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CLEOME VISCOSA: AN EFFECTIVE MEDICINAL HERB FOR OTITIS MEDIA

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ABSTRACT
Otitis media is one of the most common bacterial infectious diseases among children worldwide and is the leading cause of morbidity and mortality in critically ill patients in developing countries. Acetone, chloroform and methanol extracts of *Cleome viscosa* leaves were investigated for antimicrobial activity against pathogenic bacteria causing otitis media using disc diffusion and broth dilution method. Extracts were also evaluated for antioxidant activity using DPPH radical and phytochemicals. Among the organisms tested *S. pneumoniae* and *E. coli* were most sensitive to acetone extract followed by *S. aureus, K. pneumoniae* and *P. aeruginosa*. Effective inhibition zone range of acetone extract was 20-35 mm and MIC range was 4-32 mg/ml. Activity of other extracts was isolate dependent. Radical scavenging activity was 33 % at 50 µg/ml and 21% at 100 µg/ml concentration. Phytochemical analysis showed presence of flavonoids, tannins, saponins and alkaloids. The study shows that *Cleome viscosa* may have potential in the control of otitis media pathogens.

KEY WORDS: Medicinal plants, antimicrobial activity, otitis media.

INTRODUCTION

Diseases of ear, nose and throat i.e. upper respiratory tract affect the functioning of adults as well as children, often with significant impairment of the daily life of affected patients (Witsell *et al*., 2001). Upper respiratory tract infection includes sore throat, earache, laryngitis, common cold, sinusitis and otitis media (Sazawal and Black , 2003). Otitis media causes inflammation of the middle ear drum and the inner ear (Albert 1999). Otitis media is a complex generic term that includes the acute otitis media (AOM) and otitis media with effusion (OME) (Bowd, 2005) is highly prevalent worldwide (Ifante and Fernandez, 1993). Over 50 % of the cases of otitis media caused by bacteria also fungi, viruses, *Mycoplasma pneumoniae* and *Chlamydia trachomatis* may cause otitis media (Block, 1997). Otitis media known to be the most common childhood infection which lead annually to death of over 50,000 children under 5 years (Rovers *et al*., 2006). Microbes commonly associated with otitis media include streptococci, staphylococci, *Heamophilus, Pseudomonas, Proteus* etc (Jokipii *et al*., 1977) and are highly resistant to commonly used antibiotics (Oyeleke, 2009).

The World Health Organization (WHO, 2002) estimated that 80% of populations of developing countries rely on traditional medicines for their primary health care. Because of vast areas and variety of agro-climates in India, a large number of medicinal and aromatic plants found growing widely and several of these plants have been in use for centuries of their medicinal properties (Sahu *et al*., 1992).

Cleome viscosa Linn commonly known as "wild or dog mustard," is an annual, sticky herb belonging to family Capparaceae found as a common weed all over the plains of India and throughout the tropics of the world. The whole plant and its parts (leaves, seeds, and roots) are widely used in traditional and folkloric systems of medicine. In Asia and Africa the leaves and seeds used 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 (CSIR, 1950). The roots are a remedy for scurvy and rheumatism (Rukmini, 1978). An aqueous seed extract displayed significant analgesic activity in mice and local anaesthetic activity in guinea pigs (Parimaladevi *et al*., 2003).

MATERIALS AND METHODS

1. Collection of plant material

Healthy, disease free and mature *Cleome viscosa* leaves were collected, washed thoroughly, shade dried and then powdered with the help of blender. The powdered material was kept in airtight bottles until further use. The information about plant was gathered from traditional practitioners, tribal people and Nagarjuna medicinal plants garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

2. Preparation of plant extracts

Dry powdered plant material was extracted in Soxhlet's extractor for 6 hrs or till the plant material gets colourless using solvents petroleum ether, chloroform, methanol and acetone. All the extracts were concentrated using rotary evaporator and dry residue was preserved at 5°C in airtight bottles until further use.

