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Antibacterial and Antifungal Activity of Leaf, Stem and Root Extracts 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 its anti-diabetic property. Hence an effort was made to investigate the antibacterial activity and antifungal activity of the petroleum ether and methanolic extracts of leaf, stem and root of *Costus igneus.* Six pathogens viz. *Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Proteus vulgaris* were selected to study the antibacterial activity. Seven pathogens viz. *Aspergillus niger, Macrophomina phaseolina, Rhizoctonia solani, Ustilaginoidea virens, Alternaria alternate, Fusarium oxysporum* and *Colletotrichum capsici* were selected to study the antifungal activity. Antibacterial and antifungal activity was evaluated by using paper disc diffusion method as well as agar well diffusion method. The methanolic extracts were found to have significant activity against both gram-positive and gram-negative bacteria. The root, stem and leaf methonolic extracts significantly inhibited the growth of all the test bacteria and fungi when compared to petroleum ether extracts. The inhibition zone ranged from 8-15mm in disc diffusion method and 11-33mm in agar well diffusion method for bacteria. In the case of fungal pathogens zone of inhibition ranges from 10-13mm in disc diffusion method and 11-33mm in agar well diffusion method. High inhibition zone was observed in leaf methonolic extracts in the case of fungi, while in bacteria stem methonolic extracts showed high inhibition zone. The present study reveals that *Costus igneus* can be used as a promising tool for antibacterial and antifungal activity. The above activity has been reported for the first time from the methanolic extract of root, stem and leaf of *Costus igneus*

Keywords: *Costus igneus*, Antibacterial activity, Anti fungal activity, Methanolic extracts, Petroleum ether extracts.

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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. 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.

Costus igneus N.E.Br is a perennial rhizomatous herb belonging to the family Costaceae, which is found in tropical Africa, Asia, Australia, and North, Central and South America. It is commonly referred to as Insulin plant, spiral flag and Pushkarmula in Sanskrit (2). Costus is one of the important medicinal plants with a source of antidiabetic compounds. Apart from antidiabetic nature, they also posses antibacterial activity (3) and antioxidant properties (4). The whole plant C. igneus is used not only for its anti-diabetic property but also for increasing the longevity of life. The rhizome has been used to treat fever, rash, asthma, bronchitis, intestinal worms, ailments of eyes, stomach, neck, jaws, tongue, mouth and also for curing edema, wheezing (dyspnoea), haemorrhoids, spermaturia. Until now, C. igneus has been reported to contain resinoids, essential oil, and alkaloid named saussurine, inulin and resin (5). 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 (6,7).

Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease has been discovered. 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 (8,9). The present effort has been made to investigate the antimicrobial activity of the methanolic and petroleum extract of root, shoot and leaves of C.igneus.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh and healthy leaves, roots and stem of the Costus igneus were collected from the Botanical Garden, Department of Botany, Osmania Univrsity, Telangana, India. They were washed thoroughly with running tap water followed by rinsing with distilled water and then the different parts were separated and cut into small pieces. They were shade dried at room temperature then pulverized into powder. Powdered samples were stored in an air tight container till further use.

Preparation of plant extracts

The shade dried and powdered leaf, stem and root materials were extracted with petroleum ether and methanol. About 50gm of the each sample was extracted with 500ml solvent by using soxhlet apparatus. Further, the solvent was evaporated using a rotary vacuum evaporator and used for antimicrobial activity. The extract was stored at 4ºC and used for antimicrobial activity.

Test Organisms

Gram positive, Gram negative bacteria and fungi were used as test organisms for this study. Gram positive bacteria such as Bacillus subtilis (ATCC6633), Staphylococcus aureus (ATCC 9144), gram negative bacteria Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC25619), Klebsiella pneumoniae (ATCC 13883), Proteus vulgaris (ATCC29905) and fungi viz. Aspergillus niger (ATCC10864), Macrophomina phaseolina (ATCC 52761), Rhizoctonia solani (ATCC76167), Ustilaginoidea virens (ATCC16180), Alternaria alternate (ATCC66981), Fusarium oxysporum (ATCC52429) and Colletotrichum capsici (ATCC96158) were used. All the bacterial and fungal strains were maintained on Nutrient agar and Potato dextrose agar respectively.

Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient and PDA agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to conical flasks of nutrient broth and PDA broth that were incubated for 24 hrs for bacteria and 7days for fungus cultures at 30°C on orbital shaker. The cultures were diluted with fresh broth to achieve optical densities to 2.0 x 10^6 colony forming units (CFU/ml).

