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EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF STEM EXTRACTS OF *COSCINIUM FENESTRATUM*: AN IMPORTANT MEDICINAL PLANT OF WESTERN GHATS

Blessing Ramya J*, Reginald Appavoo M. and Irene Wilsy J.

Department of Botany & Research Centre,
Scott Christian College (Autonomous) Nagercoil, Kanyakumari- 629 003, Tamilnadu, India.

(Affiliated to Manonmanium Sundaranar University, Tirunelveli).

*Corresponding author Email address: ugiramya@gmail.com.

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ABSTRACT

Coscinium fenestratum is a large dioecious woody climbing shrub. This tree is also known as False Calumba or Tree Turmeric due to presence of yellow colored alkaloid berberine. It is considered as a critically endangered medicinal plant belongs to the family Menispermaceae. It is commonly found in Western Ghats of India. Due to its high economic and medicinal value, the species is overexploited from the wild and it is very rare and need high conservation. Ethno botanically the species has been used to treat as ulcers, skin diseases, eye disorders, inflammation, hypertension, jaundice, diabetes and snake bites. In the present study, antioxidant activity and antibacterial activity of *Coscinium fenestratum* stem was investigated. To assess the antioxidant activity, stem extracts were used. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The microbial growth in diverse situations is controlled by plant derived products. Ethanol, Petroleum ether and Aqueous extracts of the plant of *Coscinium fenestratum* stem were investigated for antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The maximum zone of inhibition was observed in *Proteus mirabilis* in Ethanol extract and minimum zone of inhibition was observed in *Pseudomonas aeruginosa* in aqueous extract.

Keywords : *Coscinium fenestratum*, Antibacterial activity, Antioxidant, DPPH.

Introduction

Coscinium fenestratum (Gaertn) Colebr is one of the most important plants used in traditional systems of medicine belonging to the family Menispermaceae. The plant is commonly known as tree turmeric or false Calumba due to its yellow stem. CF is found in Asian countries such as India, Malaysia, Vietnam, Myanmar, Singapore, Thailand, and Sri Lanka. In India, the plant is endangered and located in the Western Ghats areas, especially in high rainfall evergreen forests of Karnataka, Kerala, and Tamil Nadu (Tushar *et al.*, 2008). The plant is a woody climbing shrub with cylindrical stem. *Coscinium* consists of the dried stem of the plant and the drug occurs in large woody, cylindrical, straight pieces, some times as much as 10 centimetres in diameter. The stem of the plant is used in curing several diseases and disorders like diabetes, wounds and ulcers, fever, jaundice, snake bite, piles etc in ethnomedicine. The chief constituent of *Coscinium* is the yellow crystalline alkaloid, berberine (Sudharshan *et al.*, 2008). *C. fenestratum* is a valuable drug in the preparation of medicines. The stem extract of CF was reported to have a significant effect on simulating insulin secretion (Birdsall, 1997). *Coscinium fenestratum* is the energetic drink in fever conditions and general weakness of the body. It has the Raktha shodaka (blood purifying) quality (Vesituru 1994). It is overexploited for its medicinal importance (Kumar *et al.*, 2007). The IUCN red list of threatened plants recorded the status of *C. fenestratum* as

highly endangered in India (Schippmann, 1999). In recent years, increasing strains of microorganisms throughout the World have developed resistance to large number of antibiotics that has created immense clinical problem and made the management of infectious diseases quite complicated (Davis, 1994). The way to avoid antibiotic resistance of pathogenic species is by using plant-based compounds rather than existing synthetic antibacterial agents (Shah, 2005). The present study is aimed to analyze the antibacterial activity and antioxidant of the plant *Coscinium fenestratum*.

Materials and Methods



Kingdom	: Plantae
Class	: Magnoliopsida
Order	: Ranunculales
Family	: Menispermaceae
Genus	: <i>Coscinium</i>
Species	: <i>fenestratum</i>

Collection of plant material

A small stem of *Coscinium fenestratum* was collected from Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala were shade dried and broken into coarse material.

Antibacterial Activity

Preparation of Extract

The selected *Coscinium fenestratum* was dried under shade condition for one month and cut in to small pieces, pulverized in a grinder and store in sterile container for further use. The solvents like ethanol, aqueous and petroleum ether were used for the extraction. About 10 gram of powdered CF stem was soaked separately in 100 ml of ethanol, aqueous, petroleum ether for three to four days at room temperature in dark condition. The extracts were filtered by using Whatman No.1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator and stored at 4°C for further use. Each extracts was resuspended in the respective solvent and used for the analysis of antibacterial activity.

