EVALUATION OF ANTIBACTERIAL ACTIVITY OF *CARDIOSPERMUM HALICACABUM* AGAINST CLINICAL ISOLATES OF OTITIS MEDIA.

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Abstract

India is virtually herbarium of the world. India possesses all types of climatic conditions varying from cold temperature in the Himalayas to tropical in south for the growth of a variety of medicinal and aromatic plants. India, with her varied climatic conditions and topography has considered as "Botanical Garden of the World". In the present study the phytochemical study, antioxidant activity and *in vitro* antibacterial activity of acetone, methanol and chloroform extracts of *Cardiospermum halicacabum* leaves were investigated. *Cardiospermum halicacabum* is a climber belongs to the family Sapindaceae. The plant is a twinner, pubescent or nearly glabrous annual or perennial. This plant exhibited a wide range of biological and pharmacological properties. Phytochemical analysis revealed the presence of alkaloids, tannins, saponins and flavonoids in leaves of *C. halicacabum.* Acetone extract of *C. halicacabum* showed 24.05% free radical scavenging at 50µg/ml. The extracts were screened for *in vitro* antibacterial activity against selected otitis media pathogens including *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumonia* and *S. pneumonia* by disc diffusion method and acetone extract was predominant. Acetone extract of *C. halicacabum* inhibited 100% isolates of *S. pneumonia* and *E coli*, 90% isolates of *P. aeruginosa*, 79% *K. pneumoniae* and 97% isolates of *S. aureus* with effective zone range of 15-30 mm and MIC range was 08-32 mg/ml. Present study justifies the claimed uses of *C. halicacabum* in the Indian traditional system of medicine to treat various diseases. However, as the toxic effects of plant extracts were not tested in present study In future research is required to determine which chemicals are effective which will provide valuable clues for developing herbal drugs for treatment of otitis media.

Key words: Otitis media, *Cardiospermum halicacabum*, Antimicrobial activity.

Introduction

Medicinal plants occupied an important position in socio-cultural, spiritual and medicinal arena of rural people of India. The Indian systems of medicines i.e. Ayurveda predominantly use plant based raw materials in most of their preparations and formulations. There has been an increasing evidence of bacterial and fungal infections due to population explosion, changed environmental conditions, wastes from different sources. It may affect food with perfect nutritional value and results in reducing immunogenicity in human beings. These factors coupled with increasing resistance of microorganisms to allopathic agents, antibiotics increased toxicity in human being during prolonged treatment with several antimicrobials (Giordani *et al.*, 2001). Otitis media is highly prevalent worldwide (Ifante and Fernandez, 1993). Hearing loss was a significant sequel of chronic suppurative otitis media among the school children and it had adverse effect on their academic performance. Microbes commonly associated with otitis media include Streptococci, Staphylococci, *Heamophilus, Pseudomonas, Proteus* etc (Jokipii *et al*., 1977 and Klein, 1994). 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). Increasing antibiotic resistance to commonly used antibiotics exhibited by pathogens has led to the screening of several medicinal plants for the potential antimicrobial activity (Mukherjee *et al*., 1998). 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 (Singh *et al*., 2010). *Cardiospermum halicacabum* is a climber belongs to the family Sapindaceae. The plant is a twinner, pubescent or nearly glabrous annual or perennial. This plant exhibited a wide range of biological and pharmacological properties. Many reporters reported promising antimicrobial activity of *Cardiospermum halicacabum*.

Material and Methods

1) Ethno botanical survey

Plants were selected for this study based on their medicinal use. Leaves of *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

i) Specimen collection

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.

ii) Isolation of Pathogens

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.

iii) 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 cultures 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).

4) Preparation of inoculums

To prepare bacterial inoculums, 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) Antibacterial sensitivity testing

Antibacterial susceptibility testing of antibiotics was performed by disc diffusion method (Bauer *et al*., 1966). Antibiotic discs included gentamycin (10 µg), amoxicillin (10 µg) and ciprofloxacin (5 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. 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 on the seeded agar. The standard discs of gentamycin, amoxicillin and ciprofloxacin were used as a reference control. 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.

6) Determination of MIC

Minimum inhibitory concentration of acetone extract of *Ocimum sanctum* was determined by NCCLS method (NCCLS, 2003). In brief 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. Seventeen 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* (04 isolates) and *Escherichia coli* (03 isolates). Each isolate was originated from a different patient with clinical manifestations and was maintained on nutrient agar.

