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Original Research Article

Antimicrobial Activity of Methanolic Extract of the Rhizomes of *Costus igneus*

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ABSTRACT

The whole plant of *Costus igneus* commonly known as 'insulin plant' a member of *Costaceae* family is used for it anti-diabetic property. It was learnt that no substantial work on antimicrobial activity rhizomes of *Costus igneus* was carried out. Hence an effort was made to investigate the antimicrobial activity of the methanolic extract of rhizomes of *Costus igneus.* The rhizomes were exhaustively extracted by maceration with methanol for three days. After three days, methanol layer was decanted off and the concentrate was evaporated to dryness. The antimicrobial activity was evaluated using disc diffusion method and effect of concentration of extract on growth of bacteria culture was carried out by tube dilution technique. The methanolic extract was found to have significant activity against both gram-positive-*Staphylococcus aureus* and *Bacillus subtilis* and gram-negative bacteria- *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium* and no antifungal activity against both *Claviceps purpurea* and *Aspergillus niger*. It was also observed that as the concentration of the methanolic extract of the rhizomes of *Costus igneus* increased, there was significant inhibition seen in the growth of the culture.

Keywords: *Costus igneus;* anti-diabetic; *Costaceae; Staphylococcus aureus;* anti-microbial activity

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine.The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers an enormous potential source of new anti-infective agents. Although ancient civilization recognized the antiseptic or antibacterial potential of many plant extracts, they failed to document the preservative and curative effects of plant extracts. Medicinal plants used in traditional medicine are considerably useful and are readily available in rural areas at relatively cheaper than modern medicine.

Infectious disease can become a threat to public health in this world. Medicinal plants have a long

history of use and their use is widespread in both developing and developed countries. Multifarious biologically active compounds that are found in plants possess antibacterial properties. Plant produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [1]. According to the report of the World Health Organization, 80% of the world populations rely mainly on traditional therapies which involve the use of plant extracts or their active substances.

India has been identified as one of the top twelve mega biodiversity centres of the world with immensely rich flora of about 2000 species of medicinal plants and a vast geographical area with high production potential and varied agro-climatic conditions. For a long period of time, plants have been a valuable source of natural products for maintaining human health, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purpose has gradually increased. According to the recent studies medicinal plants would be the source to obtain a variety of drugs [3, 7].

The microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs. Antibiotics are mostly associated with side effects 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. The rapid increase in the rate of infections, antibiotic resistance in microorganisms and due to side effects of synthetic antibiotics, medicinal plants are gaining popularity over synthetic drugs. Thus, it is anticipated that phytochemicals with adequate bacterial efficacy will be used for the bacterial infections.

In recent years, the antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in plants, have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections.

Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur. 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.

Costus igneus N.E.Br (Fig. 1) *is a* perennial rhizomatous herb belonging to the family Costaceae, which is found in tropical Africa, Asia, Australia, and North, Central and South America.

Fig. 1: ***Costus igneus***

In India, it is cultivated in coastal area, Uttar Kannada district of Karnataka and Tamil nadu. In these areas, people take traditionally 2-3 leaves of this plant twice a day for the management of diabetes. It is a prostrate growing plant with spreading, rooting stems. Its leaves are slender and lance shaped with tooted, scalloped or lobed margins. They are grayish green stained with red

purple above and darker purple beneath. The tiny white flowers grow intermittently throughout the year. This plant reaches a height of 6 inches and has an indefinite spread [8,2,19].

Different types of oral hypoglycemic agents such as insulin, suphonylurea etc. are used for the treatment of this disease, but they cause side effects on continued use, resulting in growing interest in phytomedicine because of their effectiveness, fewer side effects and low costs [3]. Traditional anti-diabetic plants might provide new oral anti-diabetic compounds, which can counter the high cost and poor availability of the current medicines for many rural populations in developing countries [18]. *Costus igneus* (Spiral ginger), commonly known as 'insulin plant' a member of *Costaceae* Family and the whole plant is used for it anti-diabetic property and prevents the body from disease, protects mind and which prolongs the longevity of life. in Southern India [12].