Bacterial strains

In the present study five human pathogenic pathogens were used namely *E. coli* (MTCC 1687), *Staphylococcus aureus* (MTCC 737), *Pseudomonas aeruginosa* (MTCC 1688), *Klebsiella pneumoniae* (MTCC 7162) and *Proteus mirabilis* (MTCC 3310) obtained from MTCC Chandigarh. Stock culture were maintained in nutrient agar medium at 40°C, and then subcultured in nutrient broth at 37°C prior to each microbial test.

Disc diffusion method

The disc diffusion method was used to screen the antibacterial activity (Bauer, 1966). The sensitivity test of the Ethanol, Petroleum ether and aqueous extract were determined using agar-disc diffusion method. Media were prepared using Muller Hinton Agar poured in petridishes and inoculated with test organisms from the broth using cotton swabs. Disc impregnated with the plant extract were placed on the swabbed plate. The plates were incubated overnight at 37°C for 24 hours. Amikacin was used as positive reference standard. After incubation, the clear zone around the disc were measured and expressed in mm as a measure of their antibacterial activity.

Antioxidant activity

Chemicals

1,1-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid were purchased. For antibacterial study five bacterial pathogens, were obtained from MTCC, Chandigarh. All other chemicals and reagents used in the study were of analytical grade.

Sample preparation:

10 mg of test samples stem was dissolved in 50 ml of ethanol marked as stock and mixed well. The samples were

prepared with different concentration (20 µg, 40 µg, 60 µg, 80 µg and 100 µg) as working sample solution. Standard ascorbic acid was also prepared in a same way and made it as 100µg concentration (Ascorbic acid) as positive control. DPPH with ethanol was taken as a negative control.

Antioxidant assay (Free radical scavenging activity-DPPH)

The DPPH radical- scavenging activity was performed by using standard protocols followed by Priya *et al.* (2010). The plant extracts were diluted in ethanol to make different concentrations *viz.* 10, 20, 40, 60, 80 and 100 µg/ml. About 2 ml of each dilution was mixed with 1 ml of 0.2 mM DPPH in ethanol and mixed thoroughly. The mixture was incubated in a dark room at 20°C for 40 min. Then the absorbances were read at 517 nm using spectrophotometer with ethanol as blank. The percentage scavenging of DPPH by the plant extracts was calculated by the following formula:

$$\text{Percentage DPPH radical scavenging} = [(Ac - At) / Ac] \times 100$$

Where, Ac is the absorbance of the control (DPPH); At is the absorbance of the test sample.

Result and Discussion

The antibacterial activity results obtained in the study are depicted in Table 1 which showed the growth inhibition produced by the plant extracts of *Coscinium fenestratum* on five species of bacteria. The highest activity (zone of inhibition in diameter is about (18±0.30mm) was demonstrated by the ethanol extract of *Coscinium fenestratum* plant against *Proteus mirabilis* while the lowest activity was (8±0.28) by the aqueous extract against *Pseudomonas aeruginosa*. The antibacterial activity of ethanol showed more active than other extracts in its antibacterial activity. All the bacterial organisms screened the growth of *Proteus mirabilis* (18±0.30) and *Escherichia coli* (17±0.18) were majorly inhibited in the ethanol extract; *Klebsiella pneumoniae* (15±0.08) and *Staphylococcus aureus* (16±0.32) in Petroleum ether extract while *Escherichia coli* (12±0.08) and *Proteus mirabilis* (11±0.12) in the aqueous extract. In the present study ethanol extract showed more activity against the pathogens of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *E. coli* and *Proteus mirabilis* while *Staphylococcus aureus* in *P. ether* extract.

The free radical scavenging activity of *Coscinium fenestratum* was studied by its ability to reduce the DPPH, a stable free radical. The DPPH inhibition of stem extracts is shown in Fig. 2. In DPPH assay, the ethanolic stem extract showed more antioxidant activity. DPPH scavenging activity was ranging from 24.11±0.63% to 41.01±0.38% in the case of ethanolic stem extract, the highest scavenging activity was found at a concentration 100µg/ml and the lowest was found at a concentration of 20µg/ml. The reduction in the number of DPPH molecule can be correlated with the available number of hydroxyl groups. Hence the significant scavenging activity may be due to the presence of hydroxyl groups present in the extracts.