7) Phytochemical Analysis

The freshly prepared extracts were subjected to standard preliminary phytochemical analysis for the presence of alkaloids, flavonoids, tannin and saponins as described elsewhere (Jane and Patil, 2012).

8) Antioxidant activity

Antioxidant activity of *Ocimum sanctum* was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity. The extracts were mixed with methanol to get various concentrations as 400, 200, 100, 50, and 10µg/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 formula,

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

Results

Phytochemical analysis of *Cardiospermum halicacabum* revealed that in aceton extract alkaloids, saponins and flavonoids were present while in methanol extract only alkaloids and flavonoids were present. (Table 1). Antioxidant activity of *Cardiospermum halicacabum* in terms of radical scavenging activity in acetone extract was found to be 56.00%, 24.05% and 21.20% at 10µg/ml, 50µg/ml and 100µg/ml concentration respectively (Table 2). Table 3 indicates antimicrobial activity of standard reference antibiotics. The results indicate that otitis media pathogens were inhibited by gentamicin with inhibition zone 10-20mm, amoxicillin with 8-15 mm and ciprofloxacin with 20-35mm. Acetone extract of *C. halicacabum* inhibited 88.88% isolates of *K. pneumoniae*, 96% isolates of *S. aureus*, 89.47% isolates of *P. aeruginosa*, 100% *S. pneumoniae* and 86.36 % isolates of *E. coli* with effective zone range of 19-35 mm. Acetone extract of *C. halicacabum* inhibited 100% isolates of *S. pneumonia* and *E coli*, 90% isolates of *P. aeruginosa*, 79% *K. pneumoniae* and 97% isolates of *S. aureus* with effective zone range of 15-30 mm (Table 4, Fig 1). The 03 isolates each of *K. pneumoniae* and *E. coli*, all 03 isolates were inhibited at 16 mg/ml, with mean MIC and MIC_{70} at 16 mg/ml concentration of *Cardiospermum halicacabum* extract. *Cardiospermum halicacabum* showed MIC range of 04-08 mg/ml against four isolates of *S. aureus*. Among 04 isolates, 03 were

inhibited at 08mg/ml and 01 isolate required 04 mg/ml concentration of *Cardiospermum halicacabum* extract for inhibition. *P. aeruginosa* showed MIC range 16-32 mg/ml. Three isolates of *P. aeruginosa* were inhibited at 32 mg/ml, however 01 isolate inhibited at lower concentration of 16 mg/ml. Three isolates of *S. pneumoniae*, were tested for MIC and all 03 isolates were inhibited at 32 mg/ml. concentration of *Cardiospermum halicacabum* extract (Table 5).

Table 1. Phytochemical screening of *C. halicacabum*

Sr. no.	Constituent	Name of test/ Reagent	Acetone	Methanol	Chloroform
1.	Tannins	$FeCl_3$	-	-	-
2.	Alkaloids	Mayer's reagent	+	+	-
3.	Saponins	Frothing test	+	-	-
4.	Flavonoids	Shinoda's test	+	+	-

Table 2: Antioxidant activity of acetone extract of *C. halicacabum*

Concentration	Radical scavenging activity
10 µg/ml	56.00 %
50 µg/ml	24.05 %
100 µg/ml	21.20 %

Table 3: Sensitivity of otitis media pathogens against antibiotics

Organism (No. of isolates)	Zone of inhibition range (mm)		
	Gentamicin	Amoxycillin	Ciprofloxacin
K.pneumoniae (09)	17-18	12-14	28-30
S. aureus (25)	10-18	12-15	20-26
P. aeruginosa (19)	18-20	12-15	30-35
S.pneumoniae (28)	18-20	08-10	26-30
E. coli (22)	15-18	10-12	25-27

Table 4: Sensitivity of otitis media pathogens to *C. halicacabum* extracts

Organism (No. of isolates)	Methanol		Petroleum ether		Acetone		Chloroform	
	S	R	S	R	S	R	S	R
K.pneumoniae (09)	00	09	00	09	07	02	00	09
S. aureus (25)	00	25	00	25	24	01	00	25
P. aeruginosa (19)	00	19	00	19	17	02	00	19
S.pneumoniae (28)	00	28	00	28	28	00	00	28
E. coli (22)	00	22	00	22	00	22	00	22

S- Sensitive R- Resistant

Table 5: MIC of acetone extract of *C. halicacabum*

Plant	Organism	Isolate No.	Zone of inhibition (mm)	MIC value (mg/ml)
Cardiospermum halicacabum	*K.pneumoniae*	15	22	16
		80	25	16
	S. aureus	17	25	08
		60	30	04
		62	25	08
		87	25	08
	P. aeruginosa	70	22	16
		84	20	32
		94	20	32
	S.pneumoniae	65	20	32
		85	20	32
		93	20	32
	E. coli	09	22	16
		50	22	16
		90	22	16

