Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 7
Open Access

In Vitro Antimicrobial Activity and Phytochemical Analysis of Costusigneus Leaf and Bulb

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Cite this paper as: Sonal Jain, Dildar Husain, Divya Shrivastava,Pushpendra Singh Bundela (2024). In Vitro Antimicrobial Activity and Phytochemical Analysis of Costusigneus Leaf and Bulb. *Frontiers in Health Informatics*, *13 (7) 1175-1194*

Abstract
The present study has been carried out to estimate the qualitative analysis of the secondary metabolites from *Costusigneus* plant parts i.e. leaf and bulb using thin layer chromatography. Two different extracts (chloroform and methanol) of bulb and leaf were used to examine anti-bacterial activity against Gram positive bacteria like *Bacillus subtilis, Staphylococcus aureus* and Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* by agar well diffusion method and antifungal activity against *Candida albicans, Aspergillus niger, Penicilliumchrysogenum* and *Fusarium moniliform*. The presence of quercetin (flavonoid) in both bulb and leaf was confirmed with$R_f$values of 0.77 and 0.79, respectively whereas Kaemferol was detected in leaf withR_f 0.86 value. As phytosterol compounds, stigmasterol was identified in both leaf (R_f 0.80) and bulb (R_f 0.81) and campestrol(R_f0.30) was detected only in leaf. Diosgenin, a steroidal sapogenin was identified only in leaf with 0.56 $R_f$value. As compared to chloroform extract, methanol leaf extract gave better results against all studied bacteriaespecially against *P. aeruginosa* bacteria (15 mm) at higher concentration. In case of chloroform extract of bulb, the most prominent activity was observed against *E. coli* with an inhibition zone of 14 mm, whereas methanol extract was most efficient against *P. aeruginosa* (16mm).Both extracts of bulb and leaf showed prominent anti-fungal activity against studied fungal pathogens, but maximum inhibition zone (17mm) was observed against *P. chrysogenum*withchloroform bulb extract, followed by 16 mm inhibition zone was determined with chloroform leaf extract against *F. moniliforme.* Overall, the finding results concluded that *Costusigneus* has several bio-active compounds that play significant role in its therapeutic potential, especially in anti-microbial activity.

Keywords: *Costusigneus,* Secondary metabolites, Thin Layer Chromatography, Antimicrobial activity,Inhibition zone

Introduction

Plants have always been important various components of both conventional and modern medicine, for their utility in health and wellness (**Ghallo*et al.*, 2022**). According to the estimates from the World Health Organisation (WHO), up to 80% of the population in developing nations still relies on local medicinal plants to meet their basic medical needs. A high percentage of synthetic drugs are made from precursor compounds derived from plants, and an estimated 25% of prescription drugs and 11% of drugs considered essential by the WHO are plant-based (**Akther *et al.*, 2022**). The main benefits of choosing traditional medicine over modern medicine are their simplicity of access, effectiveness in treatment, and low cost. At this point, thousands of polyphenolic compounds, including flavonoids and phenolics, have been found in medicinal plants (**Kabir*et al.*, 2012**).

Vascular plants contain enormous variety of chemical compounds, distinct from the inter-mediates and products of primary metabolism, which vary according to family and species. Primary metabolites are highly conserved and directly

required for the growth and development of plants (**Fernie and Pichersky, 2015**). Primary metabolites are found in all plants and execute vital metabolic responsibilities, by participating in nutrition and reproduction (**Croteau*et al.,* 2000**). The medicinal properties of these plants are because of the existence of the heterogeneous group of natural metabolic products called secondary metabolites which are divergent in their structure and metabolic pathways (**Costa *et al.,* 2012**). Plant secondary metabolites are compounds that help a plant survive in a competitive environment and are not a part of the plant's regular development and progress, unlike primary metabolites **(Teoh, 2016).** These metabolites make a major contribution to the specific odours, tastes and colours of plants (**Costa *et al.,* 2012**).A simple classification of secondary metabolites includes three main groups: terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin) and nitrogen containing compounds (such as alkaloids and glucosinolates) (**Roze*et al.*, 2011**). Secondary metabolites possess a wide range of pharmacological activities and play crucial biological roles. They serve as a defence response during pathogen attack and a valuable drug resource **(Lv*et al.,* 2024).**

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases (**Bhatia *et al.*, 2010**).However, antibiotic resistance emerged shortly after the discovery of the first antibiotic and has remained a critical public health issue ever since. Managing antibiotic resistance in clinical settings continues to be challenging, particularly with the rise of superbugs, or bacteria resistant to multiple antibiotics, known as multidrug-resistant (MDR) bacteria. This rapid development of resistance has compelled researchers to continuously seek new antimicrobial agents to curb resistance **(Angelini, 2024).**The use of plants as medicines has a long history in the treatment of various diseases and to date, ~ 100,000 plant species have been tested for their medicinal use (**Schmidt *et al*., 2012; Veeresham 2012).**Research shows that medicinal plants contain bioactive compounds such as coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectins, polypeptides, and polyacetylenes, which serve as foundations for antibiotic development **(Edeoga*et al.,* 2005; Rahman and Anwar, 2007; Parvin*et al.,* 2014)**. These naturally occurring plant chemicals have demonstrated significant benefits, including antioxidant, antibacterial, and antifungal activities. They can enhance the effectiveness of existing antibiotics, helping to prevent the development of resistance **(Bribi, 2018).** Their benefits include accessibility, cost-effectiveness, and minimal side effects**(Miethke*et al.,* 2021).**

*Costusigneus*Nak (also known as blazing costus, Step ladder, Spiral flag, or Insulin plant) is a perennial, erect, spreading plant that grows to about two feet tall, with spirally arranged leaves and lovely flowers **(Darade and Dhamane, 2024).** It is a traditionally used medicinal herb which is native to Southeast Asia. The plant has been introduced into India and it is grown as an ornamental plant in south India. Insulin plant contains various phytochemical constituents like steroid, alkaloid, flavonoid, triterpene, glycoside, and saponins. Various pharmacological activities include antidiabetic effect, antiproliferative potential, antimicrobial activity, antiurolithiatic property anti-inflammatory potential, its effect on learning and memory, antioxidant activity, neuroprotective role, hypolipidemic activity (**Godi*et al.,* 2019**), anticancer etc.**(Dhone*et al.,* 2022).**Present study focuses on the qualitative estimation of secondary metabolites of *Costusigneus* bulb and leaves along with the study of their antimicrobial activity.