Goveas *et al.* (2013) showed the methanolic stem extract was moderate activity against *E. coli* (17±0.33), *P. aeruginosa* (12±0.20), *B. subtilis* (13±0.45). Methanol leaf extract had maximum activity against *S. aureus* (6.4±0.67) and lowest against *B. subtilis* (3.9±0.58). The antibacterial activity of aqueous and methanolic extracts of leaf and stem of *C. fenestratum* was assayed *in vitro* by agar disc diffusion

method against four different bacterial strains. In DPPH assay, the methanolic stem extract showed more antioxidant activity when compared to methnolic leaf extract. DPPH scavenging activity was ranging from 4.7±0.67% to 71.3±0.36% .The highest scavenging activity was found at a concentration 256µg/ml and the lowest was found at a concentration of 2µg/ml respectively.

Table 1 : Antibacterial activity of *Coscinium fenestratum* (stem) against bacterial pathogens.

No	Bacterial Pathogens	Zone of Inhibitions(mm)			
		Amikacin	Ethanol	P.Ether	Aqueous
1	<i>Escherichia coli</i>	24mm	17mm	14mm	12mm
2	<i>Staphylococcus aureus</i>	23mm	15mm	16mm	10mm
3	<i>Proteus mirabilis</i>	26mm	18mm	13mm	11mm
4	<i>Klebsiella pneumonia</i>	20mm	16mm	15mm	9mm
5	<i>Pseudomonas aeruginosa</i>	18mm	12mm	10mm	8mm

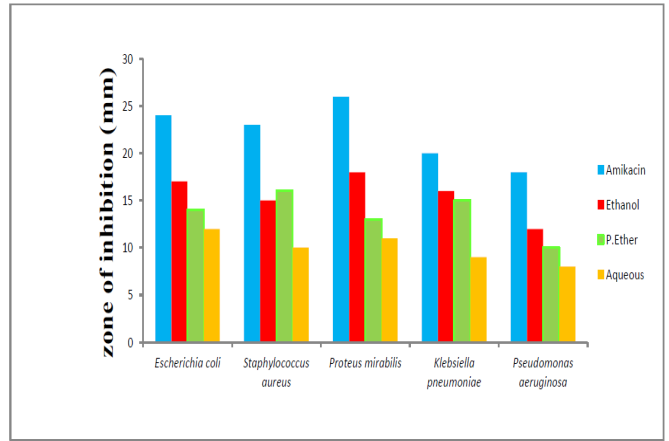


Fig. 1 : Antibacterial activity of stem extract of *C. fenestratum* against different bacterial strains.

