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Evaluation of Anti-microbial activity of methanolic extract of *Costus igneus* plant against multidrug-resistant pathogenic microorganisms

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ABSTRACT

Costus igneus plants are highly active against the majority of Gram-negative and Gram-positive microorganisms. The purpose of the current study was intended to evaluate the anti-microbial potential of methanolic extract of *Costus igneus* over multidrug-resistant bacteria, specifically to methicillin, vancomycin, carbapenems, colistin. The study also focused on the antifungal activity of the plant extract against Candida species. Phytochemical analysis was conducted to identify the presence of the active chemicals such as steroids, alkaloid, flavonoids, polyphenols, terpenoids, saponin, tannin, glycosides, quinones, coumarins and phenolic compounds using standard protocols. Anti-microbial activity of *C. igneus* was assessed through agar well diffusion technique and Minimum inhibitory concentration method (MIC) by using multidrug-resistant Gram-positive microorganisms (*Staphylococcus aureus, Enterococcus faecalis*) and multidrug-resistant Gram-negative microorganisms (*E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis, Citrobacter species*) and also *Candida albicans*. At 120mg/ml *C.igneus* plant extract concentration, maximum zone of inhibition was obtained with all the nine tested microorganisms and however the zone of inhibition was slighter with regular standard potential antibiotics like colistin, imipenem etc. Anti-microbials of plant origin possesses tremendous therapeutic potential as they can accomplish the requirements with fewer side-effects that are routinely associated with synthetic anti-microbials. In this investigation, it was established that *C. igneus* leaf extract possesses excellent anti-microbial activity which can be attributed to the occurrence of phytochemicals.

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INTRODUCTION

Medicinal plants are used traditionally for their therapeutical value from the ancient human civilization. Medicinal plants are accepted and supported all over the world when used for curing fundamental health issues. The world health organization has reported that the usage of active constituents in the plants as medicines for treating various health issues is exponentially growing. *Costus igneus* is a herbaceous plant. Alternative names include insulin plant or spiral flag or fiery *Costus*. It belongs to the

Costaceae family (Rao *et al.*, 2014). *Costus igneus* is commonly seen in Asia, Australia, Tropical Africa, North and South America, and chiefly on the Islands of Indonesia. They are widely cultivated and used in Southern India, to control diabetes due to its anti-diabetic property. It is also regarded as an ornate plant in some parts of the world (Kalailingam *et al.*, 2011; Khan *et al.*, 2014).Existence of many secondary metabolites, namely diosgenin, terpenoids, beta-carotene, alkaloids, quercetin, steroids, phenols and flavonoids are reported in different locations of the plant (Krishnan *et al.*, 2011). These can be attributed for anti-microbial activities of the plant, and anti-oxidant potentialis credited to flavonoids, β-carotene, ascorbic acid, terpenoids and α-Tocopherol. All these phytochemicals have been notified to treat numerous significant diseases like diabetes (Saraswathi *et al.*, 2010). There have been reports on many pharmacological activities of various extracts of *C. igneus*, which include antibacterial, antifungal, anti-oxidant, hypolipidemic, hepatoprotective, anti-inflammatory, antiproliferative, anti-diabetic,and many more. Many earlier investigations have demonstrated excellent anti-microbial activity in the methanolic, and ethanolic extracts of *Costus igneus* leaves. According to the established literature, the secondary metabolites of *Costus igneus* plant are highly active against both Gram-positive and Gram-negative microorganisms (Rao *et al.*, 2016; Vasantharaj *et al.*, 2013; Shaik *et al.*, 1994).