MATERIAL AND METHOD

1) Collection of plant material

The experimental plant material i.e. *Costusigneus* (leaf and bulb) was collected from Jawaharlal Nehru Agricultural University, Krashi Nagar Colony, Adhartal, Jabalpur and shade fried under room temperature.

2) Preparation of plant sample

Plant samples (leaf and bulb) of *Costusigneus* separated and washed thoroughly 2-3 times with running tap water and then air dried in the shade at room temperature for about 2-3 weeks. The dried plant samples were ground into powder form by using a homogenizer. These powdered samples were used for further experiments.

3) Qualitative analysis of phytochemical compounds

3.1 Extraction Procedure

3.1.1 Flavonoid

Each of powdered samples (10g) was extracted with 80% methanol on a water bath **(Subramanian and Nagarajan, 1969)** for 24 h. The methanol soluble fractions were filtered, concentrated *in vacuo* and aqueous fractions were fractioned by sequential extraction with petroleum ether (FrI), diethyl ether (FrII), and ethyl acetate (FrIII) separately. Each step was repeated thrice for complete extraction, fraction I was discarded in each case because it contained fatty substances, whereas fractions II and fraction III were concentrated and used for flavonoids.

Fraction III was further hydrolyzed by refluxing with 7% sulphuric acid (10 ml/gplant material for 2 h), filtered and the filtrate was extracted thrice with ethyl acetate. All ethyl acetate layers were pooled separately, neutralized by distilled water with repeated washings, and concentrated *in vacuo.* Both fractions II and III were taken up in a small volume of ethanol (2-5mL) before the chromatographic examination. Each test sample was replicated three times.

3.1.2 Phytosterol

10g dried powdered plant material was defatted in petroleum ether (60-80^{o}C) for 24 h on a water bath. Defatted material was air dried and hydrolyzed in 30% HCl (v/v) for 4 h. Each hydrolyzed sample was washed with distilled water till pH 7 was achieved and was dried later. The dried preparation was again extracted with benzene for 24 h. The extract was filtered and dried *in vacuo*. The crude extract was dissolved in benzene before chromatographic examination (**Kaul and Staba, 1968**). Each test sample was replicated three times.

3.1.3 Steroidal sapogenin

Each of the dried plantparts (10g) were macerated weighed and defatted separately in soxhlet apparatus in petroleum ether for 24 h on a water bath. Each mixture was hydrolyzed with 15% ethanolicHCl (1g/5 mL: w/v) for 4 h by refluxing on water bath **(Tomita *et al.*, 1970).** The hydrolysate was filtered and filtrate extracted 3 times with ethyl acetate. The ethyl acetate fraction of each sample was collected and washed to remove trace amount of acid by repeated washing with distilled water, dried in *vacuo*, re-formed in chloroform, filtered again and weighed. Each test sample was replicated three times.

3.2 Thin layer Chromatography

Thin glass plates (20x20 cm^2) were coated with Silica gel G (250 mμ thick). The freshly prepared plates were air-dried at room temperature; thereafter these were kept at 100°C in an oven for 30 minutes to activate and then cooled at room temperature. The freshly prepared and activated plates were used for analysis.

Each compound extract was co-chromatographed with a standard as a marker. Quercetin as flavonoid marker, stigmasterol as phytosterol and diosgenin as steroidal sapogenin were used. These plates were developed in an air-tight chromatographic chamber saturated with a solvent mixture of benzene: acetic acid: water (125:72:3; **Wong and Francis, 1968)**for flavonoid extraction, hexane: acetone (8:2; **Fazli and Hardman, 1968**) for phytosterol and chloroform, hexane and acetone (23:5:2; **Bennett and Heftmann, 1962)** for steroidal sapogenin. All developed plates were air-dried. Then flavonoid plates kept in the I_2 chamber and phytosterol and steroidal sapogeninplates were sprayed with 50% sulphuric acid **(Bennet and Heftmann, 1962),** followed by each TLC plate heated at 110˚C for 10 min. The coloured spots thus developed were noted and the retention factor (R_f) value of each spot was calculated to analysis of the secondary metabolites by using following formula:

$$R_f = \frac{\text{Distance travelled by the solute (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

4) Antimicrobial activity

4.1 Microbial Strains

Clinical laboratory bacterial isolates of *Staphylococcus aureus* MTCC 3381, *Bacillus subtilis* MTCC 10619, *Pseudomonas aeruginosa* MTCC 0424 and *Escherichia coli* MTCC 443, and fungal isolates viz. *Candida*

*albicans*MTCC 183, *Aspergillus niger*MTCC 872, *Fusarium moniliforme*MTCC 156and *Penicilliumchrysogenum*MTCC 5108 were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, India.

4.2 Determination of Antibacterial Assay

In vitro antibacterial activity of the crude extracts was studied against gram positive and gram negative bacterial strains by the agar well diffusion method **(Perez *et al.,* 1990).** Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 10 mg/mL. The Mueller Hinton agar was melted and cooled and then poured into sterile petri dishes to give a solid plate. A standardized inoculum (1.5×10^8 CFU/mL, 0.5 McFarland) prepared in sterilized 0.9% saline water was used. Wells were prepared in the seeded agar plates using sterile gel puncture. The test compound (20, 40, 60 and 80 µl) was introduced in the wells (6 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition (in mm) produced by the agent were compared with those produced by the commercial control antibiotics, Ciprofloxacin (40 µl). The experiment was performed three times to minimize the error and the mean values are presented.