Preparation of media

In vitro antimicrobial activity was screened by using Nutrient agar (NA) and Potato dextrose agar (PDA) obtained from Himedia. The required quantity of Agar media was prepared in a conical flask and sterilized by autoclaving at 121°C at 15lbs pressure for 15-20min. The sterilized NA and PDA plates were prepared by pouring 20ml of molten media and 1ml of above bacterial and fungal suspension after cyclomixing into sterile petri dishes. The plates were allowed to solidify for 15 minutes. For each bacterial and fungal strain, pure solvent (methanol, petroleum ether) was used as control.

Antimicrobial activity

Paper disc diffusion method

The Nutrient agar plates and Potato dextrose agar were prepared as mentioned above. The 1000µg/ml concentrations of extracts were loaded on sterile paper disc. The loaded disc was dried and 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 for bacteria and 72hrs for fungus. At the end of incubation period, inhibition zones formed around the disc were measured with transparent ruler in millimeter. Each extract was tested in triplicate to ensure the reliability of the result. Chloramphenicol (30g/disc) was used as the reference (positive control). A negative control was prepared with only the solvent used for extraction (10).

Agar well diffusion method

It is also known as plate hole diffusion method or cup diffusion method (11). Nutrient agar and Potato dextrose agar was used to culture the bacterial and fungal organism. The plates were inoculated with 24 h culture of respective fungi. With the help of a flamed cork borer 6 mm wells were cut and to each of the well 0.1 ml of the extract were aseptically added with the help of sterile syringe. The plates were incubated at room temperature. Inhibition zone was recorded by measuring the diameter of the zone after 72 h. Nystatin (300 g/well) was used as standard for comparison of antifungal activity (12).

RESULTS AND DISCUSSION

The potential for developing antimicrobials from higher plants appears rewarding as it leads 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 (13). Continued further exploration of plant derived antimicrobials is needed today. In the present study, the antibacterial and antifungal investigations have been done for Costus igneus N.E.Br. (root, stem, leaf) using different solvents like petroleum ether and methanol against Gram positive Bacillus subtilis, Staphylococcus aureus, and gram-negative bacteria- Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris and antifungal activity against Aspergillus niger, Macrophomina phaseolina, Rhizoctonia solani, Ustilaginoidea virens, Alternaria alternate, Fusarium oxysporum, Colletotrichum capsici. Plant extracts (leaf, stem, root) have shown potent antimicrobial activity against Gram positive, Gram negative bacteria and fungi indicating the presence of broad spectrum antimicrobial substance in the plants. The results revealed variability in inhibitory concentrations of each extract against bacteria and fungi. The earlier research reports indicated that only the rhizome have potential antimicrobial activity. But in the present study it was observed that along with the rhizome, stem and leaf methonolic extracts were also effective against all the microbes tested. The above activity has been reported for the first time from the

methanolic extract of root, stem and leaf of C. igneus. Similar type of antimicrobial properties of few medicinal plants was reported by Gothandam et al. (14).

Antibacterial activity

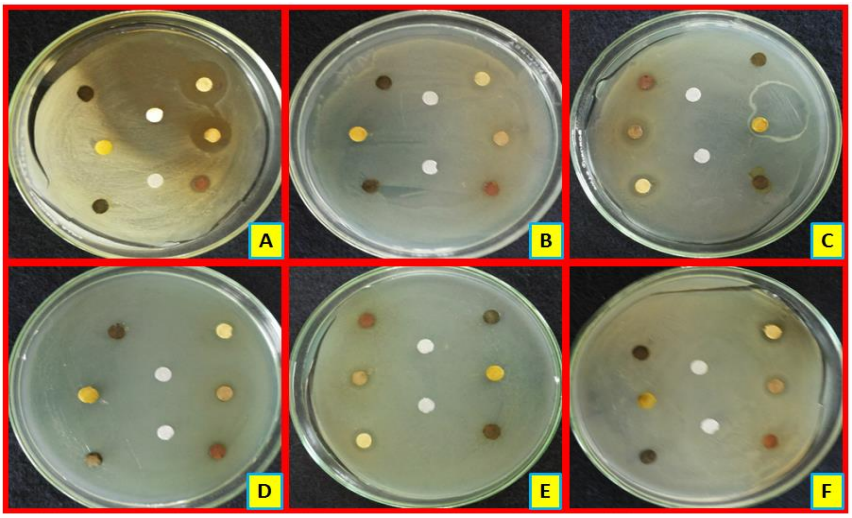
The antibacterial activity of C. igneus was carried out by both paper disc diffusion method as well as agar well diffusion method. The plant extracts were found to be inhibitory towards bacteria tested. Gram positive bacteria tested are found to be more sensitive compared to gram negative bacteria. The stem and root extracts have shown maximum inhibition zone in both methods (Figure.1). The inhibition zone ranged from 8 to15mm in disc diffusion method and 11-33mm in agar well diffusion method (Table 1, 2).