In Ayurvedic system , the rhizomes of the plant of *Costus igneus* is also considered as Bitter tonic, purgative, astringent, febrifuge, anthelmentic and expectorant. It may be also to treat fever, rash, asthma, bronchitis, intestinal worms, ailments of eyes, stomach, neck, jaws, tongue, mouth and also be used for curing fever, oedema, leprosy, wheezing (dyspnoea), haemorrhoids, spermaturia [20]. It is commonly referred to as Insulin plant, spiral flag and Pushkarmula in Sanskrit [21].

Extensive literature survey revealed the existence of many phytoconstituents like Steroids, Triterpenoids, Flavonoids, Alkaloids, Saponins and Tannins from the whole plant, leaves and other species of *Costus* [17,6].

Earlier studies on phytochemistry of leaves and roots of *C. igneus* have revealed the presence of amino acids, vitamins, carbohydrate, carotenoids, flavonoids (catechins and flavones), phthalate, phytates, saponins and tannins that contribute to the antimicrobial activity [5, 10,15]. However, no substantial work on antimicrobial activity of methanolic extract rhizomes of *C. igneus* has been carried out.

The phytochemical investigation of methanolic extract of rhizomes of *C. igneus* also led to the isolation of phthalates, flavonoids (anthrocyanidine and chalcone) etc. In the past, such compounds have shown pronounced activity against both gram-positive and gram- negative bacteria responsible for various infections [14].

The present effort has been to investigate the antimicrobial activity of the methanolic extract of the rhizomes of *C.igneus*.

MATERIALS AND METHODS

Source of Microorganisms

The antimicrobial activity of methanolic extract of the rhizomes of *C.igneus* was tested against strains that were procured from National Chemical Laboratory, Pune, India and were used for the determination of antimicrobial activity (table 1).

Equipments

Autoclave (Quality Instrument), Incubator (Quality Instrument), Cyclomixer (Remi), Colorimeter (Elico C1157), Nichrome loop, Precision Balance (Conthec), Conical flask (Borosil), Petri dish (Borosil), Pipettes (Borosil), Glass rod (Borosil), Micropipette (Finn pipette), Orbital shaking (Remi) and Refrigerator.

Collection and Authentication

The rhizomes of *C.igneus* were collected from Gomantak Ayurveda Mahavidyalaya and Research Centre, Shiroda, Goa during November 2011. The plant sample was authenticated by Prof. G. I. Hukkeri, Associate Professor in Botany, Dhempe College of Arts and Science, Miramar, Panaji, Goa [11].

Extraction process

The rhizomes of *C. igneus* were collected, washed and dried in shade. The dried rhizomes were powdered (520 gm) and exhaustively extracted by maceration with methanol for three days. After three days, methanol layer was decanted off. The process was repeated for three times. The solvent from the total extract was distilled off and the concentrate was evaporated to a syrupy consistency using rotary vacuum evaporator (25 rpm; 60°C) and then evaporated to dryness (34 g) [13].

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Microorganisms

In the present study methanolic extract of the rhizomes of *C. igneus* was tested for antimicrobial activity by disc diffusion method. Five bacterial strains used included two gram-positive- *Staphylococcus aureus (6538P)* and *Bacillus subtilis (6633)* and three gram-negative bacteria - *Escherichia coli (35218), Pseudomonas aeruginosa (19429)* and *Salmonella typhimurium(23564)*. Two fungal strains, *Claviceps purpurea* and *Aspergillus niger (10864)* were used. All the bacterial strains and fungal strains were maintained on Nutrient Agar and Saborauds Dextrose Agar respectively, were freshly sub cultured for 24-48 hrs at 37°C and 25°C respectively.

Preparation of Media

In vitro antimicrobial activity was screened by using Mueller Agar (MHA) and Saborauds Dextrose Agar (SDM) obtained from Hi media (Mumbai). The required quantity of Mueller Hinton Agar was prepared in a conical flask and sterilized by autoclaving at 121°C at 15 lbs pressure for 15-20 mins. The required number of previously cleaned and sterilized Petri dishes and large number of test tubes were taken. The saline suspension of each strain was prepared separately. The MHA and SDM plates were prepared by pouring 20 ml of molten media and 1ml of above suspension after cyclomixing into sterile Petri dishes. The plates were allowed to solidify for 5 minutes. For each bacterial and fungal strain, pure solvent (alcohol) is used as control.

Antimicrobial Agents

Streptomycin S^{25} (Hi media) and Ampicillin S^{25} (Hi media) disc were used for antibacterial studies were as Sulphamethizole S^{25} (Hi media) disc included in the study as standard reference for antifungal activity.