3. Phytochemical screening

Dry residue was dissolved in respective solvents and tested for presence of phytochemicals tannins, alkaloids, flavonoids, saponins.

Tannins : To 0.5 ml of extract solution in acetone, 1 ml of water and 2-3 drops of ferric chloride ($FeCl_3$) was added. Blue colour was observed for presence of tannins (Iyenger, 1985).

Alkaloids : To 0.5 ml of extract solution in acetone, 1 ml of 1% HCL was added and warmed the solution. After cooling mixture was filtered and treated with few drops of Mayer's reagent. The sample was then observed for the presence of brown/ red precipitate (Siddiqui and Ali, 1997).

Saponins : 0.5 ml of extract solution in acetone was added to distilled water. Presence of frothing in the solution indicated presence of saponins (Iyenger, 1985).

Flavonoids : 0.5 ml of extract solution in acetone was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated HCl was added and red colour was observed for flavonoids (Siddiqui and Ali, 1997).

4. Antioxidant activity

Antioxidant activity of *Cleome viscosa* was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities. The extracts were mixed with methanol to get various concentrations: 400, 200, 100, 50, and 10 µg/ml. From each concentration, 2 ml of extract was mixed with 1 ml of methanolic solution containing DPPH radicals, with final concentration of 0.2 mM DPPH. The contents were shaken vigorously and kept in dark for 30 min. Absorbance was measured at 517 nm. Absorbance of control was determined by replacing the sample with methanol. The scavenging activity was calculated using following formula. Antioxidant activity of medicinal plants was compared with standard antioxidant ascorbic acid.

$$\% \text{ Antioxidant activity} = \frac{1 - \text{Absorbance of sample (extract)}}{\text{Absorbance of DPPH}} \times 100$$

5. Otitis media pathogens

5. a. Specimen collection

Clinical specimens of the 100 patients suffering from otitis media infection from Shri Daryao Clinic, Amravati were collected by swabbing the affected area of ear using sterile cotton swab and immediately taken to the laboratory for bacteriological investigation.

5. b. Isolation of pathogens

Collected samples were inoculated on blood agar and incubated aerobically at 37°C for 24 hours. Isolates obtained were maintained on nutrient agar slants at 4°C until required.

5. c. Identification and biochemical characterization of bacterial isolates

Cultures from nutrient agar slants were streaked on different selective media such as EMB agar, Baird Parker Agar, *Klebsiella* selective agar, cetrimide agar and *Streptococcus* selective agar. Identification of the bacterial culture was done using staining motility, biochemical tests such as indole test, methyl red test, VP test, citrate utilization test, oxidase test, urease test, coagulase test, catalase test and bile solubility test (Cheesbrough, 1984).

5. d. Inoculum preparation

To prepare bacterial inoculum, pure culture of test organism was inoculated into 5 ml of sterile nutrient broth and incubated at 37° C for 2 to 8 hrs till moderate turbidity developed. The inoculum was standardized by matching with 0.5 McFarland turbidity standard, which corresponds to cell density approximately 10^8 CFU/ ml.

5. e. Antibacterial sensitivity testing

Antibacterial susceptibility testing of antibiotics was performed by disc diffusion method (Bauer *et al*., 1966) Antibiotic discs were purchased from M/S Himedia Mumbai and included gentamicin (10 µg), amoxicillin (10 µg, ciprofloxacin (5 µg) and chloramphenicol (30 µg). For susceptibility testing, a sterile cotton swab was dipped into the standardized inoculum and rotated firmly against the upper inside wall of the test tube to remove excess inoculum from swab. Entire sterile and dried Mueller Hinton agar surface of the plate was streaked with the cotton swab thrice, by turning the plate 60° between each streaking. Excess surface moisture was allowed to dry for not more than 15 minutes. The antibiotic disc was placed at centre on the surface of seeded agar aseptically. The plates were then left undisturbed for 30 minutes to allow diffusion of antibiotic (Dorman and Deans, 2000).

