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Studies on phytochemical and antimicrobial activity of ethanol extract of *Curcuma aromatica* and *Coscinium fenestratum*

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ABSTRACT

Preliminary phytochemical and antimicrobial activity of *Curcuma aromatica* (rhizome) and *Coscinium fenestratum* (stem) was investigated. The powdered plant materials were subjected to soxhlet extraction using ethanol as solvent. Phytochemical screening revealed the presence of flavonoids, saponins, tannins, glycosides, alkaloids and steroids in both the extracts. The ethanol extracts of the plant materials were subjected to antibacterial activity (Agar well diffusion method) and Antifungal activity (Poison food technique). The bacterial strains were seeded on media by Spread plate method and test fungi were point inoculated. Diameter of zone of inhibition was recorded (for bacteria) and colony diameter in poisoned plates (for fungi) was measured to assess the effect of extracts on test organisms. Both the extracts have shown marked antibacterial and antifungal activity against bacteria and fungi tested. Ethanol extract of *Coscinium fenestratum* was found to inhibit bacteria to more extent than ethanol extract of *Curcuma aromatica* while fungi were more inhibited by *Curcuma aromatica* extract when compared to *Coscinium fenestratum* extract. Among bacteria, *E. coli* NCIM-2065 was more inhibited by extracts followed by *Enterobacter aerogenes* NCIM-2340, *Staphylococcus aureus* NCIM-2079 and *Bacillus subtilis* NCIM-2063. The growth of *Mucor sp*. was totally inhibited by extracts of both plants at 1% concentration. *Aspergillus niger* and *Aspergillus oryzae* were found to be more sensitive to ethanol extract of *Curcuma aromatica* when compared to ethanol extract of *Coscinium fenestratum*. The antimicrobial activity of ethanolic extract of the two plant materials tested may be attributed to the phytochemicals present and the results obtained offer the scientific basis to the use of these plants in traditional medicine.

KEYWORDS

Coscinium fenestratum;
Curcuma aromatica;
Phytochemical screening;
Antimicrobial activity;
Agar well diffusion;
Poison food technique.

INTRODUCTION

Coscinium fenestratum, Colebr. belongs to the family Menispermaceae and is a critically endagered dioecious medicinal liana found in Western ghats of India. It is a native plant of India and Ceylon. The plant is widely distributed in Western ghats of India (Tamilnadu and Kerala), Malaysia and Srilanka. It is commonly known as Daru haridra in Sanskrit, Ceylon wood in English and Arisina balli and Marmanjal in Kannada. 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 diam-

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eter. 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. *Curcuma aromatica* belongs to the family Zingiberaceae and is found distributed in India, Malaysia, South Asia, and Java. It is known as Wild Yellow Ginger in EnglishIn India, it is cultivated chiefly in West Bengal, Kerala and Karnataka. It is recognized as a medical herb with strong antibiotic properties. The rhizome is used to treat several types of ailments in the body including cancer. It contains aromatic volatile oils that possess several important physiological functions[1]. The aims and objectives of this investigation were to subject ethanol extracts of *Curcuma aromatica* and *Coscinium fenestratum* to preliminary phytochemical analysis and to find out whether the extract possess antimicrobial activity.

MATERIALS AND METHODS

Collection and identificaiton of plant material

The plant materials of *Curcuma aromatica* (rhizome) and *Coscinium fenestratum* (stem) were purchased from local shops and authenticated to their identity by Department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga and voucher specimens were deposited in the department.

Extraction of crude drug- soxhlet extraction

The plant materials of *Curcuma aromatica* and *Coscinium fenestratum* were powdered mechanically. About 250g of powdered materials were subjected to soxhlet extraction and exhaustively extracted with ethanol for about 48 hours. The extract was filtered and subjected to Vacuum under reduced pressure using rotary flash evaporator, dried in dessicator and stored in airtight containers. The ethanol extracts were subjected to preliminary phytochemical analysis[2,3].

Formulation of extracts

For antibacterial activity, the ethanol extracts of *Curcuma aromatica* and *Coscinium fenestratum* were dissolved in 5% DMSO to a final concentration of 100mg/ml.

Antibacterial susceptibility test (Agar well diffusion method)

The bacterial strains namely *Escherichia coli* NCIM-2065, *Enterobacter aerogenes* NCIM-2340, *Staphylococcus aureus* NCIM-2079 and *Bacillus subtilis* NCIM-2063 were obtained from National Chemical Laboratory, Pune. The antibacterial susceptibility test was performed by Agar well diffusion method[4]. The bacterial strains were inoculated to Muller Hinton broth (Oxoid) medium and incubated. A standard size of inoculum was plated on surface of Muller Hinton agar by spread plate technique using sterile L shaped glass spreader and plates were allowed for few minutes. Using sterile cork borer, 6 mm diameter wells were bored in the agar and the extracts (100mg/ml) of both the plant materials reconstituted in 5% DMSO were transferred into the wells. The plates were allowed to stand at room temperature for two hours and then incubated at 37°C for 24 hours and zone of inhibition was measured to the nearest milimeter. Streptomycin (10 mcg/ml) was used as control drug or reference drug. The test was done in triplicates to arrive concordent results.

