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The Action of *C leome Viscosa* and *Cardiospermum halicacabum* Extracts on Fungi Associated With Otitis Media

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Abstract:

Acetone, methanol and chloroform extracts obtained from *Cleome viscosa* and *Cardiospermum halicacabum* were evaluated for their *in vitro* antifungal activity. The activity was tested against human pathogenic clinical isolates of filamentous fungi associated with otitis media by disc diffusion method. *Cleome viscosa* and *Cardiospermum halicacabum* extracts displayed highest antifungal activity with 12 and 13 mm inhibition zone respectively. Methanol extracts of *Cleome viscosa* and *Cardiospermum halicacabum* were able to inhibit 78 and 72% fungal isolates respectively while the standard antifungal agent fluconazole exhibited extremely poor antifungal activity. The results suggest the possible use of the above plants for the treatment of ear infections due to fungi.

Keywords:
Medicinal plant, bioactivity, pathogenic fungi.

Introduction:

The World Health Organization reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. The use of medicinal plants has low cost and has been used for several generations. The specific plants to be used and the methods of application for particular ailments were passed down through oral tradition. Plants with possible antimicrobial activity should be tested against some microbes to confirm the activity. The activity of plant extracts on bacteria and fungi has been studied in different parts of the world (Vuuren and Naidoo, 2010).

Otitis media is a complex generic term that includes the acute otitis media (AOM),chronic otitis media (COM) and otitis media with effusion (OME) (Bowd, 2005). 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). Occasionally, fungi, viruses, *Mycoplasma pneumoniae* and *Chlamydia trachomatis* may cause otitis media (Block, 1997). The present study deals with the screening of antifungal activity of *Cleome viscosa* and *Cardiospermum halicacabum*. *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. The pharmacological studies have shown that *Cleome viscosa* possesses various notable biological activities such as anthelmintic, antimicrobial, analgesic, anti-inflammatory, immunomodulatory, antipyretic, psychopharmacological, antidiarrheal and hepatoprotective activities (Mali, 2010). *Cardiospermum halicacabum*is a climber belongs to the family Sapindaceae and exhibit a wide range of biological and pharmacological properties (Maluventhan and Sangu, 2010).

Material and methods:

1) Ethno botanical survey

Plants were selected for this study based on their medicinal use. Leaves of *Cleome viscosa* and *Cardiospermum halicacabum* were collected from the waste fields and road sides in Amravati. Material was washed thoroughly, shade dried and then powdered with the help of blender. The powdered material was kept in airtight bottles until further use. The ethno botanical data gathered at medicinal and aromatic plants unit, Dr. P.D.K.V. Akola.

2) Preparation of plant extracts

Dry powdered plant material was extracted with solvents petroleum ether, chloroform, methanol and acetone with Soxhlet's extractor for 6 hrs or till the plant material gets colourless. The solvent was removed using a rotary vacuum evaporator to give a concentrated extract, which was then frozen and freeze-dried until use.

3) Otitis media pathogens

Clinical specimens of 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.

Samples were initially inoculated on blood agar and incubated aerobically at 37°C for 24 hours. Fungal colonies were streaked on Sabouraud Dextrose Agar (SDA) plates and incubated aerobically for 24-72 h at 30°C for fungal pathogenic species isolation. A preliminary examination of fungal colony on SDA was done through study of colony morphology.

4) Antifungal sensitivity testing

Disc diffusion method was employed for the determination of antifungal activities. Fungal spores were separately suspended in a normal saline solution and adjusted to transmittance (T) of 75–77% at 530 nm, to obtain inoculum of $1x10^6$ CFU /ml. SDA plates were seeded with spore inoculum. The dried plant extracts were dissolved in the respective solvent (acetone, methanol and chloroform) to a final concentration of 1000 µg/ml. Sterile paper discs (6 mm in diameter) were impregnated with 20 µl of the plant extracts. The discs were placed on plates, previously inoculated with fungal suspension. Negative controls were prepared using the acetone, methanol and chloroform that were used to dissolve the plant extracts. Plates were incubated at 30C° for 48 h. The diameters of the inhibition zones were measured in mm. All the tests were performed in triplicate. Clotrimazole (10 µg/disc) and fluconazole (10 µg/disc) were used as standard.

