



Original Research Article

Evaluation of antibacterial activity in three species of *Costus*

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A B S T R A C T

Keywords

Antibacterial activity;
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 inhibition zones.

The rhizome extract of three *Costus* species (*Costus speciosus*, *Costus pictus* and *Costus igneus*) were studied for their antibacterial activity against gram positive bacteria *Bacillus subtilis* (ATCC 633), *Staphylococcus aureus* (ATCC 9144) and gram negative bacteria *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC25619). Different concentrations of extract ranging from 500-2000 µg were used. The rhizome extract of three species inhibited the growth of all the test fungi. The zone of inhibition increased with increase in concentration of the test solution. High inhibition zone was observed in *C.pictus* against *S. areus*(1.26 cm) followed by *P.aeruginosa*(1.21 cm) followed by *E.coli*(1.18cm). *B.subtilis* showed low inhibition zone (1.12 cm). In *C.speciosus* the zone of inhibiton ranged from 1.25cm for *S.areus* followed by *P.aeruginosa*(1.21cm), *E.coli* (1.15 cm) and *B.subtilis* (1.10 cm). Higher activity of *C.igneus* rhizome extract was found against *S.areus* (1.20 cm), followed by *P.aeruginosa*(1.18cm), *E.coli* (1.11 cm)and *B.subtilis* (1.0 cm).

Introduction

Medicinal plants possess many antimicrobial compounds and can be utilized as anti-infectious agents. Plant based products are not associated with side effects like synthetic drugs. Therefore there has been an increased interest in the use of plants as therapeutic agents. During last few decades, many plant species were screened and plants with high bioactive compounds were identified. *Costus* is one of the important medicinal plants with a source of antidiabetic and antimicrobial compounds (Anonymous 1988).

The *Costus* species (family Costaceae), also known as spiral or crepe ginger are the plants commonly used in traditional and commercial formulations. These plants are used for their tonic, stimulant, carminative, diuretic, digestive and antiseptic properties (Sivarajan and Balachandran, 1994).The rhizomes *Costus* species are rich in steroid – diosgenin, which is anti-diabetic in nature and is used in the treatment of diabetes-mellitus (Chopra *et al.*, 1956).Apart from antidiabetic nature, they also posses

antimicrobial (Asolkar, 1992) and antioxidant properties (Vijayalaxmi and Sarada, 2008). In the present study, antibacterial activity of three species of *Costus* (*C.speciosus*, *C.pictus* and *C.igneus*) was evaluated *in vitro* using agar cup bioassay. .

Materials and Methods

Plant material

The rhizomes were collected from three different species of *Costus* i.e., *C.speciosus*, *C.pictus* and *C.igneus*. The air dried and powdered rhizomes (500 g) were extracted with petroleum ether for 3-4 hours at 60 -80°C in a Soxhlet apparatus. Different concentrations of plants extracts were evaluated for its antibacterial activity.

Test organisms

Four test organisms, *Bacillus subtilis* (ATCC 633), *Staphylococcus aureus* (ATCC 9144), *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC25619) where obtained from IICT, Hyderabad. Cultures of test organisms were maintained on nutrient agar slants and sub-cultured in petridishes prior to testing.

The readymade medium (Hi-media, 23g) was suspended in distilled water (1000ml) and heated to boiling until it dissolved completely. The medium and petridishes were autoclaved at 15 Psi for 20 min. Stock solutions were prepared by dissolving plant extract in DMSO (dimethyl sulphoxide) and different concentrations were made (50-2000 μ g).

Bio assay

Agar cup bioassay (Linday, 1962) was employed for testing antibacterial activity

of plant extract .The medium was poured into petridishes under aseptic conditions in a laminar flow chamber and left to solidify. These petridishes were inoculated with 0.5 ml of 24 hrs old cultures of test organisms. After inoculation, cups were scooped out with 7mm sterile cork borer and the lids of the dishes were replaced .To each cup different concentrations of test solutions (50-2000 μ g)were added. Zone inhibition was also studied for standard antibiotics, streptomycin and penicillin G (Table: 2).DMSO was used as a control.

Minimum inhibitory concentration (MIC) Test

MIC test was performed by broth dilution method. Different concentrations (2.0 ,1.0, 0.5, 0.25,0.125, 0.0625, 0.0312, 0.0156,or 0.0078 mg/ml) of rhizome extract were prepared in 1ml working solution of extract(2mg/ml)was added to the test tube 1, containing 1ml of nutrient broth .After thorough mixing , 1ml of solution was transferred to second test tube and the process was continued for succeeding transfers. The last test tube received no test solution and served as control.