Antimicrobial Activity

The Mueller Hinton Agar plates and Saborauds Dextrose Agar were prepared as mentioned above. The 1000µg/ml concentrations of extracts were loaded on sterile paper disc. The loaded disc was placed on the surface of medium using sterile forceps and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. For each bacterial and fungal strain, pure solvent (alcohol) is used as control. Streptomycin 25mcg/disc, Ampicillin 25mcg/disc for antibacterial studies and Sulphamethizole 25 mcg /disc for antifungal studies were used as positive control. These studies were performed in triplicate and mean values were tabulated in tables 2, 3 and 4 [3, 10, 14].

Effect of Concentration of Extract on Growth Culture by Tube Dilution Technique

The media was prepared by dissolving the specified quantities of Mueller Hinton Agar (MHA) broth in purified water. The medium was distributed in 5ml quantities in test tubes. The test tubes were closed with cotton plugs and sterilized. The test solutions were prepared by dissolving 10mg of the ethanolic extract in 5 ml of ethanol that gave 2000 µg/ml concentration. From the above solution 5 ml was transferred to a test tube containing 5 ml of Mueller

Hinton broth and the resultant concentration was half of the previous one. From the above test tube 5 ml was taken and transferred to another test tube containing 5ml of Mueller Hinton broth. This was repeated up to six dilutions. 5 ml was discarded from the last test tube. The test tubes were closed with cotton plugs. Aseptic conditions were maintained throughout the process of sample transfer to the each of test tubes. Concentration of test compound in the test tubes was tabulated in Table 5 [3,9,16].

Following the above method one set of the test tubes were prepared and used to inoculate a different bacterial culture (10^7 cells/ml approximately All the test tubes were incubated at 37°C for 24 hours on shaking incubator to ensure the uniformity of mixing). A positive control and negative control were also maintained to confirm the nutritive and sterility properties of the prepared medium respectively. Presence or absence of growth of organisms was observed by noting the turbidity and activity of the extract against each organism was seen [3,9.16].

RESULTS AND DISCUSSION

The leaves of *Costus igneus* and other species of *Costus* have been extensively studied but however it was seen that no substantial work had been carried out on rhizomes of *Costus igneus* Methanol was used as a solvent in preparing the extract of *Costus igneus* as it is one of the best and most preferred solvent used in extraction of phytoconstituents. The methanolic extract of the rhizomes of *C. igneus* was subjected to antimicrobial studies. The results indicated that the methanolic extract showed broad spectrum antibacterial activity against both gram-positive- *Staphylococcus aureus* and *Bacillus subtilis* and gram-negative bacteria - *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium* which is indicated by the zone of inhibition (Tables 2 and 3). The results also indicated that the methanolic extract of rhizomes of *C. igneus* showed no antifungal activity against both *Claviceps purpurea Aspergillus niger* (Tables 2 and 4).

Effect of Increase in Concentration of Methanolic Extract of the Rhizomes of *C. Igneus* on the Bacterial Cultures (Fig.2 & 3)

It was observed that as the concentration of the methanolic extract of the rhizomes of *C. igneus* increased, there was significant inhibition seen in the growth of the culture as was evident from the drastic decline in absorbance values. Decline in the absorbance indicated the decrease in the number of bacteria thus resulting in a less turbid solution. The methanolic extract of *C. igneus* showed a significant decline in growth of *Bacillus subtilis* and was seen in case of and in the concentration range between 62.5 -1000 µg/ml (Tables 2 and 6). A sharp decline in of growth of *Staphylococcus aureus, Salmonella typhimurium,* and *Pseudomonas aeruginosa* with the methanolic extract, were as in case *Escherichia coli* the extract showed a gradual decline in the growth followed by sharp decline in the concentration range between 250-1000µg/ml. (Refer Tables 2 and 6).

