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ANTIBACTERIAL ACTIVITY IN THE RHIZOME EXTRACTS OF *COSTUS SPECIOSUS* (KOEN.)

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Hexane, methanol and water extracts of leaf and rhizome extracts of *Costus speciosus* used by Indian traditional healers for treating skin diseases, diabetes, jaundice, snake bites and / or anti-inflammatory properties was screened for *in vitro* antibacterial activities against pathogens isolated from infected burn patients For evaluating antibacterial activity, the disc-diffusion assay was used against burn pathogens. Minimal inhibitory concentration values were determined with a microdilution assay. No antibacterial activity was recorded with water extracts. The disc-diffusion method showed significant zone of lysis against all the pathogens studied and results are comparable to the conventional antibiotic cream namely Silver Sulphadiazine (SSD).

Keywords : Antibacterial activity; Burn injuries; Diosgenin.

Introduction

Research in antibacterial activity of higher plants in India started seriously in fifties and gained momentum in seventies. Many workers conducted a large scale screening of Indian plants for their biological activity. *Costus speciosus* (Koen.) Sm (family Zingiberaceae) commonly known as *"Spiral ginger"* is a rhizomatous perennial herb with pinkish white flowers in reddish bracts. It is distributed below 1500 m altitude in tropical forests throughout India. The plant is ornamental and the rhizome is a source of an antihelmintic compound and an alternative source of diosgenin[1]. It is also used locally for diabetes and jaundice. The root extract act as an astringent, aphrodisiac, depurative, purgative and useful in catarrhal fever, coughs, skin diseases and snake bites[2-7]. The plant is conventionally propagated by vegetative techniques using rhizome and sucker segments and through seeds, which are very slow for large-scale plantation.

Bacterial colonization of burned and devitalized tissue is inevitable and invasive bacterial infection is still one of the major problems in the treatment of burn victims. Approximately 70-80% of hospital deaths are reported to be due to infections[8]. Burn patients are at high risk for nosocomial infections due to multi-resistant bacterial species and a high proportion of which was due to Gram-negative organisms[9]. Burns remain a huge public-health issue, in terms of morbidity and long-term disability throughout the world especially in the developing countries[10]. The most common pathogens causing serious infection in burn patients include *Proteus, Coliform* species, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis and Clostridium* and *Streptococcus pyogenes*[11]. Hence the present study is aimed at evaluating the antibacterial effect of crude extracts of, leaf and rhizomes of *C. speciosus* on burn pathogens and comparing the efficiency against conventional antibiotic cream namely Silver Sulphadiazine (SSD).

Materials and Methods

Preparation of Plant extracts : Rhizomes of *Costus speciosus* (Koen.) Sm, collected from the Western Ghat Hills near Belgaum - Panaji Highway road, Belgaum, Karnataka, India, were washed thoroughly in Tween - 20 for 15 min, and then under running tap water for 2 hour. Whole leaf and rhizomes were surface sterilized sequentially with the solutions of 70% ethanol (5 min) and 0.1% $HgCl_2$ (2 min) before rinsing thoroughly with sterilized distilled water. Rhizomes used in the screening procedures was dried in an oven at 50°C and stored at room temperature until extraction. Dried leaf and rhizomes were ground to a powder. Two separate samples of 1 g were extracted with 10 ml water (polar) and methanol (moderately polar), respectively, and 4 g samples of plant material were extracted with 40 ml hexane (non-polar). Extraction was done by sonication for 30 min in an ultrasound bath after which the plant extracts were macerated overnight. The plant extracts were filtered through Whatman No. 1 filter paper into pill vials. The clear filtrates were air dried under a fan and the residues were resuspended in their extracting solvents to give 100 mg residue ml^{-1} for the antibacterial assay.

Screening of Antibacterial activity : The test pathogenic

organisms in the investigations of antibacterial activity namely *Shigella, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas, Bacillus subtilis, Salmonella* were procured from the Bacterial collections of Burn ward Unit, Department of Microbiology, Medical College, Mumbai, India. Tenfold serial dilution of overnight MH broth cultures were prepared and 100 µl of each dilution were spread onto MH agar plates using a glass spreader. The plates were incubated overnight at 37°C and colonies were counted using a colony counter. Following the assumption that each living bacterial cell will grow into a separate colony on the plate, the number of cells present per milliliter of the original overnight cultures was calculated. The optical density (OD) at 600 nm for each dilution was determined using spectrophotometer, and used to indicate numbers of bacterial cells in cultures for the antibacterial screening and MIC determination.