4.3 Determination of Antifungal Assay

In vitro anti-fungal activity of the sample extracts was investigated by agar well diffusion method **(Bonjar*et al.*, 2005).** The fungi were subcultured onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile phosphate buffer saline (PBS) and adjusted to a concentration of 10^6 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. Wells of 6 mm in diameter were punctured in the culture media using sterile gel puncture. 20, 40, 60 and 80 µl of fresh extracts was administered to fullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). Ketoconazole (40 µl) was used as antifungal positive control. The All experiments were made in triplicate and means were calculated.

5. Statistical Analysis

Each treatment was conducted in three replicates and the results were presented as mean ± standard error [SE].

$SE = SD / \sqrt{N}$

Here, SD denotes Standard deviation and N denotes number of observations or sample sizes.

Result and Discussion

1) Qualitative analysis of phytochemical compounds

1.2 Flavonoid 0.80, 0.88 and0.77

Flavonoids contain hydroxyl functional groups, identified as a class of antioxidant compounds in plant that act as free radical terminators (**Shahidi*et al.,* 1992; Das *et al.,* 1990; Younes*et al.,* 1981**). In the current study thin layer chromatography was performed to identify the flavonoid compounds present in the tested bulb sample of *Costusigneus.* As shown in Figure 1, spots of flavonoid compound on TLC plate were observed at 0.86and0.77R_f values, respectively from FR-II and FR-III. According to Table 1, FR-II and FR-III have kaempferol and quercetin as flavonoid compounds, respectively because 0.86 $R_f$resemble to kaempferol compound that has 0.84 R_f value and 0.77 R_f correlate to quercetin compound (0.79 R_f value). In case of leaf sample the tested flavonoid compounds were observed at 0.79 R_f values, respectively as visible in Figure 1 with FR-II and FR-III. According to Table 2, both FR-II and FR-III have quercetin.In a study conducted by **Reddy *et al.,* 2018** the methanolic leaf extract of *C.igneus*showed the presence of quercetin with $R_f$value of 0.56 as compared with the standard R_f value. **Muthukumar*et al.,* 2019** made a quantitative estimation of total flavonoid content in *C.igenus* leaf extract which was quantified to be 58.3±0.2837 mg/gm. Likewise, in case of **Khanday*et al.,* 2019** the estimation of total flavonoid contents of the different leaf extracts of *C. igneus* revealed that hexane extract was found to contain maximum total flavonoid content with 535.48 mg/g extract followed by acetone (481.11 mg/g extract) and hot water extract (59.07 mg/g extract).

Table 1: Chromatographic and Physico-chemical characteristics of isolated Flavonoids from bulb of *Costusigneus*

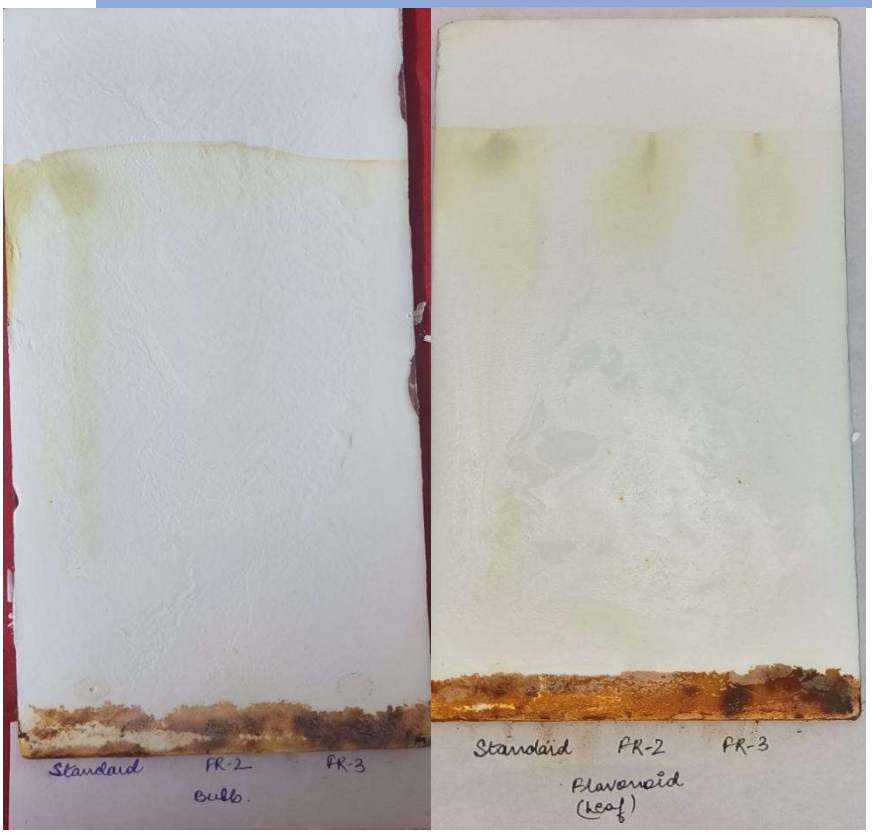
lated compounds	**value**	**ysical appearance**	
		y light	**Vapour (R_1)**
empferol	6	N-YW	V-BN
ercetin	7	N-YW	V-BN

Abbreviations: S_1 – Benzene: acetic acid: water (125: 72: 3), R_1 – I_2 Vapour, YW – Yellow, BN – Brown, GN – Green.