For antibacterial susceptibility testing of plant extracts the sterile disc of 6 mm diameter (SD067, Hi-Media, Mumbai) was impregnated with 20µl of plant extract (200 mg/ ml). The discs were then placed at centre on the seeded agar. The plates were incubated at 37° C for 24 hrs. The assessment of antibacterial activity was done by measuring the diameter of the growth inhibition zone formed around disc. Test was done in duplicate.

5. f. Determination of MIC

Minimum inhibitory concentration of acetonic extract of *Cleome viscosa* was determined by NCCLS method (NCCLS, 2003). Stock solution of plant extract (1024 mg/ml) was prepared in respective solvent and vigorously shaken for about 1 min. The stock solution was then stored in refrigerator until use. Sixteen well characterized clinical isolates of otitis media pathogens were selected for MIC determination by broth macrodilution method. The clinical isolates included *Klebsiella pneumoniae* (03 isolates), *Staphylococcus aureus* (04 isolates), *Pseudomonas aeruginosa* (03 isolates), *Streptococcus pneumoniae* (03 isolates) and *Escherichia coli* (03 isolates). Each isolate was originated from a different patient with clinical manifestations and was maintained on nutrient agar.

RESULTS

Phytochemical screening of *Cleome viscosa* extracts in different solvents like acetone, methanol and chloroform were performed to detect the presence of active biomolecules such as alkaloids, tannins, flavonoids, etc. Alkaloid, tannins and flavonoids were present in methanol, acetone and chloroform extracts of *Cleome viscosa* while saponins were present only in acetone extract (Table 1). Antioxidant activity in terms of radical scavenging activity of *Cleome viscosa* in acetone extract was found to be 29, 33 and 21 % at 10 µg/ml, 50 µg/ml and 100 µg/ml

respectively (Table 2). 100 % isolates of *K. pneumoniae, S. aureus, S. pneumoniae* , *E.coli* and 68 % isolates of *P. aeruginosa* were resistant to amoxicillin. In case of *E. coli* and *S. aureus* 100% isolates were resistant to gentamicin while other organisms were totally sensitive. Similarly ciprofloxacin inhibited the otitis media pathogens except *E. coli* (Table 3).

Antimicrobial inhibition zones were ranged from 10-35 mm. Methanol and chloroform extracts were less effective. Methanolic extract of *Cleome viscosa* inhibited 33.33 % *K. pneumoniae*, 32 % *P. aeruginosa* and 85.71 % *S. pneumoniae* isolates. However, chloroformic extracts inhibited 37 % isolates of *P. aeruginosa*. None of the isolates of *K. pneumoniae, S. aureus, S. pneumoniae* and *E.coli* were inhibited by chloroformic extract (Table 4). On the other hand acetonic extracts were highly effective and inhibited 77.77 % *K. pneumoniae*, 88 % *S. aureus*, 63 % *P. aeruginosa*, 100% *S. pneumoniae* and *E. coli* isolates. Petroleum ether extracts were totally ineffective (Fig. 1). Acetone extract of *Cleome viscosa* showed a narrow MIC range (16-32 mg/ml) against isolates of *K. pneumoniae*. Of the 03 isolates of *K. pneumoniae*, 02 isolates were inhibited at 16mg/ml and remaining 01 isolate was inhibited at 32 mg/ml concentration. Mean MIC of 21 mg/ml and MIC_{70} of 32 mg/ml was observed for *K. pneumoniae. Cleome viscosa* showed MIC range of 04-08 mg/ml with mean MIC of 07 mg/ml and MIC_{70} of 08 mg/ml against isolates of *S. aureus*. Among 04 isolates of *S. aureus*, 03 were inhibited at 08 mg/ml while 01 isolate inhibited at low concentration (04 mg/ml). Isolates of *P. aeruginosa*, *S. pneumoniae* and *E. coli* were inhibited at 16 mg/ml concentration of *Cleome viscosa* extract (Table 5).

