIC.

RESULTS AND DISCUSSION

The results of antibacterial activity of the extracts of *Cleome viscosa* is summarized in Table-1. Both methanol and aqueous extracts have showed good antibacterial activity against all seven bacteria. Methanol extracts were more active than the aqueous extracts. Out of the two doses (500µg and 750µg) of extracts, higher doses showed greater antibacterial activity. There is no overcoming of growth in the zone of inhibition with prolonged incubation.

TABLE 1: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXTRACTS OF *CLEOME VISCOSA*

Name of the bacteria	**Crude extracts**	**Diameter of Zone of the Inhibition(mm)**		
		Extract Concentrations		**Ampicillin**
		500µg	**750µg**	**30µg**
Escherichia coli	Methanol	12±0.4	16±0.4	22±0.4
	Aqueous	--	--	
Klebsiella pneumoniae	Methanol	18±0.5	23±0.6	21±0.2
	Aqueous	16±0.5	18±0.8	
Proteus vulgaris	Methanol	23±0.2	22±0.6	21±0.6
	Aqueous	17±0.7	20±0.1	
Pseudomonas aeruginosa	Methanol	25±0.2	28±0.6	23±0.6
	Aqueous	16±0.7	20±0.1	
Bacillus subtilis	Methanol	18±0.9	21±0.5	19±0.1
	Aqueous	--	--	
Staphylococcus aureus	Methanol	12±0.4	14±0.4	23±0.6
	Aqueous	--	--	
Streptomyces pneumoniae	Methanol	--	--	16±0.6
	Aqueous	--	--	

--: no activity
Values were expressed in Mean± SEM of three individual experiments.

All the bacterial species showed a fairy high degree of sensitivity to methanol extract. Methanol extract showed promising results against *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus* except *Srteptomyces pneumoniae* was found to be resistant. The aqueous extract was found effective against *Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*. Methanol extract found to be highest antibacterial activity against *Pseudomonas aeruginosa* (28mm), with the aqueous extract exhibited highest antibacterial activity against *Bacillus subtilis* (21mm). *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Streptomyces pneumoniae* were found resistant to the aqueous extract. Both methanol and aqueous extracts did not show any activity against *Streptomyces pneumoniae*.

From the MIC values (Table-2), it was observed that methanol extract showed significant inhibitory activity followed by aqueous extract. The MIC values for methanol extract were <3.125µg/ml for *Pseudomonas aeruginosa*, while 6.25µg/ml against *Proteus vulgaris* and 50µg/ml against *Bacillus vulgaris* and *Klebsiella pneumoniae*. The MIC values of aqueous extract were 50µg/ml for *Pseudomonas aeruginosa* and *Proteus vulgaris* and 100µg/ml for *Klebsiella pneumoniae* and *Bacillus subtilis* and other organisms showed feeble inhibition.

TABLE 2: MIC OF METHANOL AND AQUEOUS EXTRACTS OF *CLEOME VISCOSA*.

Name of the bacteria	MIC (µg/ml)	
	Methanol extract	Aqueous extract
Escherichia coli	100	>100
Klebsiella pneumoniae	50	100
Proteus vulgaris	6.25	50
Pseudomonas aeruginosa	<3.125	50
Bacillus subtilis	50	100
Staphylococcus aureus	100	>100
Streptomyces pneumoniae	>100	>100

The antibacterial activity of methanol extract of *Cleome viscosa* was higher than those of tested standard (synthetic) antibiotic, Ampicillin. Compared to this antibiotic (30µg/ml) methanol extract showed a broad spectrum activity against *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Bacillus subtilis*, and in some cases better level of antibacterial activity. The aqueous extract had narrow spectrum of activity against tested bacteria.

Methanol extract exhibited more antibacterial activity than corresponding aqueous extract. This may also be as a result of their phytochemical constituents. The presence of only phenols and tannins may not have been enough to exert an inhibitory activity on the pathogens. On other hand, the components may not have in themselves the ability to cause any inhibition on the growth of the test organisms.

It has been reported that bioactive natural products present in plants can be used as drugs and biological or pharmacological tools [17]. Flavonoids from *Erythrina burtii* were active against Gram positive and Gram negative bacteria [18]. Flavonoids and tannins have also been found to be responsible for the antimicrobial activities of some 45 medicinal plants in India [19].

CONCLUSION

This study was investigated *in vitro* antibacterial activity. *Cleome viscosa* showed significant and higher antibacterial activity with low MIC values may serve as sources for compounds with therapeutic potency. This study may provide promising baseline information for the potential use of these crude extracts in the treatment of bacterial infections. Therefore, this is a good candidate for phytochemical and pharmacological investigations to discover new broad spectrum bioactive compounds.

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