Antifungal susceptibility test (Poison food technique)

In the study, two species of the genus *Aspergillus* (namely *Aspergillus niger, Aspergillus oryzae) and Mucor sp.* were selected as target fungi which are known to cause opportunistic mycotic infections in susceptible individuals. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[5]. The antifungal activity was assessed using Poison food technique[6]. The test fungi was allowed to grow in Sabouraud's dextrose agar plates poisoned with extracts of *Curcuma aromatica* and *Coscinium fenestratum* (1% ethanol extract). The test fungi were inoculated by Point inoculation method where the spore suspension of test fungi were taken with the help of inoculation needle and touched the centre of the medium. The effect of extract on fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate and comparing with control (plates not poisoned with extract). The experiment was done in triplicate and average reading was recorded.

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RESULTS AND DISCUSSION

TABLE 1 reveal the phytochemical screening of ethanol extracts of *Curcuma aromatica* and *Coscinium fenestratum*. Flavonoids, saponins, tannins, glycosides, alkaloids and steroids were detected in both the extracts but triterpenoids were detected in *Curcuma aromatica* extract but not detected in *Coscinium fenstratum* extract. TABLE 2 and TABLE 3 depict antibacterial and antifungal activity of ethanol extracts of test plants respectively. Both the extracts have shown marked antibacterial and antifungal activity against bacteria and fungi tested. Ethanol extract of *Coscinium fenestratum* was found to inhibit bacteria to more extent than ethanol extract of *Curcuma aromatica* while fungi were more inhibited by *Curcuma aromatica* extract when compared to *Coscinium fenestratum* extract. Among bacteria, *E.coli* NCIM-2065 was more inhibited by extracts followed by *Enterobacter aerogenes* NCIM-2340, *Staphylococcus aureus* NCIM-2079 and *Bacillus subtilis* NCIM-2063. The growth of *Mucor sp*. was totally inhibited by extracts of both plants at 1% concentration. *Aspergillus niger* and *Aspergillus oryzae* were found to be more sensitive to ethanol extract of *Curcuma aromatica* when compared to ethanol extract of *Coscinium fenestratum*.

Medicinal plants are important elements of traditional medicine in virtually all cultures. Medicinal plants which have been used by human being to treat common infectious diseases are important elements to traditional medicine. In India, a number of plant extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological activities[7]. The antimicrobial activities of the plants may be attributed to to the phytoconstituents present in them such as flavonoids, phenolics and polyphenols, tannins, alkaloids, quinones, triterpenoids, sesquiterpenoids etc. These phytochemicals have shown to possess antimicrobial activities against wide range of microorganisms[2]. Curdione, neocurdione, curcumol, tetramethyl pyrazine and (R)-(+)-1,2-hexadecanediol were isolated from *C.aromatica* with resin D-101 silica gel column and thin-layer chromatography[8]. Camphor (26.94%), ar-curcumene (23.18%) and xanthorrhizol (18.70%) were found in the essential oil of *C.aromatica*[9]. The most notable volatile oils of *C.aromatica* Salisb. (characterized by GC and GC-MS) being germacrene-D, curzerene, germacrone, curzerenone, xanthorrhizol, curcuphenol and hydroxyisogermafurenolide[10]. Three new sesquiterpenes, isozedoarondiol, methylzedo arondiol and neocurdione, were isolated from *C.aromatica* Salisb[11]. *C.aromatica* ethanol extract, when subjected to mosquito repellent activity, was found to provide biting protection against mosquito and thus it could be applied as an effective personal protection measure against mosquito bites[12]. *C.aromatica* was

TABLE 1: Preliminary phytochemical analysis of ethanol extracts of *Curcuma aromatica* and *Coscinium fenestratum*

Phytochemical group	*Curcuma aromatica*	*Coscinium fenestratum*
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+
Alkaloids	+	+
Steroids	+	+
Triterpenoids	+	-

'+' detectable, '-' undetectable

TABLE 2: Antibacterial activity (Agar well diffusion method) of ethanol extract of *Coscinium fenestratum* and *Curcuma aromatica*

Test bacteria	Zone of inhibition in mm		
	Streptomycin (10 mcg/ml)	*Coscinium fenestratum* extract	*Curcuma aromatica* extract
Escherichia coli	26	25	18
Enterobacter aerogenes	22	22	17
Bacillus subtilis	19	14	13
Staphyococcus aureus	20	19	17

Extract concentration- 100mg/100ml

TABLE 3: Antifungal activity (Poison food technique) of ethanol extract of *Coscinium fenestratum* and *Curcuma aromatica*

Test fungi	Colony diameter in cm		
	Control	1% ethanol extract of *Curcuma aromatica*	1% ethanol extract of *Coscinium fenestratum*
Aspergillus niger	4.6	0.3	2.1
Aspergillus flavus	3.5	0.2	0.3
Mucor sp.	5.7	0.0	0.0

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found to have therapeutic potential for the prevention of hyperglycemia associated diabetic complications[13]. The *Coscinium fenestratum* extract was found to produce strong inhibition zones against *Propionibacterium acnes* and Phytochemical screening revealed the presence of alkaloid which could be responsible for activity[14]. Antibacterial activity of *Coscinium fenestratum* was found to be mainly due to the presence of berberine[15].

CONCLUSION

Infectious diseases still represent an important cause of mortality and morbidity among humans especially in developing countries. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness. The antimicrobial activity of extracts could be attributed to the presence of novel phytochemicals in it. Use of these herbal compounds could confer protection against drug resistant microorganisms. The study was done *in vitro*. Further studies in animal models could possibly reveal the antimicrobial activity of plant extracts *in vivo*. The results of this study offer a scientific basis to the ethnomedicinal use of the plants.

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