Table 2: Chromatographic and Physico-chemical characteristics of isolated flavonoids from leaf of *Costusigneus*

lated compounds	**value**	**ysical appearance**	
		y light	**Vapour (R_1)**
Quercetin	9	N-YW	V-BN

Abbreviations: S_1 – Benzene: acetic acid: water (125: 72: 3), R_1 – I_2 Vapour, YW – Yellow, BN – Brown, GN – Green.



(a) **(b)**

Figure 1: Detection of flavonoid compounds in *Costusigneus*a) bulb b) leaf

1.2.1.2 Phytosterol

Phytosterols are plant-derived sterols that have similar physiological functions with cholesterol in vertebrate animals **(Zaloga, 2015)**. More than 100 types of phytosterols and 4000 other types of triterpenes have been found according to the literature. β-Sitosterol, campesterol, stigmasterol, brassicasterol, and Δ5-avenasterol are the main phytosterols in plants **(Miras-Moreno *et al.,* 2016)**. In current study the phytosterol were detected from both leaf and bulb samples of *C. igenues*(Table 3, Figure 2). In leaves the analyzed R_f values were 0.80 and 0.30 respectively in FR-II and FR-III. FR-II was estimated to be stigmasterolthat R_f value is 0.83 whereas FR-III was detected to be campesterol (0.29 R_f value). Likewise in bulb stigmasterol was detected with R_f value of 0.81 that is nearest value of standard (Table 4). In a study of**Khanday*et al.,* 2019,** the estimation of phytosterol contents of the different leaf extracts of *C. igneus*was found highest in acetone extract followed by hexane and hot water extracts. Quantitative characterization of phytosterols constituent of *C.lucanusianus* stem by **Peters *et al.,* 2022** was estimated to be 26.97 mg/100g which included Cholestanol, Tinyaloxin, Daucosterol, Ergosterol, Campesterol, Stig-Massterol, Savenasterol and Sitosterol. Likewise preliminary phytochemical screening of ethanolic extract of leaves of *C.pictus* by **Colaco*et al.,* 2018** showed moderate presence of phytosterols.

Table 3: Chromatographic and Physico-chemical characteristics of isolated phytosterols from leaf of *Costusigneus*

lated compounds	**value**	**Color After Spray**

gmasterol	0	Y
mpesterol	0	Y

Abbreviations: S_1 - Hexane : acetone (8 : 2), R_1 - 50% H_2SO_4, GY – Grey.

Table 4: Chromatographic and Physico-chemical characteristics of isolated phytosterols from bulb of *Costusigneus*

lated compounds	**value**	**Color After Spray**
gmasterol	1	Y

Abbreviations: S_1 - Hexane : acetone (8 : 2), R_1 - 50% H_2SO_4, GY – Grey.

Figure 2: Detection of phytosterol compounds in *Costusigneus*bulb and leaf

1.2.1.3 Steroidal saponin

Saponins, one type of bioactive compound, are amphitheatric glycosides with one and more hydrophilic sugar and

hydrophobic steroidal and terpenoid part. The former is known as steroidal saponin, and the latter is called terpenoid saponins (**Porte *et al.,* 2022).** They are mainly distributed among monocotyledonous families: Amarillidaceae (Agapanthus, Allium), Asparagaceae,Costaceae (Costus), Dioscoreaceae (Dioscorea), Liliaceae(Fritillaria, Lilium), Melanthiaceae (Paris), Smilacaceae (Smilax). Although it is uncommon, steroidal saponins can also be found in some dicotyledonous angiosperms, such as: Fabaceae (Trigonella), Zygophyllaceae (Tribulus, Zygophyllum), Solanaceae (Solanum, Lycopersicon, Capsicum), Asteraceae (Vernonia), and Plantaginaceae (Digitalis) **(Faizal and Geelen, 2013; Rahman *et al.,* 2017; Lanzotti, 2005; Sobolewska*et al.,* 2016; Wang *et al.,* 2018).**In current study the steroidal saponin (diosgenin) was detected from leaf sample of *C. igneus*only (Table 5). In leaves the analyzed R_f values was 0.56 with a green spot and this denoted the presence of diosgenin compound in leaf because R_f values is nearest to the standard (0.58 R_f).whereas in bulb no steroidal saponin was detected. In a study conducted by **Pazhanichamy*et al.,* 2012**diosgenin was isolated and purified from sapogenin extract of rhizome of *C. igneus* by repeated silica gel column chromatography followed by preparative TLC with $R_f$being 0.27. **Ismail *et al.,* 2015** reported a new spirostane steroidal saponin, govanoside A along with three known compounds borassoside E, pennogenin and diosgenin which were isolated from rhizomes of *Trillium govanianum.* In a study by **Lebot*et al.,* 2019** steroidal saponins were quantified in three species of *Dioscorea*i.e D. *cayenensis*, *D. esculenta* and *D. rotundata*. Mean total saponins contents were 42.15 mg/g in *D. cayenensis,* 17.65 mg/g in *D. esculenta* and 17/44 mg/g in *D.rotundata,* with accessions presenting very high values in *D. cayenensis* (78.31 mg/g) and *D. rotundata* (69.65 mg/g).

Table 5: Chromatographic and Physico-chemical characteristics of isolated steroidal saponin from leaf *Costusigneus*

lated compounds	**value**	**Colour After Spray**	
			R_2
osgenin	6	N	N

Abbreviations: S_1 – Chloroform: hexane : acetone (23: 5 : 2), R_1 - 50% H_2SO_4, GN - Green.