Many bacterial species Viz, *Enterococcus* species and *Streptococcus* species etc. are concerned in the pathologic process of the skin, gastrointestinal, respiratory, urogenital diseases and exhibited resistance to virtually all traditional antibiotics. Clinical isolates of *Staphylococcus aureus* are the primary cause of nosocomial infections, as they have developed increased resistance to an array of routinely used antibiotics. Among many, organisms of grave concern are Vancomycin-resistant enterococci (VRE) and Methicillin-resistant *Staphylococcus aureus* (MRSA). HA-MRSA strains are more often multidrug-resistant. The standard treatment for severe MRSA infections include administration of vancomycin, however, vancomycin-resistant *S. aureus* (VRSA) have been recently notified. Expression of the mecA gene encoding low-affinity penicillin-binding protein PBP_2 is in charge of resistance to β-lactam drugs and their derivatives (Fauci *et al.*, 2012). Gram-negative bacilli (GNB) are well known contributory agents of both and hospital and community-associated infections leading to high morbidity and mortality rates. It can be ascribed to their ability to acquire and disseminate resistant genes, and this has been identified to deteriorate the antibiotic treatment. Beta-lactam class of antibiotics, aminoglycosides, peptide derivatives etc. generally prescribed for treating GNB infections. Conversely, for resistant strains, polymyxins and colistin are used rarely where the patient is not acknowledging to primary or secondary line drugs. Nevertheless, very few studies have communicated the prevalence of colistin and polymyxin resistant strains. Multidrug-resistant Enterobacteriaceae producing broad-spectrum Beta-lactamases (ESBLs) and acquired AmpC-type cephalosporinases, carbapenemases have been already popular worldwide as nosocomial pathogens (Pitout, 2010; Okoche *et al.*, 2015; Kontopoulou *et al.*, 2010). Nevertheless, susceptibility testing should be performed on all clinical isolates to help determine the optimal selection of anti-microbial therapy. Over recent years, there has been a drastic increase in the frequency of treatment failures in candidiasis, chiefly in immune-compromised patients receiving long term antifungal therapy with azole compounds, which has posed a severe problem in its successful use in chemotherapy.

The current investigation was designed to evaluate the anti-microbial potential of methanolic extract of *Costus igneus* against multidrug-resistant bacteria, specifically to methicillin, vancomycin, carbapenems, colistin. The study also focusses on the antifungal activity of the plant extract against Candida species.

MATERIALS AND METHODS

Collection of plant material and preparation of leaf extract

Leaves of *Costus igneus* plant were identified and gathered from Ambasamudram, Tirunelveli, Tamil Nadu, India. Fresh leaves collected were gently cleaned and dried under shade. The dried leaves were prepared into powder using a mechanical pulverizer. The coarsely powdered samples were extracted with methanol using a Soxhlet apparatus.

Phytochemical analysis

Phytochemical testing is a preliminary screening test to identify the secondary metabolites. Phytochemical analysis was done to determine the existence of principal active chemical components, namely alkaloid, flavonoids, polyphenols, terpenoids, saponin, tannin, glycosides, steroids, quinones, coumarins and phenolic compounds.

Table 1: Comparison of Antibiotic Susceptibility of *Costus igneus* leaf extract with various standard antibiotics of tested microorganisms

Microorganisms	Antibiotics	Zone of inhibition with antibiotics in mm (Resistant or sensitive to antibiotics)	Zone of inhibition with *C. igneus* in mm				
			Concentration of plant extract (mg/ml)				
			10	30	40	50	120
E. coli	Ertapenem	12 mm (Resistant)	12mm	14	14	14	16mm
	Piperacillin/ Tazobactam	11 (Resistant)					
	Cotrimaxazole	No zone (Resistant)					
	Ampicillin	No zone (Resistant)					
	Ceftazidime	No zone (Resistant)					
	Amoxy-clav	No zone (Resistant)					
	Imipenem	No zone (Resistant)					
	Colistin	14 (Sensitive)					
Pseudomonas aeruginosa	Amikacin	**19** (Sensitive)	15	15	15	15	17
	Piperacillin/ Tazobactam	9 (Resistant)					
	Ceftazidime	No zone (Resistant)					
	Cefepime	No zone (Resistant)					
	Colistin	**15** (Sensitive)					
	Amoxycillin/ clavulanic acid	8 (Resistant)					
	Imipenem	**24** (Sensitive)					
Klebsiella pneumonia	Ampicillin	No zone (Resistant)	14	14	14	14	16
	Ertapenem	No zone (Resistant)					
	Cotrimaxazole	No zone (Resistant)					
	Ceftazidime	No zone (Resistant)					
	Piperacillin	No zone (Resistant)					
	Tazobactam	No zone (Resistant)					
	Vancomycin	No zone (Resistant)					
	Colistin	15 (Sensitive)					
Staphylococcus aureus	Penicillin	No zone (Resistant)	14	14	15	16	18
	Cefoxitin	No zone (Resistant)					
	Erythromycin	No zone (Resistant)					
	Teicoplanin	6 (Resistant)					
	Chloromphenicol	No zone (Resistant)					
	Imipenem	No zone (Resistant)					
	Ampicillin/ sulbactum	No zone (Resistant)					
	Vancomycin	6 (Resistant)					
	Cotrimaxazole	5 (Resistant)					