Result and discussion:

Out of 18 isolates acetone extract of *Cleome viscosa* inhibited 10 isolates (55%) with inhibition zone range of 7-11 mm.; methanol extract exhibited inhibition zone range of 7-12mm and inhibited 14 isolates (78%) while chloroform extract showed inhibitory activity against 08 isolates (44%) and zone range was 8-14 mm (Table 1, Fig 1). All the three extracts inhibited more than 07 fungal isolates. Methanol extract inhibited highest number of fungal isolates while acetone extract showed moderate and chloroform extract showed minimum activity.

Out of three extracts of *Cardiospermum halicacabum* acetone extract inhibited 09 isolates (50%) and zone range was 7-11 mm, while methanol extract showed highest range of zone of inhibition ranging 8-13mm and inhibited 13 isolates (72%). Chloroform extract produced inhibition zone of 7-12 mm and inhibited 61% isolates (Table 2, Fig 2). All three extracts inhibited more than 06 isolates of fungus. Methanol extract showed maximum activity by inhibiting highest number of fungal pathogens followed by chloroform extract which showed moderate activity and acetone extract demonstrated least activity. Antifungal agents Flucanozole and Clotrimazole were used as standard. Clotrimazole inhibited 17 isolates with inhibition zone range of 9-23 mm while fluconazole inhibited only 2 isolates with zone range of 7-10mm.

Fungal diseases represents a critical problem to health and they are one of the main cause of morbidity and mortality worldwide (CSIR, 1998). In humans, fungal infections range from superficial to deeply invasive or disseminated and have increased dramatically in recent years. The treatment of mycoses has lagged behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available. Therefore, a search for new antifungal drugs is extremely necessary (Fortes *et al* 2008).

Sudhakar *et al.*, (2006) tested the ethanolic extracts of the leaves and flowers of *Cleome viscosa* for antimicrobial activity and showed broad-spectrum antimicrobial activity against number of bacterial pathogens but leaf extract of *Cleome viscosa* showed moderate activity against pathogenic fungi. In present study methanol and acetone extract showed prominent activity against fungal isolates. Wake *et al.*, (2011) studied ethyl acetate, petroleum ether and chloroform extract of *Cleome viscos a*against bacteria with inhibition zone range 12-17 mm while against fungus with 12-14mm.Reddy *et al.*, (2010) tested antimicrobial activity of methanol and chloroform extracts of *Cardiospermum halicacabum* against six bacterial and two fungal species and reported broad-spectrum antimicrobial activity against all tested organisms. From the results it is revealed that fungal isolates were resistant to the routine antifungal drug fluconazole, while sensitive to plant extracts used in present investigation.

Results of the present work confirm the use of these plants in folk medicine for the treatment of otitis media and underlines the importance of these plants in the discovery of new bioactive compounds. Further phytochemical research is needed to identify the active principles responsible for the antifungal effects of these medicinal plants.

Table 1: Inhibitory effect of *Cleome viscosa* on fungal species isolated from otitis media patients

Isolate No.	Inhibition zone (mm)				
	CVA	CVM	CVC	CLO	FLU
03	-	11	14	11	-
04	-	07	-	23	-
05	07	09	-	15	07
11	09	11	09	11	-
12	11	12	-	15	10
23	-	-	-	-	-
24	-	07	-	18	-
26	07	08	-	19	-
30	-	-	-	21	-
37	-	-	-	18	-
44	-	09	-	23	-
46	11	08	08	13	-
61	11	11	09	11	-
67	08	09	08	09	-
74	09	11	09	12	-
75	07	12	08	11	-
76	11	11	11	11	-
79	-	-	-	15	-