In the present study has shown that the rhizome extract of *C. igneus* has a potent antibacterial property against majority of organisms tested. Due to a rapid increase in the rate of infections and antibiotic resistance in microorganisms medicinal plants are gaining popularity over these drugs. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

Table 1: List of test microorganisms procured form NCL- Pune

A) **BACTERIA**	**TYPE**	**NCIM No.**	**ATCC No.**
Staphylococcus aureus	Gram-positive bacteria.	2079	6538P
Bacillus subtilis	Gram-positive bacteria.	2063	6633
Escherichia coli	Gram-negative bacteria.	-	35218
Pseudomonas aeruginosa	Gram-negative bacteria.	2036	19429
Salmonella typhimrium	Gram- negative bacteria.	2501	23564
B) FUNGUS			
Aspergillus niger	Fungus.	616	10864
Claviceps purpurea	Fungus.	1046	-

Table 2 : Antibacterial/ Antifungal activity of methanolic extract of the rhizomes *Costus igneus*

Microorganism	**Anti-Microbial / Antifungal Activity**	**Percent inhibition of growth at concentration of 500 µg/ml (%)**
Staphylococcus aureus	+ve	29.41
Bacillus subtilis	+ve	57.14
Escherichia coli	+ ve	48.27
Pseudomonas aeruginosa	+ ve	30.43
Salmonella typhimrium	+ve	47.16
Aspergillus niger	- ve	-
Claviceps purpurea	-ve	-

Table 3: Antibacterial activity of methanolic extract of the rhizomes *Costus igneus* by disc diffusion method

Microorganism	**Zone of inhibition in mm**		
	***C. ingeus* rhizomes methanolic extract**	**streptomycin control**	**Ampicillin control**
Staphylococcus aureus	40	22	22
Bacillus subtilis	60	27	30
Escherichia coli	40	10	10
Pseudomonas aeruginosa	43	27	32
Salmonella typhimrium	46	26	10

Table 4: Antifungal activity of methanolic extract of the rhizomes *Costus igneus* by disc diffusion method

Microorganism	**Zone of inhibition in mm**	
	***C. ingeus* rhizomes methanolic extract**	**Sulphamethizole control**
Aspergillus niger	-	-
Claviceps purpurea	-	42

Table 5: Concentration of test compound in the test tubes

Dilution	1	2	3	4	5	6
Concentration µg/ml	1000	500	250	125	62.5	31.25

Table No 6: Percentage of inhibition in growth of cultures by methanolic extract of the rhizomes *Costus igneus* on bacterial cultures

Concentration µg/ml	Percentage of (%) Inhibition in Growth of Cultures				
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
250	17.64	42.85	12.06	21.74	39.62
500	29.41	57.14	48.27	30.43	47.16

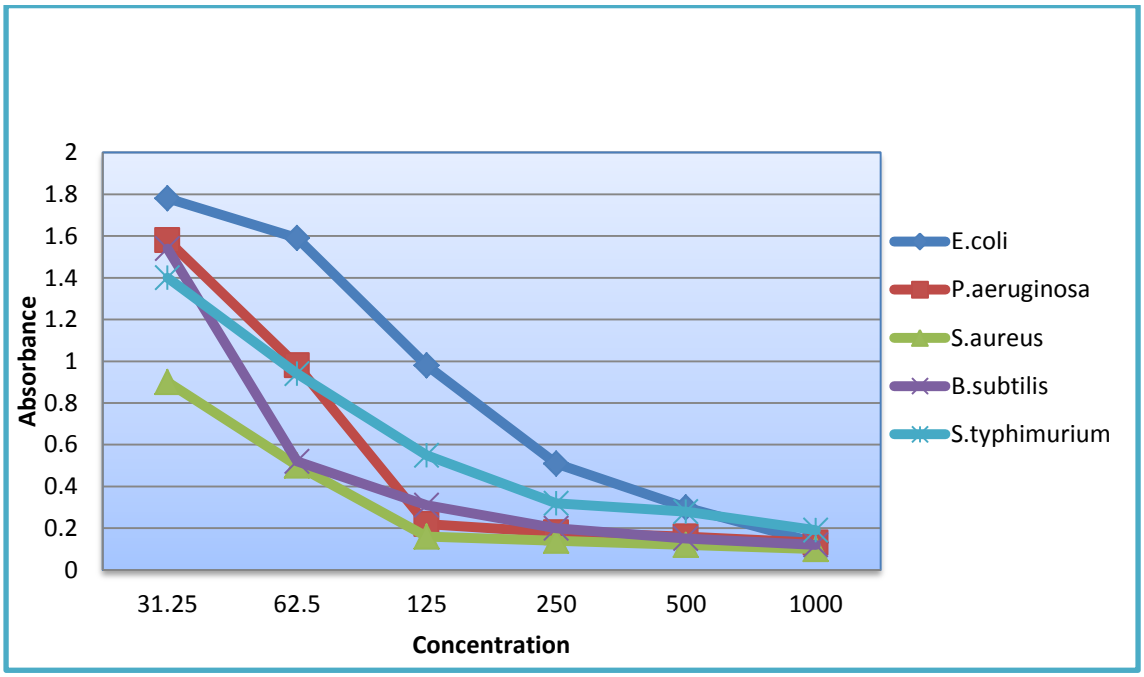

Fig. 2: Effect of concentration of methanolic extract of *C.igneus* on the growth of 5 different bacterial culrures