Disc diffusion method

As shown in the Figure. 1 high inhibition zone was observed in methonolic extracts of stem against B. subtilis (19mm) fallowed by E.coli (14), S. aureus (12mm), P.vulgaris (11mm), P.aeruginosa (10mm), K. pneumoniae (10mm) by disc diffusion method. Rhizome methonolic extracts also showed maximum inhibition zone against B. subtilis and E.coli (15mm), followed by P.aeruginosa (12mm), S. aureus (11mm), P. vulgaris (11mm) and K. pneumoniae (10mm) by disc diffusion method. Methonolic extracts of leaf showed least inhibition zones against bacteria i.e. it showed only 8mm zone against B. subtilis, E.coli. Remaining organisms failed to show zone of inhibition by disc diffusion method. None of petroleum ether extracts showed inhibition zones against bacteria by disc diffusion method (Table 1,2).

Agar well diffusion method.

High inhibition zone was observed in stem methonolic extracts against K. pneumoniae (33mm) fallowed by P. vulgaris (30mm), B. subtilis (25mm), P.aeruginosa (23mm), S. aureus (22mm), and E.coli (20mm) by agar well diffusion method. Methonolic extracts of root also showed high inhibition zones against bacteria i.e S. aureus (30mm), P.aeruginosa (30mm), K. pneumoniae (25 mm), E.coli (24), B. subtilis (22mm), P. vulgaris (21mm) by agar well diffusion method (Figure.1). Methonolic extracts of leaf also showed high inhibition zones against bacteria i.e. P.aeruginosa (28mm), fallowed by K. pneumoniae (22 mm), P. vulgaris (20mm), S. aureus (12mm), E.coli (12mm) and B. subtilis showed least (11mm) zone of inhibition (Table 1,2).



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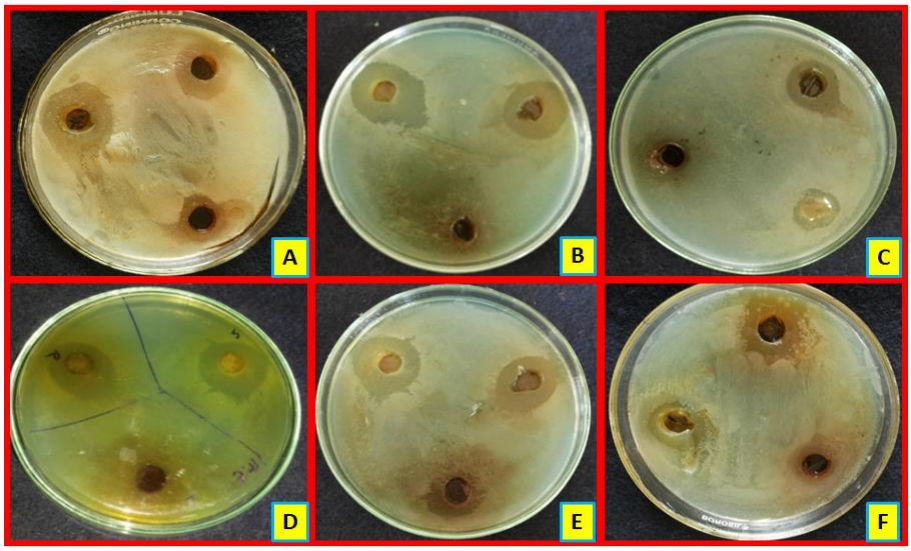


