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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE LEAF ESSENTIAL OIL OF *COSTUS PICTUS* D. DON. FROM SOUTH INDIA

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ABSTRACT

The medicinal plant *Costus pictus* D. DON. (Zingiberaceae family) is well known for its antihyperglycemic and insulin secretory activity. In this study, the leaf essential oil of *Costus pictus* was tested for antibacterial activity against ten bacterial strains: *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method. The leaf oil exhibited pronounced activity against all tested microorganisms and the activity was quite comparable with the standard antibiotics screened under similar conditions. The remarkable antibacterial activity exhibited by the leaf oil can be attributed to the synergic effect of the antimicrobial agents present in it. This study shows that *Costus pictus* leaf essential oil can be used as a potential external antiseptic and can be incorporated into the drug formulations.

Key words: *Costus pictus*, Leaf essential oil, Antibacterial activity, Agar well diffusion method, Standard antibiotics, Drug formulation.

INTRODUCTION

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to the report of the World Health Organisation, 80% of the world populations rely mainly on traditional therapies which involve the use of plant extracts or their active substances[1]. The microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs[2]. Antibiotics are sometimes associated with side effects[3] whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature[4]. All these data high lights the need for new alternative drug regimens.

Costus pictus D. DON. (Zingiberaceae family) is a native of south and Central America. It is widely cultivated in south India and also run wild in many places. This is a recent introduction from America as a herbal cure for diabetes, hence commonly called as 'insulin plant.' Though not clinically proved, it is widely used as a remedy for diabetes[5]. Powdered leaves of the medicinal plant *Costus pictus* known to possess therapeutic effect, when supplemented to streptozotocin induced diabetic rats, is found to reduce blood glucose level by 21% after 15 days of supplementation[6]. The methanolic leaf extract of *Costus pictus* is used to lower blood glucose level in alloxan induced diabetic rats[7]. The antihyperglycemic and insulin secretory activity of an aqueous extract of *Costus pictus* leaf is investigated in streptozotocin induced diabetic rats[8,9]. Beena Jose and L. Joji Reddy[10] isolated the essential oils from stem, leaf and rhizome of *Costus pictus* by steam distillation and quantified using GC-MS.

Microbiological assays are used for the quantitative determination of antibiotics and inhibitory chemical agents and also the determination of the sensitivity of the microorganisms to these agents. Synthetic chemicals have their side effects and the development of bacterial resistance to the presently available antibiotics has necessitated the search for new antimicrobial agents. So we look into the nature as an ally and resource in finding new strategies to combat diseases of plants, animals and human beings. Most of the previous chemical investigations on *Costus pictus* have focused mainly on the evaluation of anti-diabetic effect of the plant. So far no data about the antibacterial activity of *Costus pictus* leaf essential oil has been reported. In this work, the antibacterial property of the *Costus pictus* leaf oil was checked against ten pathogenic bacteria namely *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*.

The Gram-positive bacterium *Staphylococcus aureus* is mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning. The Gram-positive bacteria *Bacillus cereus* cause food borne illness in humans, *Enterobacter faecalis* cause inflammation of inner layer of the heart and *Streptococcus faecalis* is responsible for urinary tract and kidney infections. The Gram-negative bacterium *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia. Gram-negative bacteria such as *Klebsiella pneumoniae* cause pneumonia and urinary tract infections. *Proteus vulgaris*, the Gram-negative bacteria, cause wound infections and pneumonia. The Gram-negative *Salmonella paratyphi* causes bacterial enteric fever and *Pseudomonas aeruginosa* causes kidney infection. *Serratia marcescens* is responsible for septicemia, meningitis, wound and eye infections [11].

MATERIALS AND METHODS

Plant material

The stems, leaves and rhizomes of *Costus pictus* were collected in December 2009 from Thrissur district of Kerala, South India and authenticated by Dr. A.K. Pradeep, Dept. of Botany, Calicut University. Voucher specimen is deposited in the specially maintained herbarium, Department of Chemistry, Calicut University.

Antibacterial activity

The essential oils from the stems, leaves and rhizomes of *Costus pictus* were extracted by steam distillation and analysed by GC-MS[10] (table 1). Out of the three oil samples, leaf oil (yield 1.6%) was examined for antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Enterobacter faecalis* and *Streptococcus faecalis* and Gram-negative bacteria such as *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella paratyphi* and *Serratia marcescens*.

Microbial strains

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were *Bacillus cereus* (MTCC-1305), *Enterobacter faecalis* (MTCC-5112), *Salmonella paratyphi,*

(MTCC-735), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-729), *Streptococcus faecalis* (MTCC-439), *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647) and *Serratia marcescens* (MTCC-86).