Continued on next page

Table 1 continued

Microorganisms	Antibiotics	Zone of inhibition with antibiotics in mm (Resistant or sensitive to antibiotics)	Zone of inhibition with *C. igneus* in mm				
			Concentration of plant extract (mg/ml)				
			10	30	40	50	120
Salmonella typhi	Ampicillin	No zone (Resistant)	11	11	11	14	15
	Ceftriazone	No zone (Resistant)					
	Nalidixic acid	No zone (Resistant)					
	Teracycline	No zone (Resistant)					
	Azithromycin	No zone (Resistant)					
	Imipenem	13 (Resistant)					
	Chloramphenicol	11 (Resistant)					
	Colistin	14 (Sensitive)					
Enterococcus faecalis	Tetracycline	10 (Resistant)	15	15	15	15	16
	Ampicillin	No zone (Resistant)					
	Amoxy-clav	No zone (Resistant)					
	Vancomycin	No zone (Resistant)					
	Teicoplanin	No zone (Resistant)					
	Chloromphenicol	No zone (Resistant)					
	Linezolid	No zone (Resistant)					
Proteus mirabilis	Colistin	18 (Sensitive)	7	8	9	12	13
	Imipenem	12 (Resistant)					
	Amoxycillin/ Clavulanic acid	12 (Resistant)					
	Cotrimaxazole	9 (Resistant)					
	Ampicillin	No zone (Resistant)					
	Cefuroxime	No zone (Resistant)					
	Ciprofloxacin	No zone (Resistant)					
Citrobacter species	Colistin	17 (Sensitive)	16	17	17	18	18
	Imipenem	15 (Resistant)					
	Amoxycillin/ Clavulanic acid	No zone (Resistant)					
	Cotrimaxazole	No zone (Resistant)					
	Ampicillin	No zone (Resistant)					
	Cefuroxime	No zone (Resistant)					
	Ciprofloxacin	No zone (Resistant)					
Candida albi-cans	Fluconazole	10 (Resistant)	10	12	13	15	16

In this present study, several phytochemical constituents were evaluated qualitatively using standard protocols (Adamu *et al.*, 2018; Wakawa *et al.*, 2018; Shukla *et al.*, 2015).

(Achi *et al.*, 2017) To test the alkaloid present in the leaf extract, Mayer's test was performed in which a drop of Mayer's reagent was added to the sample and formation of creamy white precipitate notifies the test as positive. The presence of flavonoids in the leaf extract was determined by adding a pinch of Magnesium, 0.5ml of alcohol and a few drops of a concentrated HCl to the sample and the manifestation of red colour reveals the presence of flavonoids. The presence of polyphenols can be estimated qualitatively by Wiefferering test and development of reddish-violet precipitate confirms the presence of polyphenols. Terpenoids identification can be made by dissolving the sample with 2ml of chloroform and 2ml of H_2SO_4, and the formation of reddish-brown colour reveals the presence of terpenoids.

For testing the saponins, frothing test was used. The leaf extract sample was shaken with 10 ml of distilled water in a test tube. The froth formation and its persistence in a water bath for 5minutes, convey the presence of saponins. The ferric chloride test performed detection of tannins in the leaf extract. For identifying glycosides in leaf extract, it was initially dissolved with glacial acetic acid, to which few drops of concentrated sulphuric acid and ferric chloride were added, and development of reddish-brown colour at the junction of two layers and a bluish-green colour in the top layer communicates the presence of Glycosides. Steroids in leaf extract are identified by dissolving 0.5ml methanolic extract in 2ml of acetic anhydride, and 2ml sulphuric acid was added.

The change of colour from violet to blue or green indicates the presence of steroids. For quinones detection, 1ml of leaf extract was mixed with 1ml of concentrated H_2SO_4 and red colour formation intimate the presence of quinones. For coumarins identification, 1ml of the sample was mixed with 1ml of 10% NaOH and the generation of intense red colour confirms the presence of coumarins. Phenolic compounds identification in a leaf extract was made with Ferric chloride test.