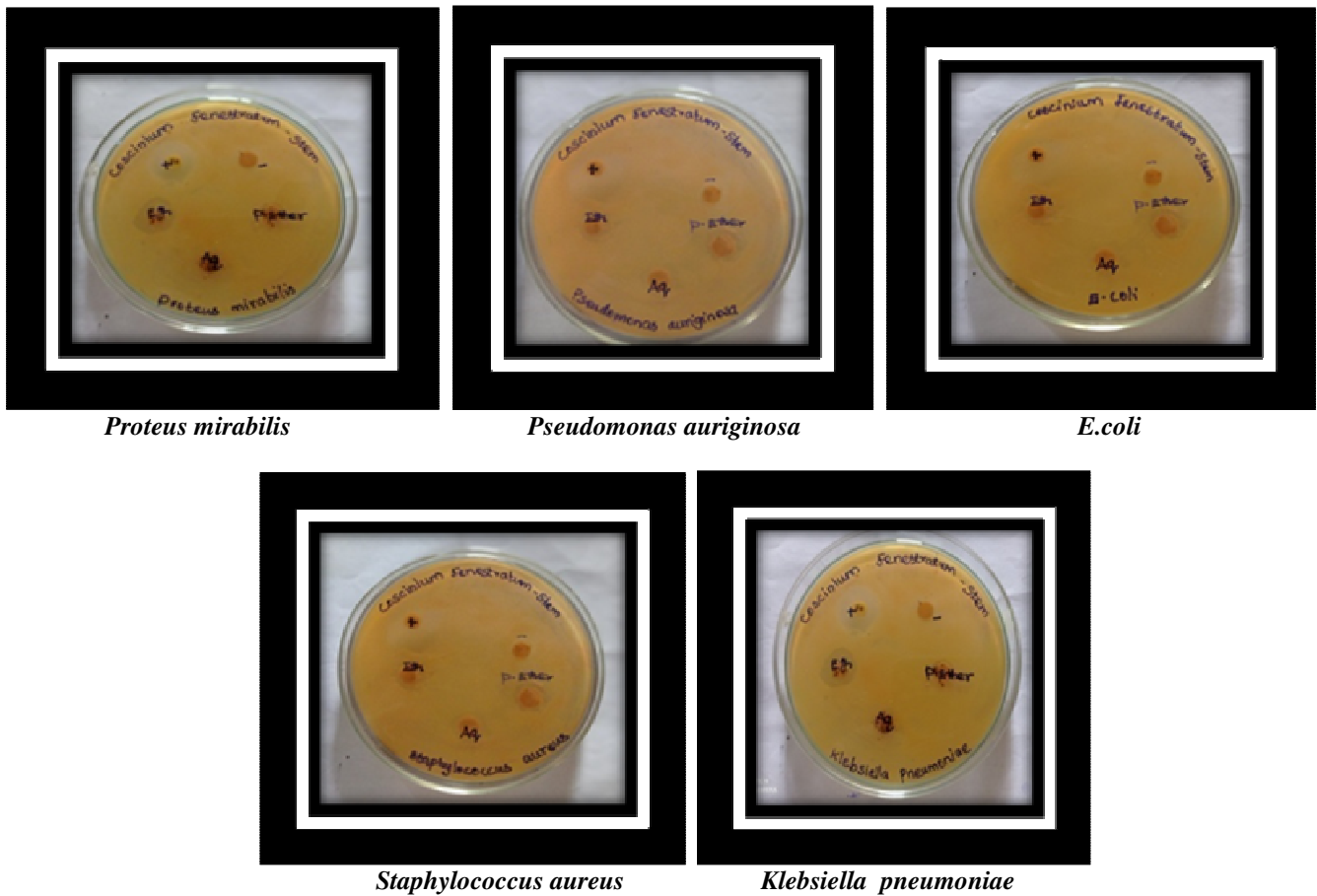


PLATE 1: Inhibition Zone in *Coscinium fenestratum* (stem) using different solvent Disc Diffusion Method

Table 2 : Inhibition percentage of anti-oxidant potential of ethanolic extract of *Coscinium fenestratum* (stem) by DPPH scavenging assay

S. No	Sample Code	Concentration	OD value at 520 nm (in triplicates)			Mean OD	% of Inhibition
1.	Blank	-	0.96	0.95	0.97	0.96	
2.	Control (Ascorbic acid)	100 µg /ml	0.027	0.027	0.026	0.03	97.22
3.	Stem	20 µg /ml	0.81	0.808	0.807	0.57	41.02
		40 µg /ml	0.728	0.728	0.728	0.73	24.17
		60 µg /ml	0.716	0.714	0.712	0.71	25.63
		80 µg /ml	0.64	0.666	0.663	0.66	31.63
		100 µg /ml	0.691	0.69	0.69	0.69	28.09

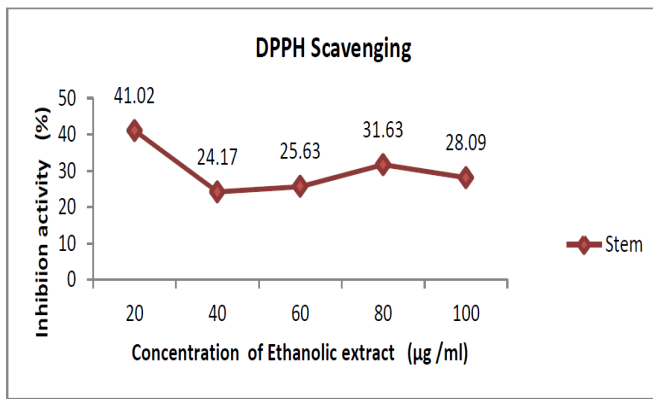


Fig. 2 : DPPH radical scavenging activity of different concentrations of *C.fenestratum* stem extracts.

Conclusion

The present study supports the traditional use of *Coscinium fenestratum* and indicated that the plant contains some major bioactive compound that inhibits the growth of respiratory microorganism thereby proving very effective source of derived drugs. The herbal based phytomedicine have large therapeutic applications since they can have less side effects when compared with synthetic antimicrobials.

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