Figure 3: Detection of steroidal saponin in *Costusigneus*bulb andleaf

2) Antimicrobial activity

2.1 Antibacterial activity

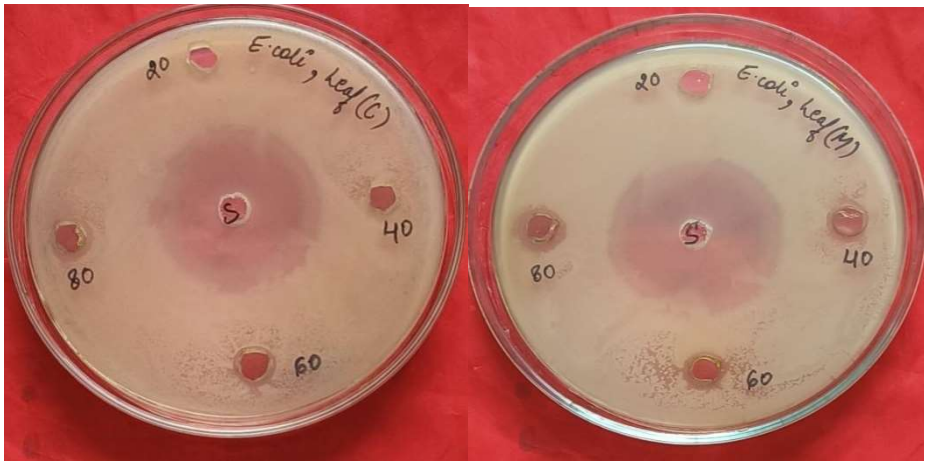
In anti-bacterial activity, the chloroform extracts of *C. igneus* leaves (Table 6) showed maximum inhibition of 12mm both against *P. aeruginosa* as well as *S. aureus* at higher concentration whereas11mm inhibition zone observed against *B. subtilis*. Least zone of inhibition was shown against*E. coli* (9mm). On the other hand, the methanol extracts of *C. igneus* leaves showed maximum zone of inhibition against *P. aeruginosa* bacteria (15 mm) at 80 µl concentration followed by *S. aureus* (13mm) and minimum inhibition against *E. coli* and *B. subtilis* with 11mm inhibition zone. As compared to chloroform extracts, leaves methanol extract gave better results against all studied bacteria.

In case of anti-bacterial activity of *C. igneus*bulb extracts (Table 7), the chloroform extract showed the most prominent activity against *E. coli* with an inhibition zone of 14 mm at higher concentration followed by *P. aeruginosa* (12mm), *S. aureus* (11mm) and least by *B. subtilis* (10mm) whereas in case of methanol extract *P. aeruginosa* was most efficient with an inhibition zone of 16mm at 80 µl concentration followed by *S. aureus*(15mm), *E. coli* (10mm) and *B. subtilis* (8mm) in order of inhibition zone. Both extracts were almost equally efficient against the tested bacteria in bulb.**Kala, 2014** studied antibacterial activity of petroleum ether, methanol, ethyl acetate, acetone and chloroform extracts of *Costusigneus* parts (leaf and root) against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* The highest activity was shown by *B. subtilis* in methanol extract of leaf with 17mm zone of inhibition whereas lowest was observed in acetone root extract by *P. aeruginosa* with 6mm inhibition zone. **Jaini*et al.*, 2020** reported that the anti-bacterial activity of ethanol and chloroform extracts of *C.pictus*leaves showed potential zone of inhibition against *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus* spp., *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, proteus mirabilis* and *Streptococcus mutans.* The highest activity was shown by *Lactobacillus* spp.(23mm)in ethanol extract and lowest by *Pseudomonas aeruginosa*

(13mm) similarly; in chloroform extract the highest activity was shown by *Lactobacillus sps*(20mm) and lowest by both *Bacillus subtilis, Streptococcus mutans*with 10mm inhibition zone. Furthermore, **Akani*et al.*, 2020** revealed that *S. aureus* and *E. coli* were both inhibited by the aqueous leaf extract of *C. afer* with zone diameter of 16 mm and 15 mm, respectively as well as the ethanolleaf extract with diameter of 18mm and 15 mm, respectively.

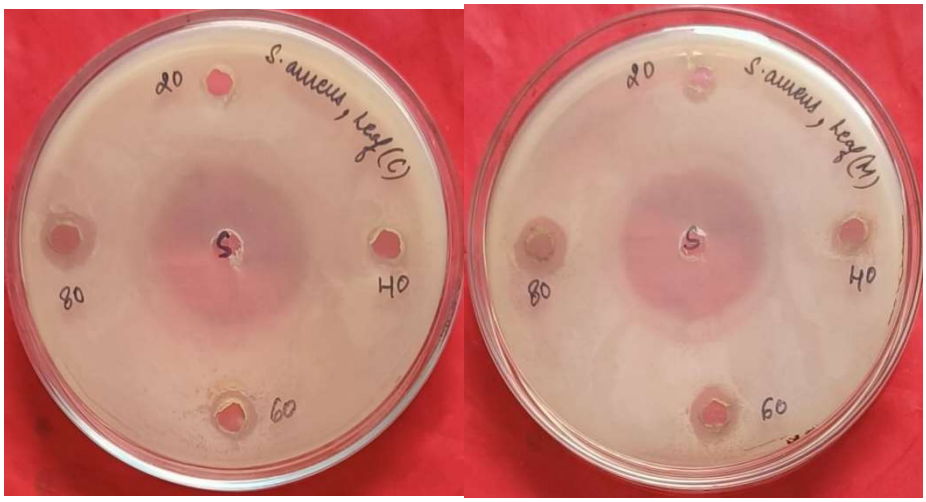
Table 6: Antibacterial activity of *Costusigneus*leaves against pathogenic bacteria

Bacteria	Extract	Inhibition zone (mm)				
		Standard	20 µL	40 µL	60 µL	80 µL
	Chloroform	30	nil	Nil	7	9
E. coli	Methanol	30	nil	7	8	11
	Chloroform	29	nil	7	8	12
S. aureus	Methanol	29	7	9	12	13
	Chloroform	27	nil	7	9	11
B. subtilis	Methanol	27	nil	Nil	7	11
	Chloroform	28	7	8	11	12
P. aeruginosa	Methanol	28	7	8	13	15