Evaluation of Anti-microbial activity of *Costus igneus*

In the present study, the anti-microbial activity of *C. igneus* was conducted through agar well diffusion technique and Minimum inhibitory concentration method (MIC) by using multidrug-resistant Gram-positive and Gram-negative microorganisms and also Candida albicans. The study was performed during the period Jan to April 2019 in the Central research laboratory, GITAM Institute of Medical Sciences and Research, Visakhapatnam. The cultures were isolated from various OP and IP patient samples like blood, urine, CSF, endotracheal aspirates, pus, skin scrapings, throat swabs etc.The specimens were processed and examined in the Department of Microbiology using various standard microbiology protocols. The samples were tested to notice, isolate and identify the pathogens using microscopy, Gram staining, and by culturing on blood agar, Mac Conkey agar, CLED agar etc. and by performing biochemical tests (Amjad *et al.*, 2011).

Antibiotic susceptibility testing (AST)

Antibiotic susceptibility testing was performed according to CLSI guidelines using Mueller-Hinton agar (MHA) plates using antibiotics concentration approved by the WHO experts committee on biological standardization. The inoculated plates were incubated at 37°C for 16-18 hrs. The cultures were tested with known antibiotic discs of standard concentration purchased from Himedia Laboratories Pvt. Ltd., Mumbai. The inhibition zone was calculated according to CLSI guidelines (CLSI, 2016) (Collee *et al.*, 1996).

Methanolic extract of *Costus igneus* leaf was taken in the concentration of 10mg/ml, 30mg/ml, 40mg/ml, 50mg/ml and 120mg/ml. The test organisms (*Proteus mirabilis, Klebsiella pneumoniae, E.coli, Pseudomonas aeruginosa, Citrobacter species, Staphylococcus aureus, Salmonella typhi and Enterococcus faecalis*) were standardized with 0.5 McFarland standard, and the test organisms were seeded on sterile MHA plates. *Candida albicans* was seeded on sterile MHA containing 0.5 μl/ml methylene blue and 2% glucose. Wells were cut using sterile borer, and different concentration of *Costus igneus* leaf extract was added to the wells. Further, the plates were incubated at 37°C for 16-18 hrs. The obtained zone of inhibition was calculated and reported in millimetres.

In the Modified Hodge test, the ability of the microbial isolates to produce carbapenemase was tested and permits the growth of standard strain *E.coli* ATCC 25922 towards a carbapenem disc. The test results were noted based on the examination of clover leaf pattern indentation. The procedure was performed according to the protocol of Amjad *et al.* (2011).

For recognition of colistin-resistant organisms, the E-test method was employed. A colistin E-strip (concentration ranging from 0.06 to 1,024 μg/ml) was applied to each MHA plate and incubated at 35°C for 16-20h. The observations were recorded as MIC

Table 2: Antimicrobial activity with Minimum Inhibitory Concentration method

Microorganisms	MIC concentration of test compound (mg/ml)	Antimicrobial susceptibility (S/R)
S. typhi	10	Sensitive (S)
	30	Sensitive
	40	Sensitive
	50	Sensitive
	120	Sensitive
S. aureus	10	Resistant (R)
	30	Resistant
	40	Resistant
	50	Resistant
	120	Sensitive
E.coli	10	Resistant
	30	Resistant
	40	Resistant
	50	Resistant
	120	Sensitive
P. aeruginosa	10	Resistant
	30	Resistant
	40	Sensitive
	50	Sensitive
	120	Sensitive
E. faecalis	10	Resistant
	30	Sensitive
	40	Sensitive
	50	Sensitive
	120	Sensitive
K. pneumonia	10	Resistant
	30	Sensitive
	40	Sensitive
	50	Sensitive
	120	Sensitive
Proteus mirabilis	10	Sensitive
	30	Sensitive
	40	Sensitive
	50	Sensitive
	120	Sensitive
Cirobacter species	10	Sensitive
	30	Sensitive
	40	Sensitive
	50	Sensitive
	120	Sensitive
Candida albicans	10	Sensitive
	30	Sensitive
	40	Sensitive
	50	Sensitive
	120	Sensitive