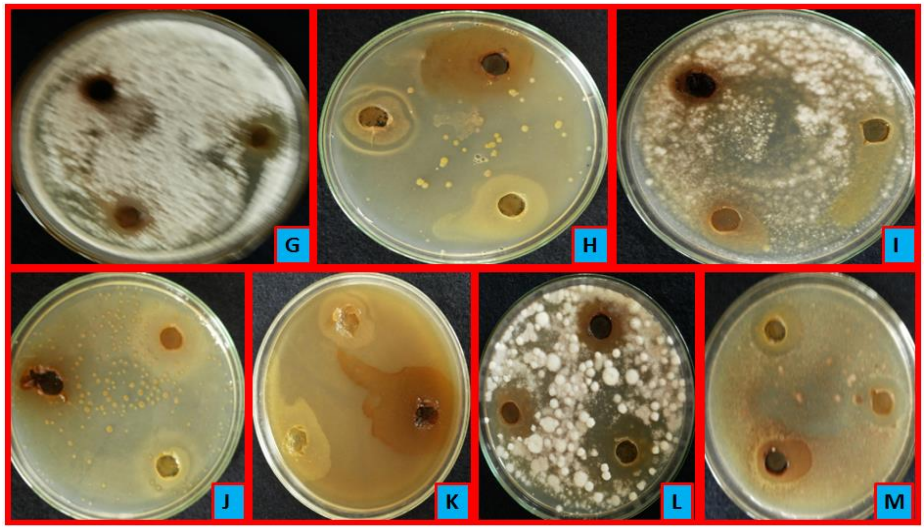
Figure 1: Antibacterial activity of C. igneus by Paper disc diffusion method and Agar well diffusion method. A- Bacillus subtilis, B-Staphylococcus aureus, C-Escherichia coli; D- Pseudomonas aeruginosa; E-Klebsiella pneumonia, F-Proteus vulgaris

Only S. aureus showed inhibition zone with petroleum ether extract of stem. Remaining organisms failed to show zone of inhibition by agar well diffusion method. Similar, work on antibacterial studies using root extract of C.igneus was reported by Arjun Nagaranjan et al. (15). Studies on antimicrobial activities of different medicinal plants has been reported worldwide by many workers (16-18).

Antifungal activity

Disc diffusion method

In the case of fungi, there was much difference between the inhibitory zone recorded in disc diffusion and agar well diffusion method (Table 1, 2). The stem and root extracts showed maximum inhibition zone in agar well method (Figure.2). The zone of inhibition ranged from 10 to 13mm in disc diffusion method. Methonolic extracts of stem and root samples were showed least inhibition zone against M. phaseolina (12, 10mm) and U. virens (13, 10mm) by disc diffusion method. Some of the extracts were ineffective in this study, probably they do not possess antimicrobial properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol, Petroleum ether or the drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants (19, 20).



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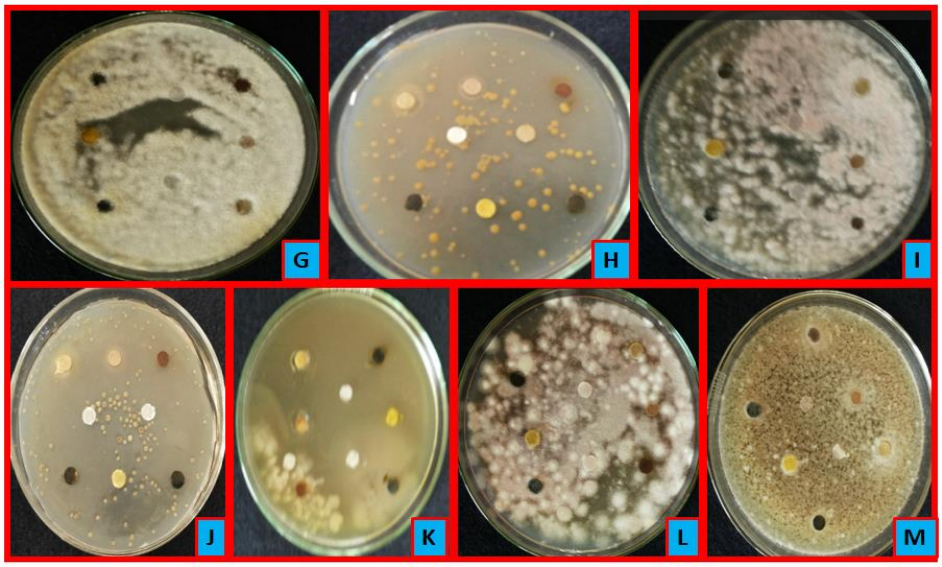

Figure 2: Antifungal activity of C. igneus by Paper disc diffusion method and Agar well diffusion method. G-Aspergillus flavus, H-Macrophomina phaseolina, I-Rhizoctonia solani, J-Ustilaginoidea virens, K- Alternaria alternate, L-Fusarium oxysporum, M-Colletotrichum capsici.