CVA, C. *viscosa* acetone extract; **CVM**, *C. viscosa*methanol extract; **CVC**, *C. viscosa*cholroform extract; **CLO**, Clotrimazole; **FLU**, Fluconazole

Table 2: Inhibitory effect of *Cardiospermum halicacabum* on fungal species isolated from otitis media patients

Isolate No.	Inhibition zone (mm)				
	CHA	CHM	CHC	CLO	FLU
03	08	-	-	11	-
04	-	09	-	23	-
05	-	09	-	15	07
11	09	11	09	11	-
12	07	11	11	15	10
23	-	-	-	-	-
24	-	-	-	18	-
26	-	09	09	19	-
30	-	07	07	21	-
37	-	-	-	18	-
44	-	08	08	23	-
46	11	13	12	13	-
61	09	08	08	11	-
67	07	09	09	09	-
74	11	11	11	12	-
75	08	11	11	11	-
76	08	11	11	11	-
79	-	-	-	15	-

CHA, *C. halicacabum* acetone extract; **CHM**, *C. halicacabum*methanol extract; **CHC**, *C. halicacabum* cholroform extract; **CLO**, Clotrimazole; **FLU**, Fluconazole.

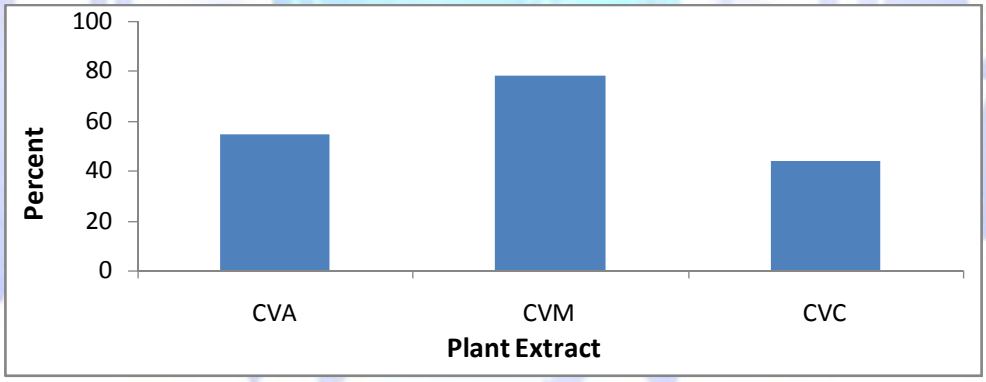

Figure 1: Percent inhibition of fungal isolates by C. *viscose*

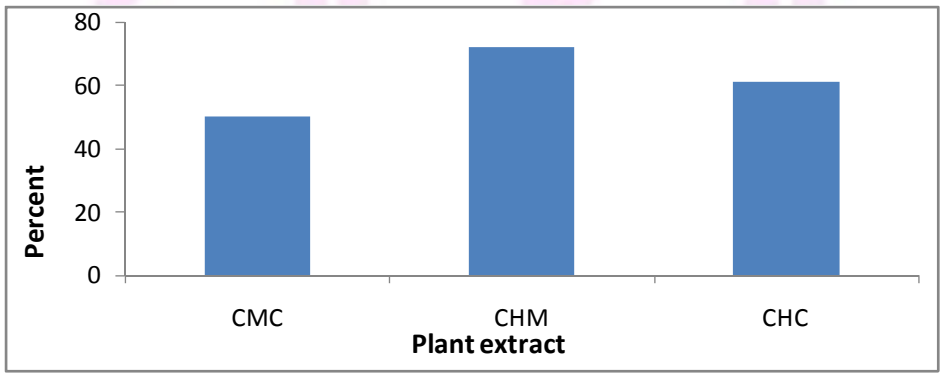

Figure 2: Percent inhibition of fungal isolates by *C. halicacabum*

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