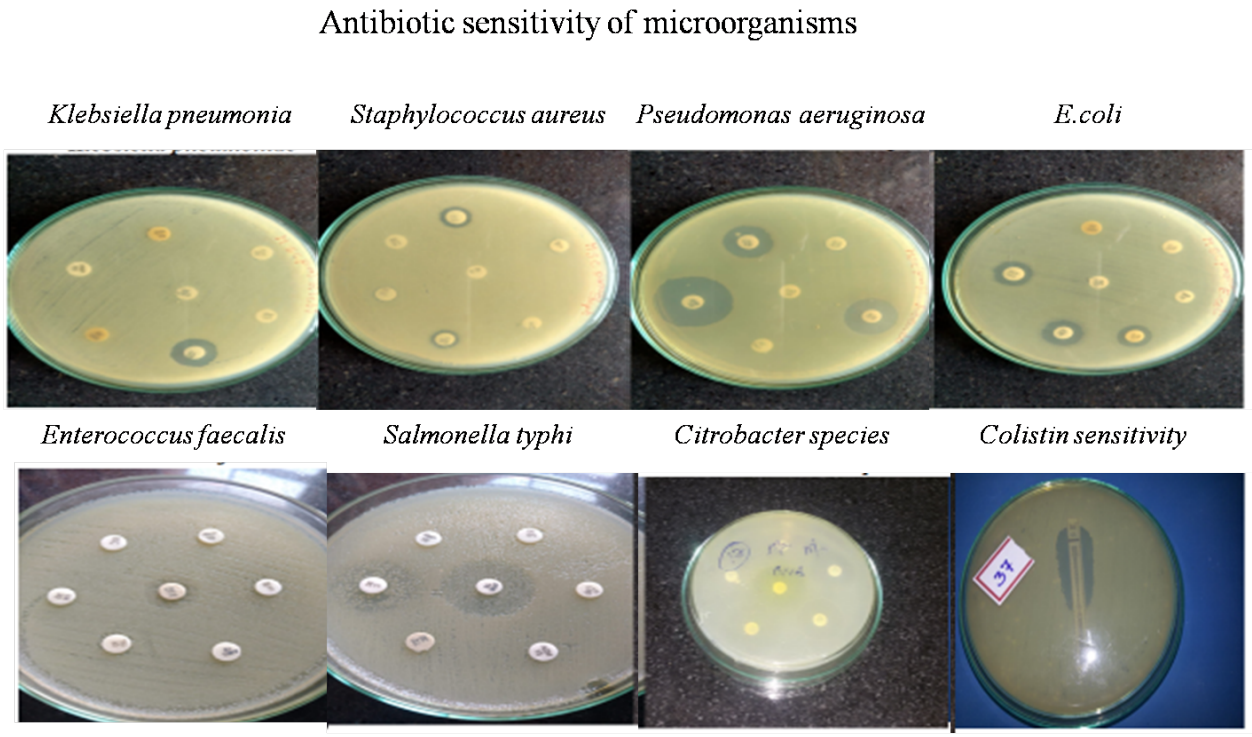

Figure 1: Antibiotic susceptibility pattern of different tested microorganisms

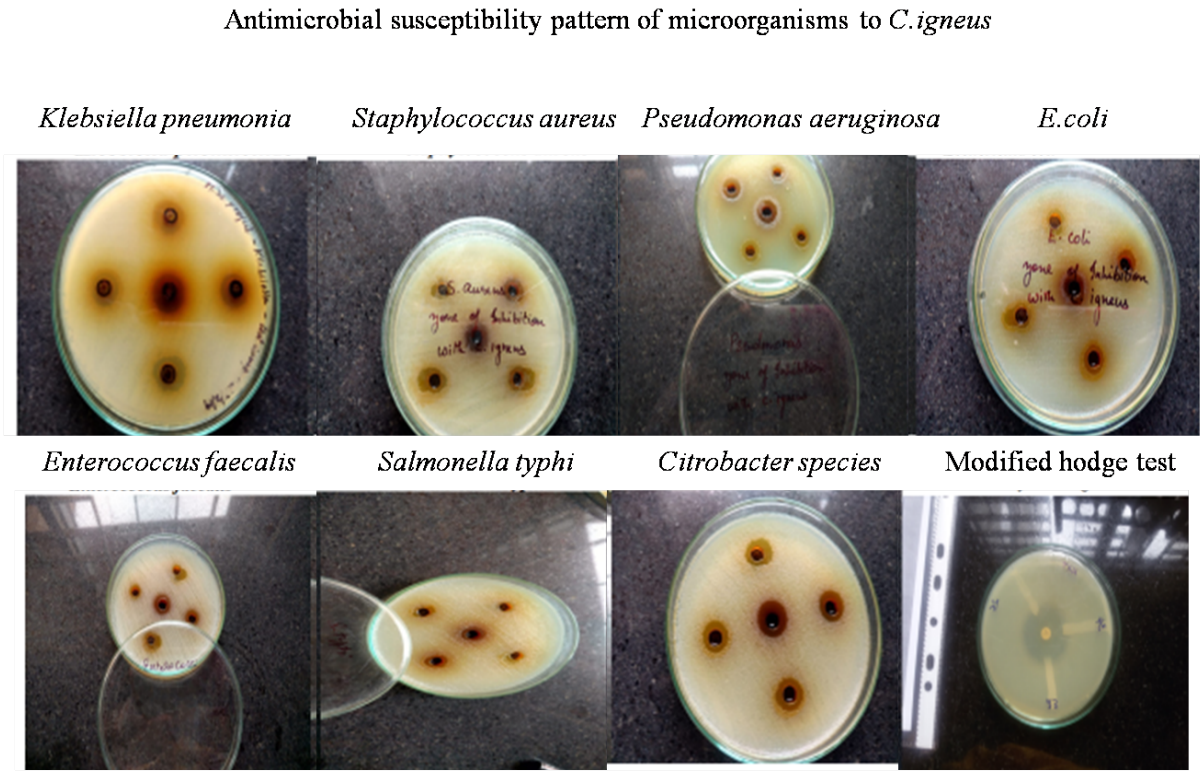

Figure 2: Antimicrobial susceptibility pattern of different test microorganisms to *C.igneus* leaf extract

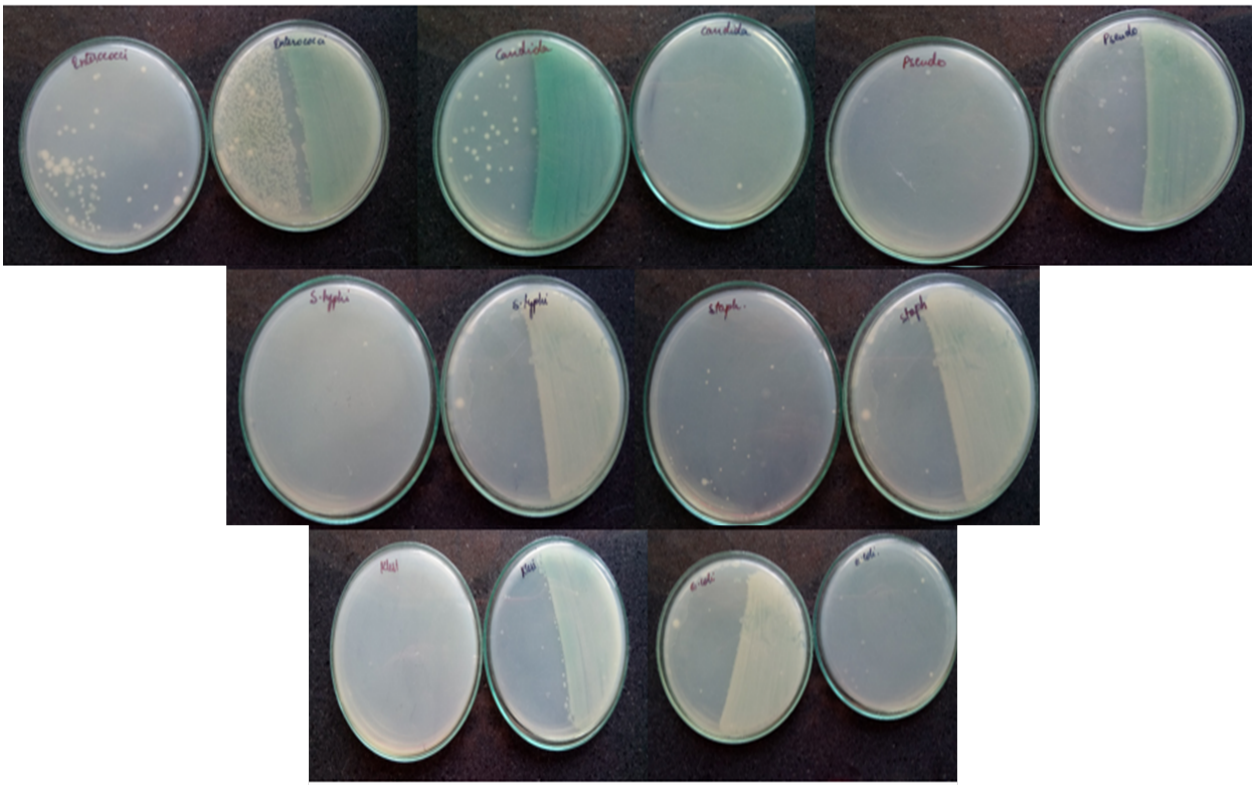

Figure 3: Minimum Inhibitory concentration of different tested microorganisms to *C.igneus* leaf extract

where inhibition of growth intersected the E-strip. A $\geq$4 μg/ml colistin concentration was used as the breakpoint to select as resistant isolates (Gales *et al.*, 1997).