Agar well diffusion method

The inhibition zone ranged from 11to33mm in agar well diffusion method (Table 1, 2). High inhibition zone was observed in leaf methonolic extracts against A. alternate (33mm), followed by M. phaseolina (30mm), F. oxysporum (24mm), C. capsici (23mm), R. solani (15mm), U. virens (15mm), A. flavus (11mm). High inhibition zone was also observed in stem and root methonolic extracts against A. alternate (32,23mm), followed by M. phaseolina (30, 25mm), F. oxysporum (25,22mm), C. capsici (23,20mm), R. solani (20,22mm), U. virens (29,26mm), A. flavus (20,13mm) by agar well diffusion method. The leaf extracts were found to be more inhibitory towards fungi than bacteria (Figure. 1). Similar type of studies was done by Malleswari et al. (21) and Kala (22) with Coleus forskohlii by using different solvents.

Table 1: Antibacterial activity of Costus igneus by Paper disc diffusion and Agar well method

Solvents	Part	B.s	S.a	E.c	P.a	K.p	P.v
		Paper disc diffusion method-Inhibition zone (in mm)					
Methonol	Leaf	8	-	8	-	7	-
	Stem	19	12	14	10	10	11
	Root	15	11	15	12	10	11
Petrolium ether	Leaf	-	-	-	-	-	-
	Stem	-	-	-	-	-	-
	Root	-	-	-	-	-	-
Solvents	Part	B.s	S.a	E.c	P.a	K.p	P.v
		Agar well diffusion method -Inhibition zone (in mm)					
Methonol	Leaf	11	12	12	28	22	20
	Stem	25	22	20	23	33	30
	Root	22	30	24	30	25	21
Petrolium Ether	Leaf	-	-	-	-	-	-
	Stem	-	23	-	-	-	-
	Root	-	-	-	-	-	-

B.s- Bacillus subtilis, S.a-Staphylococcus aureus, E.c-Escherichia coli; P.a- Pseudomonas aeruginosa; K.p, Klebsiella pneumonia, P.v-Proteus vulgaris

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Table 2: Antifungal activity of Costus igneus by Paper disc diffusion and Agar well method

Solvents	Part	A.n	M.p	R.s	U.v	A.a	F.o	C.c
		Paper disc diffusion method-Inhibition zone (in mm)						
Methonol	Leaf	-	-	-	-	-	-	-
	Stem	-	12	-	13	-	-	-
	Root	-	10	-	10	-	-	-
Petrolium Ether	Leaf	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-
Solvents	Part	A.f	M.p	R.s	U.v	A.a	F.o	C.c
		Agar well diffusion method -Inhibition zone (in mm)						
Methonol	Leaf	11	30	15	15	33	24	23
	Stem	20	30	20	29	32	25	23
	Root	13	25	22	26	23	22	20
Petrolium Ether	Leaf	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-
	Root	-	-	-	-	-	-	-

A.n-Aspergillus flavus, M.p-Macrophomina phaseolina, R.s-Rhizoctonia solani, U.v-Ustilaginoidea virens, A.a-Alternaria alternate, F.o-Fusarium oxysporum, C.c-Colletotrichum capsici.

There was not much difference observed between the inhibitory zones of bacteria and fungi recorded in disc diffusion and agar well diffusion method. The present study has shown that the rhizome, stem and leaf methonolic extracts of Costus igneus has potent antibacterial and antifungal properties. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains (23). Hence, there is possibility of developing this plant as a source of herbal antibiotic and further studies are needed for isolation and purification of bioactive constituent.

CONCLUSION

Costus igneus contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. The methanolic extracts (leaf, stem and root) of Costus igneus were used to study antibacterial and antifungal activities. The root, stem and leaf methonolic extracts strongly inhibited the growth of all the test bacteria and fungi. High inhibition zone was observed in leaf methonolic extracts in the case of fungi, while in bacteria stem and root methonolic extracts showed high inhibitory zones. The above activity has been reported for the first time from the methanolic extract of stem and leaf of C. igneus. These results open the possibility of finding new clinically effective antioxidant drugs and could be useful in understanding the relationship between traditional cures and modern medicines. Further research is necessary to identity the antimicrobial compounds and also to determine their full spectrum of efficacy. This study encourages the cultivation of this highly valuable medicinal plant to meet the increasing demand from traditional medicinal system.

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