TABLE 1: Phytochemical screening of *Cleome viscosa*

Phytochemical	Methanol	Acetone	Chloroform
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	-	+	-
Flavonoids	+	+	+

TABLE 2 : Antioxidant activity of acetone extract of *Cleome viscosa* leaves

Concentration	Radical scavenging activity
10 µg/ml	29%
50 µg/ml	33%
100 µg/ml	21%

TABLE 3: Sensitivity of otitis media pathogens to antibiotics

Organism (No. of isolates)	Antibiotic	No. of isolates resistant	No. of isolates sensitive
K. pneumoniae (09)	Gentamicin	00	09
	Amoxicillin	09	00
	Ciprofloxacin	00	09
S. aureus (25)	Gentamicin	25	00
	Amoxicillin	25	00
	Ciprofloxacin	00	25
P. aeruginosa (19)	Gentamicin	00	19
	Amoxicillin	13	06
	Ciprofloxacin	00	19
S. pneumoniae (28)	Gentamicin	00	28
	Amoxicillin	28	00
	Ciprofloxacin	00	28
E. coli (22)	Gentamicin	22	00
	Amoxicillin	22	00
	Ciprofloxacin	09	13

TABLE 4: Sensitivity of otitis media pathogens to *Cleome viscosa* extracts

Organism	Methanol		Petroleum ether		Acetone		Chloroform	
(No. of isolates)	S	R	S	R	S	R	S	R
K.pneumoniae (09)	03	06	00	09	07	02	00	09
S. aureus (25)	00	25	00	25	22	03	00	25
P. aeruginosa (19)	06	19	00	19	15	10	07	12
S.pneumoniae (28)	24	04	00	28	28	00	00	28
E. coli (22)	00	22	00	22	22	00	00	22