Table 1: Essential oil composition of stems, leaves and rhizomes of *Costus pictus*

Identified compounds	Percentage composition		
	stem	leaf	rhizome
cis-Linalool oxide	2.53	--	--
Linalool	1.16	--	8.48
Linalyl propanoate	6.03	--	--
Decanoic acid	0.34	--	--
4-Ethoxy ethyl benzoate	4.44	--	--
Dodecanoic acid	5.62	3.96	16.56
γ-Eudesmol	3.21	--	--
α-Eudesmol	3.55	--	--
Tetradecanoic acid	4.82	--	10.20
9,12-octadecadienoic acid	18.33	--	7.74
Octadecanoic acid	2.94	--	--
4-ethoxy Phenol	3.06	--	--
4-vinyl-2-methoxy Phenol	1.5	--	--
Isophytol	1.02	--	--
1,1-diethoxy Ethane	--	0.31	--
cis-3-Hexenol	--	1.41	--
2-ethoxy Butane	--	1.25	--
2-Pentanol	--	22.48	--
Tetradecane	--	0.34	--
β-Ionone	--	8.69	--
α-Ionone	--	8.01	--
n-Nonadecane	--	2.94	--
Farnesyl acetone	--	7.04	0.84
α-Terpineol	--	--	4.44
9,12-Octadecadien-1-ol	--	--	1.83

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10ml of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20ml) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1ml of bacterial suspension was taken and poured into Petri plates containing 20ml nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Screening for antibacterial activity

The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 µl of the leaf essential oil (at various concentrations) were added in each well. The essential oil of required concentrations 10%, 5% and 1% were prepared by dissolving the oil into appropriate quantities of DMSO, which did not influence the growth of bacteria was used as a negative control. The plates were then incubated at 37^oC overnight and examined for zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm[12]. All the assays were performed in triplicate and expressed as average values. The antibacterial spectra of the *Costus pictus* leaf essential oil showing zone of inhibition in millimetres, for Gram-positive and Gram-negative bacteria and the inhibition zones formed by standard antibiotics and those of negative control are listed in table 2.

Table 2: Inhibition zones formed by *Costus pictus* leaf essential oil, standard antibiotics and negative control

Microorganisms	Diameter of inhibition zones(mm/50µl)							
	C. pictus leaf oil			Tob (10 µg)	Gen (10 µg)	Oflo (10 µg)	Cip (10 µg)	Control (DMSO)
	10%	5%	1%					
Bacillus cereus	40	34	24	28	32	34	30	-
Enterobacter faecalis	34	28	20	26	32	32	26	-
Salmonella paratyphi	32	26	22	25	30	28	30	-
Staphylococcus aureus	22	18	14	26	28	24	24	-
Escherichia coli	32	28	22	26	28	24	24	-
Streptococcus faecalis	32	28	22	30	36	32	34	-
Proteus vulgaris	40	32	28	26	30	24	32	-
Klebsiella pneumonia	28	24	20	26	32	32	26	-
Pseudomonas aeruginosa	24	20	18	26	24	32	28	-
Serratia marcescens	28	24	22	24	32	30	30	-

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip: ciprofloxacin

Used concentrations: 50µl of 10%, 5% and 1% of the leaf essential oil of *Costus pictus* in DMSO

RESULTS AND DISCUSSION

Costus pictus leaf essential oil at various concentrations were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria strains and the oil exhibited marked activity against all tested microorganisms. As can be seen from table 2, the leaf oil (10%) showed pronounced activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus faecalis* and *Klebsiella pneumoniae* (22-40mm/50µl inhibition zone). The activity of the *Costus pictus* leaf oil (10%) against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi* and *Proteus vulgaris* (32-40mm/50µl inhibition zone) was found to be higher than that of the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin (10µg each) screened under similar conditions. The inhibitory effect of 10% leaf oil of *Costus pictus* on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Serratia marcescens* was quite comparable with the standard antibiotics whereas its activity against *Klebsiella pneumoniae* and *Streptococcus faecalis* was comparatively less. The activities of 5% (18-32mm/50µl inhibition zone) and 1% (14-28mm/50µl inhibition zone) of the leaf oil samples were also studied against various pathogenic bacteria and were found to be active on all microorganisms tested. The activity of 1% oil was rather less, as the activity is dose dependent, yet it showed pronounced activity on *Proteus vulgaris* (28mm/50µl inhibition zone) and was found to be higher than the standard antibiotics ofloxacin (10µg) and tobramycin (10µg) screened under similar conditions.

As the leaf oil exhibited pronounced antibacterial activity comparable with standard antibiotics, it can be used as an external antiseptic in prevention and treatment of bacterial infections. The major components of the leaf essential oil such as fatty acids [13,14] and ionones[15] are reported to have antimicrobial activities**.** The remarkable antibacterial activity exhibited by the *Costus pictus* leaf oil can be attributed to the synergic effect of the antimicrobial agents present in the oil.

CONCLUSION

The essential oil from the leaf of *Costus pictus* showed varying degrees of antibacterial activity on the microorganisms tested. The variation of the susceptibility of microorganisms towards the essential oils could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the essential oils. Due to the emergence of the antibiotic resistant pathogens, plants are being looked upon as an excellent alternate to combat the spread of multi drug resistant microorganisms.

From the above experiment it can be inferred that *Costus pictus* leaf essential oil showed significant activity against Gram-positive and Gram-negative bacteria. The activity of leaf oil was found to be quite comparable with the standard antibiotics screened under similar conditions. So the oil can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*, which have developed resistance to antibiotics. The incorporation of this oil into the drug formulations is also recommended. This study demonstrated that the essential oil from the leaf of *Costus pictus* is as effective as modern medicine to combat pathogenic microorganisms.

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