Minimum Inhibitory Concentration (MIC)

Broth dilution method was conducted for determining Minimum inhibitory concentration. The sample was prepared in the concentration of 10mg/ml, 30mg/ml, 40mg/ml, 50mg/ml and 120mg/ml. The sterile nutrient broth was prepared. The inoculum was prepared with 10^5 CFU/ml. The prepared inoculum was diluted in the ratio 1:100 (prepared inoculum: broth medium). In the clean, dry test tube 1ml of different concentration of the sample was added, followed by 1 ml of test organism inoculum. Broth and test organism without sample served as control. The content was mixed well and incubated at 37° C for 16 – 18 hours. After incubation, the content in the tube was seeded on sterile nutrient agar plates and incubated at 37° C for 16 – 18 hours. The obtained colonies were reported as CFU.

RESULTS

Costus igneus leaf extract was subjected to preliminary phytochemical analysis, and the results revealed the existence of phytochemicals like flavonoids, steroids, alkaloids, terpenoids, tannins, quinones, polyphenols, phenols, saponins, glycosides and coumarins. This indicates that *C.igneus* leaf extract has a potential anti-microbial activity.

Methanolic extract of *C. igneus* leaf was tested to evaluate its anti-microbial activity against medically necessary isolated bacteria including two strains of Gram-positive bacteria (*Staphylococcus aureus and Enterococcus faecalis*) and six strains of Gram-negative bacteria (*E. coli, S. typhi, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Citrobacter* species) and one yeast spp. (*Candida albicans*) using agar well diffusion method, Modified Hodge test and colistin 'E' strip method. Evaluation of the anti-microbial activity of the plant extract in different concentrations and with standard antibiotics at particular specified levels according to CLSI guidelines were illustrated in Table 1 and Figure 1 and Figure 2. The results of anti-microbial susceptibility had revealed that all the nine tested pathogenic microorganisms are multidrug-resistant isolates to different classes of antibiotics like penicillin and other beta-lactam drugs, cephalosporins, aminoglycosides, monobactams etc. Modified Hodge test had determined that all the tested. Gram-negative bacteria except *Pseudomonas aeruginosa* are carbapenemase-producing microorganisms capable of resistant to carbapenem families of antibiotics like imipenem, meropenem

and ertapenem. However, all six tested. Gram-negative bacteria are sensitive to colistin because the MIC was <4μg/ml. Colistin is generally referred to as the last line of drug of choice for the multidrug-resistant organism because of its nephrotoxicity. The results reported that the plant extract was potentially functional in suppressing the growth of pathogenic bacteria and fungi with variable potency.

Table 1 illustrated that the organism *E. coli* showed maximum anti-microbial susceptibility to standard antibiotic colistin (14 mm), whereas *C. igneus* showed maximum susceptibility at a higher concentration 120 mg/ml with a zone of inhibition 16mmwhich was higher than the dominant standard drug-like colistin. Pseudomonas aeruginosa showed maximum zone of inhibition to antibiotics like amikacin (19mm), imipenem (24 mm) and colistin (15mm), whereas *C. igneus* showed maximum susceptibility at a higher concentration 120 mg/ml, i.e. 17mm zone of inhibition which was higher than colistin but lesser to other antibiotics like amikacin and imipenem. *Klebsiella pneumoniae* isolated from the sputum sample showed maximum zone of inhibition towards *C. igneus* leaf extract at the concentration of 120 mg/ml (16 mm) when compared to standard drug colistin (15 mm). This indicates that *C.igneus* was more effective than colistin antibiotic. *Staphylococcus aureus* showed resistant to all the tested antibiotics and a multidrug-resistant isolate. However, it showed maximum anti-microbial susceptibility to *C. igneus* leaf extract at the concentration of 120 mg/ml (18 mm). *S. typhi* showed maximum zone of inhibition towards colistin (14mm), but the zone size was lesser slightly when compared to *C. igneus* leaf extract (15mm) at the concentration of 120mg/ml. *Enterococcus faecalis* isolated in the present study was a multidrug-resistant organism and resistant to all tested antibiotics. Still, it showed good anti-microbial activity with *C.igneus*, i.e. 16mm zone of inhibition at 120 mg/ml. *Proteus mirabilis* showed less activity to *C.igneus* extract 13mm zone at 120 mg/ml plant extract concentration than colistin showed a maximum area of inhibition 18mm. *Citrobacter* species showed a maximum zone of inhibition towards colistin (17mm), but the zone size was lesser slightly when compared to *C. igneus* leaf extract (18mm) at the concentration of 120mg/ml. *Candida albicans* showed maximum inhibition zone against *C. igneus* at the concentration of 120 mg/ml (16 mm) when compared to fluconazole (10 mm) as the standard testing drug.