S- Sensitive R- Resistant

FIGURE 1: % inhibition of bacterial isolates by *Cleome viscosa*

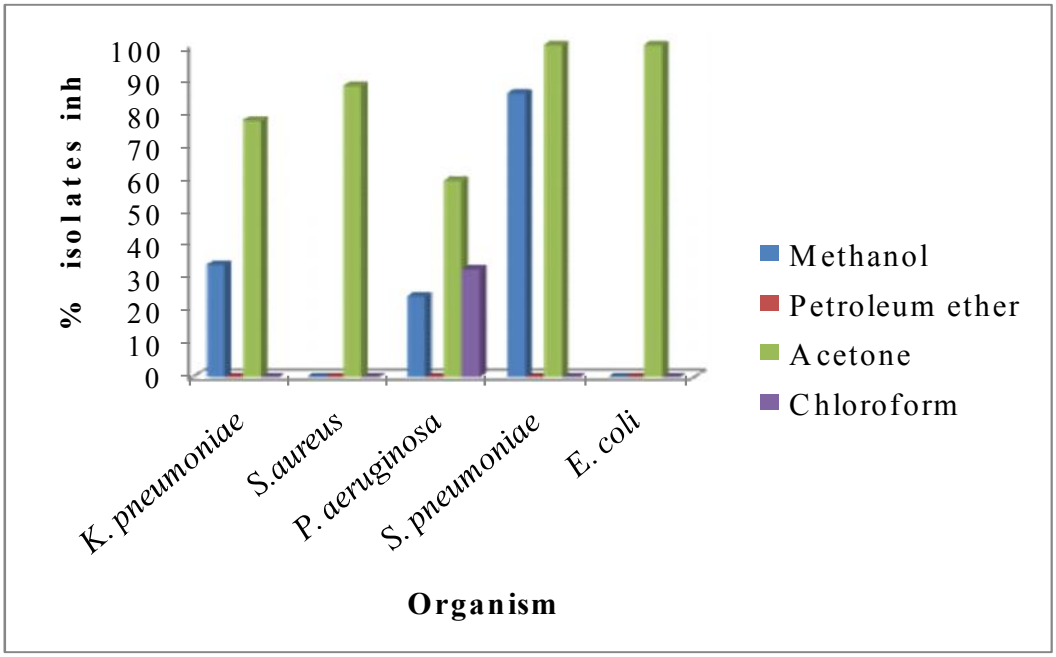

TABLE 5: Minimum inhibitory concentration of acetone extract of *Cleome viscosa*

Organism	Isolate No.	Zone of inhibition (mm)	MIC (mg/ml)
	80	25	16
K.pneumoniae	81	25	16
	89	20	32
	17	25	08
S. aureus	63	30	04
	72	25	08
	90	25	08
	66	25	16
P. aeruginosa	80	25	16
	97	25	16
	14	22	16
S.pneumoniae	65	22	16
	93	22	16
	65	25	16
E. coli	89	25	16
	93	25	16

DISCUSSION

The leaves of Cleome viscosa which are mostly used as a source of medication in traditional medicines was considered to examine the properties of the plant. A wide variety of phytoprinciples are present in *Cleome viscosa* (Mali, 2010). The results revealed that *Cleome viscosa* contained alkaloids, tannins, saponins and flavonoids. These phytochemicals may have contributed for antimicrobial activity of *Cleome viscosa* against otitis media pathogens. This result is in analogy with previous reports of Koche *et al.,* (2010) who reported presence of alkaloids, flavonoids, tannins, saponins and terpenoids in the leaves of *Cleome viscosa*. Siddiqui *et al.,* (2009) also reported the presence of alkaloids and tannins but absence of flavonoids and saponins in *Cleome viscosa.* The variations in flavonoids and saponins may be associated with varity of plant, geographical conditions, methods of extraction and solvent used. In the present study, acetone extract of *Cleome viscosa* leaves showed 33 % antioxidant activity at 50 µg/ml concentration. In contrast Koppula *et al.,* (2011) reported 0.42 µ moles/ml activity at 100 mg/ml concentration. Antioxidant activity is known to be associated with tannins, flavonoids and phenolic compounds. Tannins, alkaloids, flavonoids of *Cleome viscosa* may have contributed for strong antioxidant property.

Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. In the present study acetonic extract of *Cleome viscosa* exhibited remarkable activity against *S. pneumoniae* and *E. coli* followed by *K. pneumoniae, S. aureus* and *P. aeruginosa*. However, methanolic extract resulted in maximum activity against *S. pneumoniae* followed by *S. aureus* and *K. pneumoniae*. Crude methanolic extract of *C. viscosa* exhibit in vitro antibacterial activity against *Staphylococcus saprophyticus, Shigella sonnie, Salmonella typhi, Vibrio cholerae, Streptococcus epidermidis, Shigella flexneri* and *Staphylococcus aureus*

with inhibition zone range of 10.76-16.34 mm (Bose *et al*., 2011). Saradha and Rao (2010) reported promising activity of methanolic extract against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. In the present work also methanolic extract exhibited significant activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. Sudhakar *et al.,* (2006) reported ethanol extract of *Cleome viscosa* leaves with broad spectrum antimicrobial activity against *E. coli, P. vulgaris* and *P. aeruginosa* and moderate activity against pathogenic fungi. MIC values of *Cleome viscosa* were 16-32 mg/ml with mean MIC of 21 mg/ml and MIC_{70} of 32 mg/ml. MIC range also suggest that *S. aureus* was inhibited at a concentration of 4-8 mg/ml. However, other microbes required 2-3 fold more concentration of *Cleome viscosa* for inhibition.

CONCLUSION

The herb *Cleome viscosa* showed broad spectrum antimicrobial action towards otitis media pathogens. These results have correlation with claims made by traditional practitioners for control of ear infections. Due to development of antibiotic resistance in otitis media pathogens, it is expected to boost the use of *Cleome viscosa* in therapeutic management of ear infections caused by bacterial pathogens. Further study on separation and purification of active biomolecules will contribute in utilization of *Cleome viscosa* globally.

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