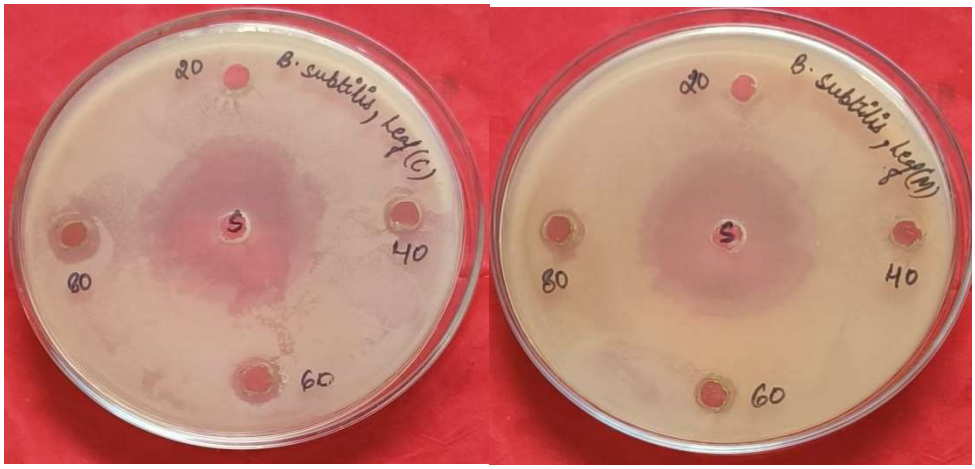
(a) **(b)**

Figure 4: Antibacterial activity of *Costusigneus*leaves against *Escherichia coli* (a) Chloroform extract (b) Methanol extract



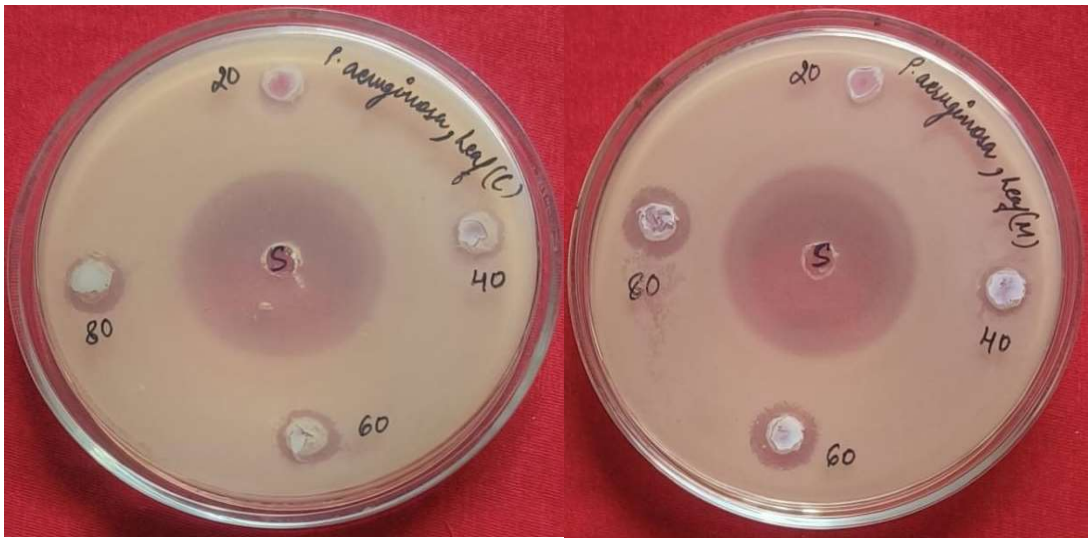
(a) (b)

Figure 5: Antibacterial activity of *Costusigneus*leaves against *Staphylococcus aureus* (a) Chloroform extract (b) Methanol extract



(a) (b)

Figure 6: Antibacterial activity of *Costusigneus*leaves against *Bacillus subtilis* (a) Chloroform extract (b) Methanol extract



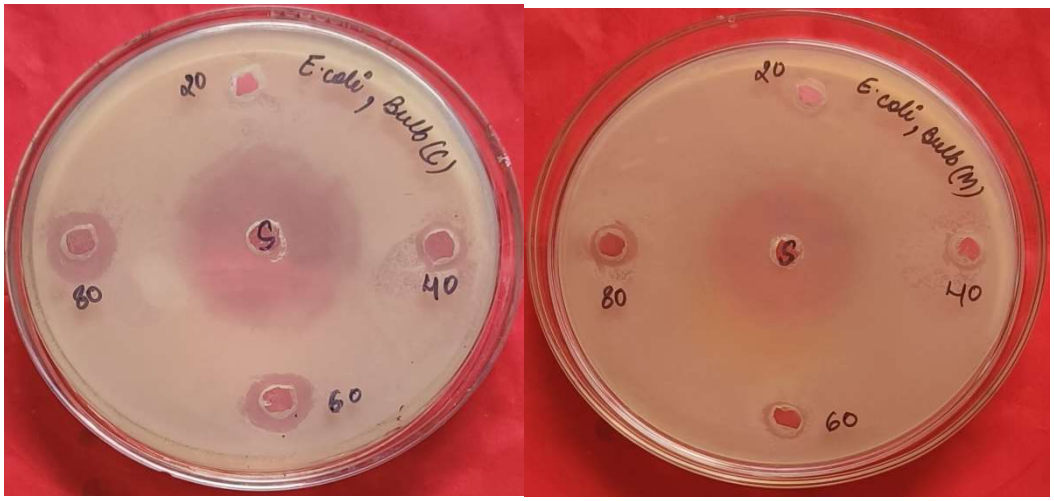
(a) (b)