For both tests, culture was kept in an incubator at 26°C and results were observed after 24 h and 3-4 replicates were maintained for each treatment.

Results and Discussion

The antimicrobial activity at different concentrations (500-2000 μ g) of the extract of *Costus species* is tabulated in table-1. The concentrations from 500 μ g-2000 μ g exhibited antimicrobial activity against all the test organisms. Below and beyond this concentration activity was not seen. The

Table.1 Antibacterial activity of rhizome extracts of *Costus* species.

Test bacteria	Zone of inhibition in cms							
	Concentration of extract in μg							
	500	700	1000	1200	1400	1600	1800	2000
	Activity of <i>C.speciosus</i>							
<i>E.coli</i>	-----	0.18	0.4	0.56	0.74	0.9	0.95	1.15
<i>P.aeruginosa</i>	0.25	0.39	0.56	0.65	1.13	1.14	1.18	1.21
<i>B.subtilis</i>	0.18	0.25	0.38	0.4	0.58	0.74	0.8	1.1
<i>S.areus</i>	0.25	0.4	0.45	0.72	1.04	1.21	1.24	1.25
				Activity of <i>C.igneus</i>				
<i>E.coli</i>	-----	0.17	0.39	0.52	0.6	0.78	0.92	1.11
<i>P.aeruginosa</i>	0.2	0.22	0.48	0.64	0.75	0.89	1.1	1.18
<i>B.subtilis</i>	0.17	0.21	0.28	0.56	0.62	0.83	0.97	1
<i>S.areus</i>	0.19	0.23	0.41	0.53	0.69	0.85	1.05	1.2
				Activity of <i>C.pictus</i>				
<i>E.coli</i>	-----	0.46	0.53	0.53	0.66	0.78	0.85	1.18
<i>P.aeruginosa</i>	-----	0.21	0.24	0.65	0.74	0.84	1.08	1.21
<i>B.subtilis</i>	0.18	0.21	0.38	0.55	0.63	0.81	0.94	1.12
<i>S.areus</i>	0.2	0.25	0.41	0.54	0.69	0.79	1.12	1.26

Table.2 Activity of antibiotics

Concentration in μg	Zone of inhibition in cms			
	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>B.subtilis</i>	<i>S.aureus</i>
Penicillin G (100)	---	---	1.6	2.1
Streptomycin (100)	2.9	3.4	---	----

zone of inhibition increased with concentration from 500-2000 μ g of test solution. 2000 μ g concentration showed maximum activity on all the test organisms.

The minimum inhibitory concentration (MIC) test was done by broth dilution method to know the lowest concentration of extract required for complete inhibition of bacterial growth. The rhizome extract of *C.igneus* and *C.speciosus* exhibited MIC of 0.25mg /ml for *P.aeruginosa*, *B.subtilis*, *S.aureus* and 0.5mg/ml for *E.coli*. Whereas MIC of 0.25mg /ml for *B.subtilis*, *S.aureus* and 0.5mg/ml for *E.coli* , *P.aeruginosa* was exhibited by *C.pictus*. All the four organisms tested were sensitive to the test solution. However the sensitivity varied among them. High inhibition zones were observed in *S.aureus* (1.26 cm) followed by *P.aeruginosa* (1.21 cm) followed by *E.coli* (1.18cm). *B.subtilis* showed low inhibition zone (1.12 cm) in *C.pictus*.

In *C.speciosus* the zone of inhibition ranged from 1.25cm for *S.aureus* followed by *P.aeruginosa* (1.21cm), *E.coli* (1.15 cm) and *B.subtilis* (1.10 cm). Higher activity of *C.igneus* rhizome extract was found against *S.aureus* (1.20 cm), followed by *P.aeruginosa* (1.18cm), *E.coli* (1.11 cm) and *B.subtilis* (1.0 cm).

Similar work on antibacterial studies using root extract of *C.igneus* on *P.aeruginosa*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Salmonella* sp. was reported (Arjun Nagarajan et al., 2011).Studies on antimicrobial activities of different medicinal plants has been reported worldwide by many workers (Samy, 2005; Palombo & Semple, 2001; Ahmad et al., 2001). Antibacterial activity of *Curcuma longa* varieties against different strains of

bacteria (Shagufta Naz et al ., 2010), rhizome extract of *Acorus* (sabita et al., 2003), leaf extract of *Mikania triangularis* (Cruz et al.,1996), root and leaf extract of *Withania somnifera* (Mahesh and Satish,2008) have been reported.

In the present study has shown that the rhizome extract of three *Costus* species has a potent antibacterial property. Hence, there is possibility of developing these plants as a source of herbal antibiotic and further studies are needed for isolation and purification of bioactive constituent.

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