The disc diffusion assay[12] was used in the antibacterial screening procedure. MH agar (Biolab) base plates were prepared using sterile 90 mm Petridishes. MH agar at 48°C was inoculated with a MH broth culture (106-10^8 bacteria CFU ml^{-1}) of each bacterial species and poured over the base plates to form a homogenous layer. Filter paper discs (Whatman No 3 and 6 mm in diameter) were sterilized by autoclaving. Ten microliter of plant extract (100 mg ml^{-1}) was applied per filter paper discs so that each disc contained 1 mg of material. The discs were air-dried and placed onto the seeded top layer of the MH agar plates. Each extract was tested in quadruplicate (four discs per plate), with a Silver Sulphadiazine (1mg ml^{-1}) disc as a reference or positive control. Air dried solvent (hexane, methanol and water) saturated discs were used as negative controls. The plates were evaluated after incubation at 37°C for 24h after which the zones of inhibition around each disc were measured. The ratio between the diameter of the inhibition zones (mm) produced by plant extracts and the inhibition zone around the disc with Silver Sulphadiazine (mm) was used to express antibacterial activity. The activity of Silver Sulphadiazine was included in this equation to adjust for plate-to-plate variations in the sensitivity of a particular bacterial strain[13].

The microplate method of Eloff[14] was used with slight modifications to determine the MIC values for plant extracts with antibacterial activity. Residues of plant extracts that were active in the disc-diffusion assay were dissolved at 50 mg ml^{-1} with the extracting solvent in the case of methanol and water. Hexane extracts were resuspended in acetone. All extracts were initially tested at 12.5 mg ml^{-1} in 96-well microtitre plates and serially diluted twofold after which 100 µl bacterial culture (approximately 10^6 bacteria CFU ml^{-1}) were added to each well.

The antibiotic Silver Sulphadiazine was included as standard in each assay. Extract-free solution was used as blank control. The microplates were incubated overnight at 37°C. As an indicator of bacterial growth, 40µl p-iodonitrotetrazolium violet (INT) (Sigma) dissolved in water were added to the microplate wells and incubated at 37°C

Table1. Antibacterial activity of various plant parts of *C. speciosus* with the diffusion and microdilution assays (MIC recorded in mg^{-1}).

Plants parts used	Extract	Bacterica used							
		B.s.		E.c.	K.p.	S.a.			
		Dif	MIC	Dif	MIC	Dif	MIC	Dif	MIC
Rhizome	Hexane	0.3±0.04	0.44±0.05	0.25±0.02	0.32±0.034	0.29±0.02	0.35±0.03	0.21±0.01	0.30±0.04
	Methanol	0.4±0.06	0.43±0.76	0.32±0.03	0.54±0.049	0.30±0.03	0.36±0.03	0.31±0.03	0.45±0.04
	Water	0	0	0	0	0	0	0	0
Leaf	Hexane	0	0	0	0	0	0	0	0
	Methanol	0	0	0	0	0	0	0	0
	Water	0	0	0	0	0	0	0	0

All the experiments repeated three times

Values represent the mean ±SE of 3 independent experiments

Bacteria tested; **B.s.**, *Bacillus subtilis*; **E.c.**, *Escherichia coli*; **K.p.**, *Klebsiella pneumoniae* ; **S.a.** *Staphylococcus aureus*

Dif, Results obtained in the disc-diffusion assays. Antibacterial activity is expressed as the ratio of the inhibition diameter around the extract to the inhibition zone around the reference neomycin antibiotic. The symbol 0 indicates no activity that no inhibition zone around the extract discs.

MIC, Results obtained in the microdilution assays. Antibacterial activity is expressed as the minimum inhibitory concentration (mg ml^{-1}), **0= MIC** not determined

for 30 min. MIC values were recorded as the lowest concentration of extract that completely inhibited bacterial growth. Since the colorless tetrazolium salt is reduced to a red colored product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT.

Results and Discussion

The antibacterial activity of crude extracts (hexane, methanol and water extracts at a concentration of 100 mg ml^{-1}) showing positive results is presented in the table 1. In total 6 extracts belongs to different plant parts of *C. speciosus* were tested in the present investigation. The extract of leaf part of *C. speciosus* exhibited no antibacterial activity. Whereas the extract of rhizomes showed antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) as well as Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) respectively. The water extracts presented no antibacterial activity against burn pathogens (Table 1). The results obtained from the present study suggested that rhizome extracts of *C. speciosus* possess a positive antibacterial activity. This may be due the presence of terpenoids, or flavonoids which possess significant antifungal, antibacterial and anti-insect activities[15-18]. On the other hand antibacterial activity might be due to the presence of diosgenin. The antibacterial activity of these extracts was found to be comparable to the antibacterial activity exhibited by conventional antibacterial cream SSD.

Another interesting observation is high MIC values recorded with both hexane and methanol extracts of rhizomes (Table 1). Reasons for high MIC values could be that the extracts tested are still in an impure form, or that active compound/s is present in very low concentrations. Nevertheless certain of the plant extracts warrant further investigation using bioassay-guided fractionation to characterize the active constituents. Therefore, the results of this study support to a certain degree, the traditional medicine uses of the plants evaluated and reinforce the concept that the ethanobotanical approach[19] to screening plants as potential sources of bioactive substances is successful. As infections being a major cause of morbidity and mortality in burn patients, these herbal extracts may prevent infection that leads to high risk of sepsis, and thereby prevents the prolongation of inflammatory phase. This is our first report of antibacterial activity found in the rhizome extracts of *C. speciosus*. Therefore, further detailed study is very much needed for the isolation and identification of active compounds responsible for the antibacterial activity particularly against the most burn pathogens.

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