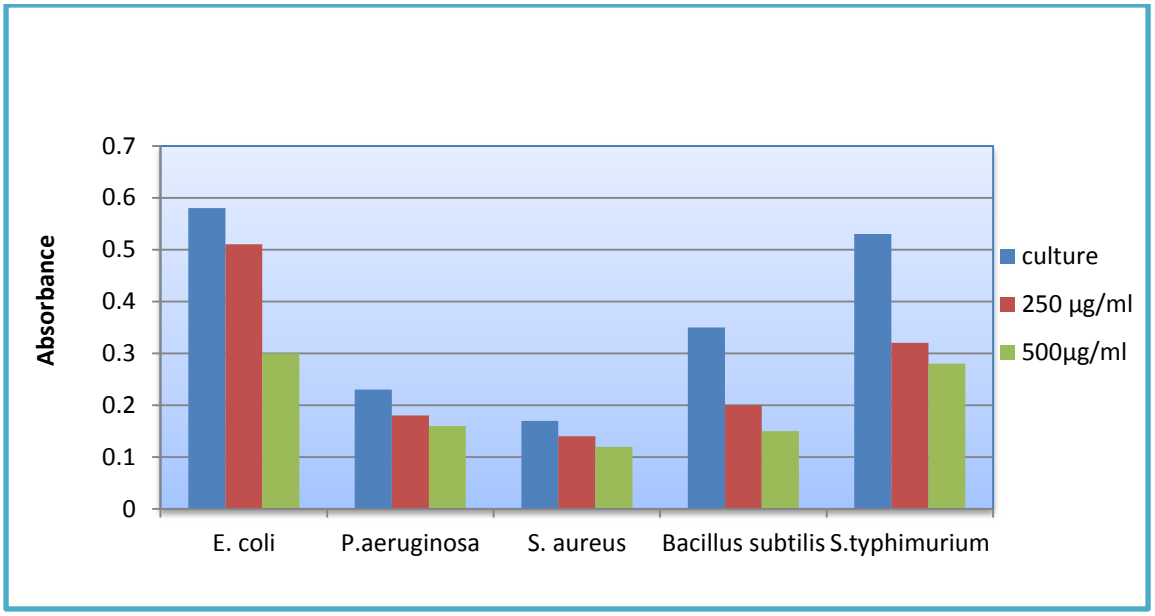

Fig.3: Comparitive effect of extract of *C. Igneus* on various bacterial cultures

The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful sources for new antimicrobial agents. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy.

The chemical investigation of methanolic extract of rhizomes of *C. igneus* led to the isolation of 4 components namely β- sitosterol; 4'-propenoxy 7-hydroxyl anthocyanidine and 6, 4' dihydroxy 3' propenchalcone; Dibutyl phthalate (DBP); Di (2-ethyl hexyl) phthalate (DEHP). Phthalates, flavonoids and others which possible contribute to the antimicrobial activity of extract [4]. 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. However, the present study of antimicrobial evaluation of *Costus igneus* forms a primary platform for further phytochemical and pharmacological studies.

CONCLUSION

The methanolic extract of the rhizomes of *C. igneus* was subjected to the antibacterial studies. The results indicate significant activity against both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium).* The antifungal study revealed no antifungal activity against both *Claviceps purpurea* and *Aspergillus niger.* It has been observed that as the concentration of the methanolic extract of the rhizomes of *C. igneus* increases, there is significant inhibition seen in the growth of the cultures. This is indicated by drastic decline in the absorbance values and confirmed by plate counts, thus exhibiting strong activity. The present extract exhibits promising antibacterial and no antifungal acitivity. The above activity has been reported for the first time from the methanolic extract rhizomes of *C. igneus*

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