Figure 7: Antibacterial activity of *Costusigneus*leaves against *Pseudomonas aeruginosa* (a) Chloroform extract (b) Methanol extract

Table 7: Antibacterial activity of *Costusigneus*bulb against pathogenic bacteria

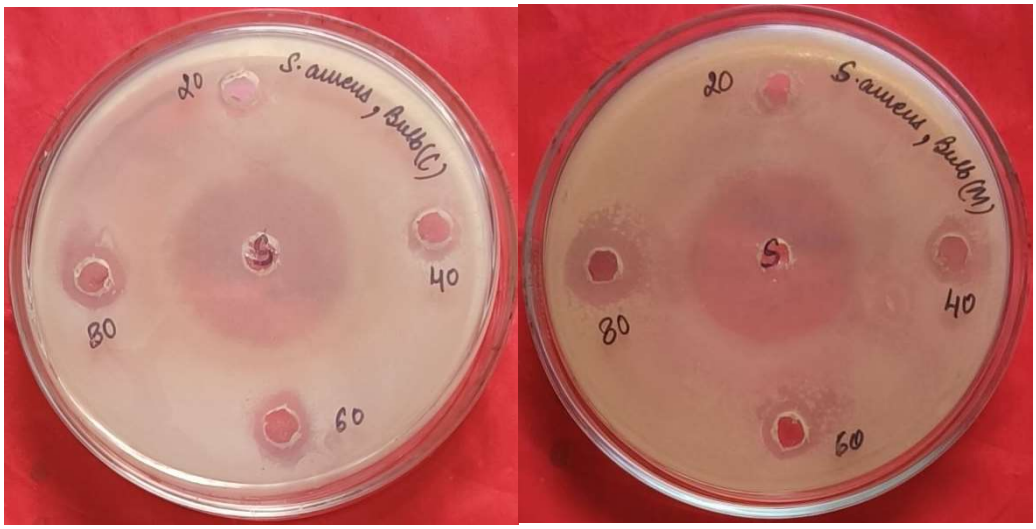
Bacteria	Extract	Inhibition zone (mm)				
		Standard	20 µL	40 µL	60 µL	80 µL
E. coli	Chloroform	30	nil	9	12	14
	Methanol	30	nil	7	8	10
S. aureus	Chloroform	29	7	9	10	11
	Methanol	29	7	10	11	15

	Chloroform	27	nil	nil	7	10
B. subtilis	Methanol	27	nil	nil	7	8
	Chloroform	28	nil	9	10	12
P. aeruginosa	Methanol	28	12	14	15	16



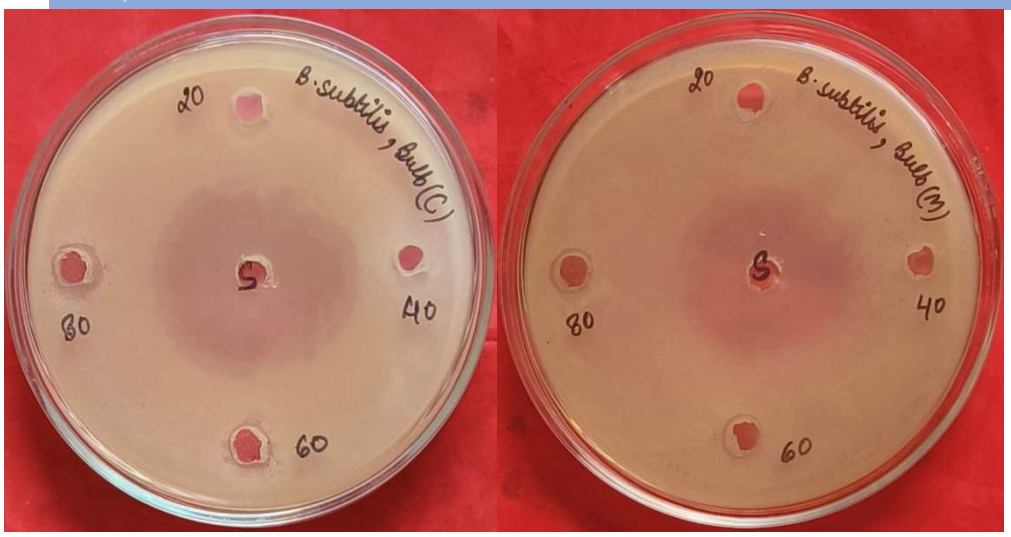
(a) **(b)**

Figure 8: Antibacterial activity of *Costusigneus*bulb against *Escherichia coli* (a) Chloroform extract (b) Methanol extract



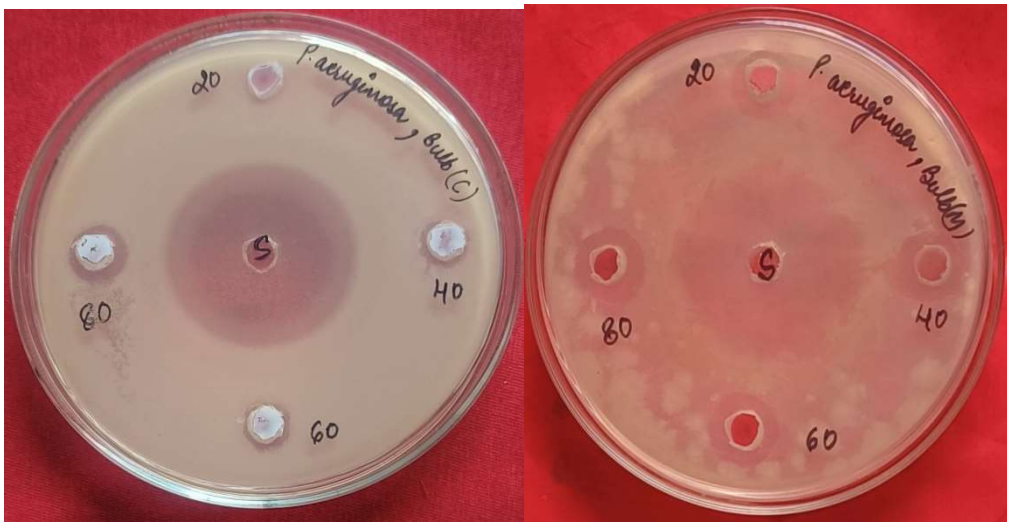
(a) **(b)**

Figure 9: Antibacterial activity of *Costusigneus* bulb against *Staphylococcus aureus* (a) Chloroform extract (b) Methanol extract