By testing the anti-microbial activity of methanolic leaf extract of *C.igneus* through MIC method (Table 2 and Figure 3), the results revealed that 10mg/ml was the MIC concentration for *S.typhi* where the complete growth of the organism was inhibited. The MIC concentration for the *S.aureus* and *E.coli* was120mg/ml, where the maximum increase was inhibited. The MIC concentration for *P. aeruginosa* was 40 mg/ml. For *E. faecalis* and *K. pneumoniae*, the MIC was 30mg/ml, where there was no growth identified. *Proteusmirabilis* and *Citrobacter* species showed a MIC value of 10mg/ml. The yeast *Candida albicans* showed MIC concentration of 10 mg/ml.

DISCUSSION

In the present scenario, the prevalence of multidrug-resistant organisms is mounting, thus compromising the treatment of several infectious diseases. Hence, there is a continuous need for the discovery of novel and useful drugs against existing antibiotic-resistant pathogens. Plant extracts are the richest sources of secondary metabolites. According to the World Health Organization estimation, approximately 80% of the world population uses plant extracts or their active components as a part of traditional therapies. In the current investigation, methanolic extract of *C. igneus* expressed higher anti-microbial activity towards the multidrug-resistant Gram-positive and Gram-negative microorganisms like *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *E.coli*, *Proteus mirabilis*, *Citrobacter* species and *Candida albicans* concerned in the pathogenesis of skin, respiratory, urogenital, gastrointestinal, diseases and are challenging to virtually all of the traditional and present generation antibiotics. The results of the phytochemical analysis of the plant extract in the present study had revealed that leaves contain components like alkaloids, tannins, flavonoids, saponins, terepenoids etc. The anti-microbial properties and other biological properties of *C.igneus* leaf extract were due to the presence of the phytochemicals as mentioned above, which are valuable for the therapeutic index. At 120mg/ml plant extract concentration, maximum zone of inhibition was obtained with all the nine different tested microorganisms and however, the area of inhibition was higher with standard potential antibiotics like colistin, imipenem etc., This indicates that *C.igneus* have good anti-microbial activity than existing antibiotics. The study conducted by Vasantharaj *et al.* (2013) proved that methanol extract of *Costus igneus* showed opulent antibacterial activity against *Escherichia coli, Bacillus cereus, Streptococcus pyrogens, staphylococcus, Pseudomonas aeruginosa* and *Salmonella typhi*. Arjun Nagarajan et al. reported similar related work on antibacterial studies by using plant extracts of *C. igneus* on *Klebsiella pneumonia, Proteus Vulgaris, Salmonella* and *P. aeruginosa* species. The anti-

microbial activity screening also shows good results at *Bacillus subtilis, Candida parapsilosis* at a lower concentration. The present study was in accordance to the past studies however the microorganisms tested for the anti-microbial activity are multidrug-resistant organisms and *C.igneus* methanolic leaf extract generated good anti-microbial activity compared to the standard antibiotics used routinely for antibiotic therapy against MDR isolates. The sensitivity of *C.igneus* against pathogenic microorganisms causing different types of infections was more remarkable than the medicines like colistin whose usage is generally restricted in treating the diseases because of their nephrotoxicity to human beings. Hence anti-microbials derived from plant parts such as *C.igneus* can act as alternative treatment options for treating MDR infections.

CONCLUSION

Anti-microbials from plant origin have significant therapeutic potential as they can be better substitutes with fewer side effects that are frequently linked with synthetic anti-microbials. In the current research work, we have found that *C. igneus* leaf extract possesses admirable anti-microbial activity which can be due to the presence of phytochemicals. Further exploration of plant-derived anti-microbials is considered necessary today to trim down the usage of powerful drugs which pose bad side effects.

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Conflict of interest

Conflict of interest declared none.

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