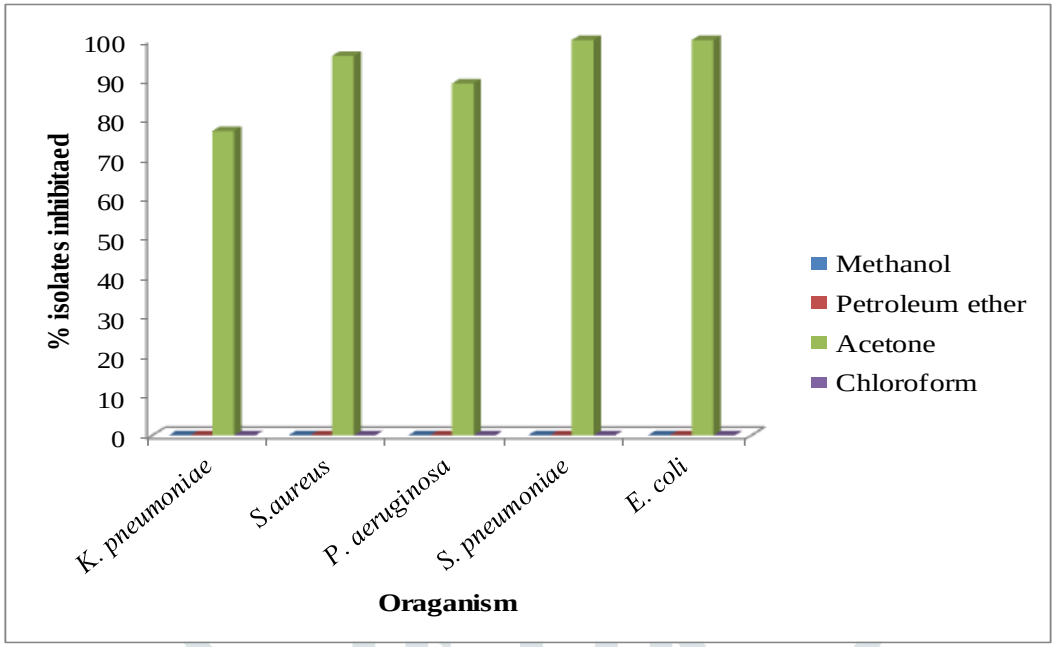

Fig. 1 Sensitivity of bacterial isolates to *C. halicacabum*

Discussion

Antibiotic resistance has become a global concern in recent years. This problem is of great significance especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents (Mahesh and Satish, 2008). Due to a rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs (Babu and Subhasree, 2009). Plants consists of many secondary metabolites such as alkaloids, phenolic compounds etc. which possesses antimicrobial properties (Vijaya K and Ananthan S, 1997). In the present study phytochemicals like alkaloids, saponins and flavonoids were present in acetone and methanol extracts. Acetone extracts of *Cardiospermum halicacabum* produced significant activity against all tested pathogens of otitis media. Methanol and chloroform extracts showed no activity. This result is in analogy with Viji and Marugesan (2010) who reported *K. pneumoniae, E. coli, P. aeruginosa* were more susceptible towards acetone extract of *Cardiospermum halicacabum.* Sughuna and Brindha (2011) reported promising activity of ethanolic extract of *Cardiospermum halicacabum* against *E. coli* and *S. aureus.* However, chloroform extract showed no activity against *E. coli* and *P. aeruginosa*. Few researchers have also reported antibacterial and antifungal potential of *Cardiospermum halicacabum* (Samy and Ignacimuthu, 2000; Rao *et al.,* 2006).

Conclusion

Acetonic extract of *Cardiospermum halicacabum* was the only potential antimicrobial against all otitis media pathogens as extracts of this plant in other solvents were totally ineffective. This finding is suggestive of extracting bioactive components only in acetone. Mean MIC revealed that *S. aureus* was highly sensitive to *Cardiospermum halicacabum* inhibited by 4 mg/ml concentration. These findings can form the basis for further studies to toxicity testing, isolate active compounds, elucidate the structures, and evaluate them against wider range of resistant bacterial strains of OM patients with the goal to find new therapeutic principles. Plant used in present study may also represent an economic and safe alternative to treat otitis media.

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