(a) **(b)**

Figure 10: Antibacterial activity of *Costusigneus*bulb against *Bacillus subtilis* (a) Chloroform extract (b) Methanol extract



(a) **(b)**

Figure 11: Antibacterial activity of *Costusigneus*bulb against *Pseudomonas aeruginosa* (a) Chloroform extract (b) Methanol extract

2.2 Antifungal activity

As shown in Table8 antifungal activity of leaf extract of *C. igneus* was highest against *F. moniliforme*followed by *C. albicans***.** Highest 16 mm inhibition zone was observed against *F. moniliforme*was observed with chloroform extract at maximum concentration thereafter 15 mm with methanol extract. The growth of *C. albicans* was inhibited up to 12 mm diameter by chloroform extract and 11 mm diameter by methanol extract. To inhibit *A. niger* growth, chloroform leaf extract was effective than methanol, while *P. chrysogenum*showed resistance to both extracts only higher concentration of chloroform extract showed significant results. However, bulb extract was showed more anti-fungal activity as compared to the leaf extract as shown in Table 9. Maximum fungal inhibitory diameter (17 mm) was recognized with chloroform extract against *P. chrysogenum.* Inhibition zone 13 mm was observed with methanol extract against *C. albicans*and with chloroform extract against *A. niger*. Furthermore, both bulb extracts showed same zone of inhibition (12 mm) against *F. moniliforme.* In the study of **Onanuga and Oloyede, 2022**rhizome extracts (hexane, ethyl acetate

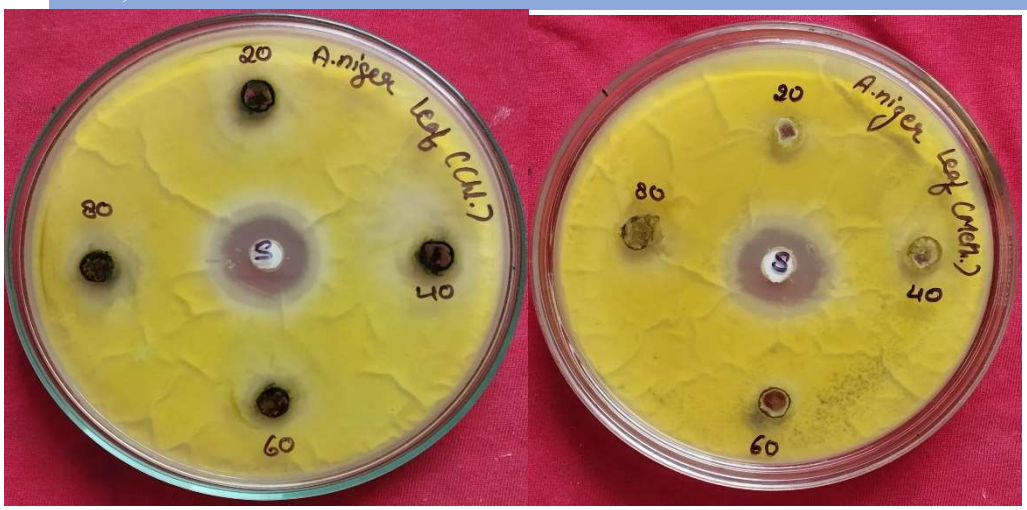
and methanol) of *C. igneus*showed antifungal activity against all the tested organisms (*C. albicans*, *A. niger, R sotolonifer* and *P. notatum*) at all concentrations (12.5, 25, 50 and 100 µg/ml) while the leaf aqueous extract showed activity only against *C. albicans* (12.0 mm) and *A. niger* (10.0 mm) at the minimum concentration of 25 µg/ml.**Damam*et al.*, 2017** observed high inhibition zone in leaf methanol extracts of *C. igneus*against *A. alternate* (33mm), followed by *M. phaseolina* (30mm), *F. oxysporum* (24mm), *C. capsici* (23mm), *R. solani* (15mm), *U. virens* (15mm), *A. flavus* (11mm). Stem and root methanol extracts showed high inhibition zone 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.**Duraipandiyan and Ignacimuthu, 2011** revealed significant antifungal property of *C. speciosus* rhizome ethyl acetate fraction against *A. niger* and *C. albicans.***Rao *et al.*, 2016**showed high anti-fungal activity (1.2 cm) of *Costusigneus* leaf extract against *Candida parapsilosis* at low concentration (2.5µg/ml), followed by 1.1 cm against *Trichophyton rubrum*, 1.0 against *Candida albicans* and 0.9 against *Aspergillus niger*.

Table 8: Antifungal activity of *Costusigneus*leaves against pathogenic fungus

Fungus	Extract	Inhibition zone (mm)				
		Standard	20 µL	40 µL	60 µL	80 µL
C. albicans	Chloroform	30	Nil	8	10	12
	Methanol	30	Nil	8	9	11
A. niger	Chloroform	20	8	9	11	13
	Methanol	20	Nil	Nil	Nil	Nil
P. chrysogenum	Chloroform	25	Nil	Nil	9	10
	Methanol	25	Nil	Nil	Nil	Nil
F. moniliforme	Chloroform	28	8	11	13	16
	Methanol	28	9	10	12	15

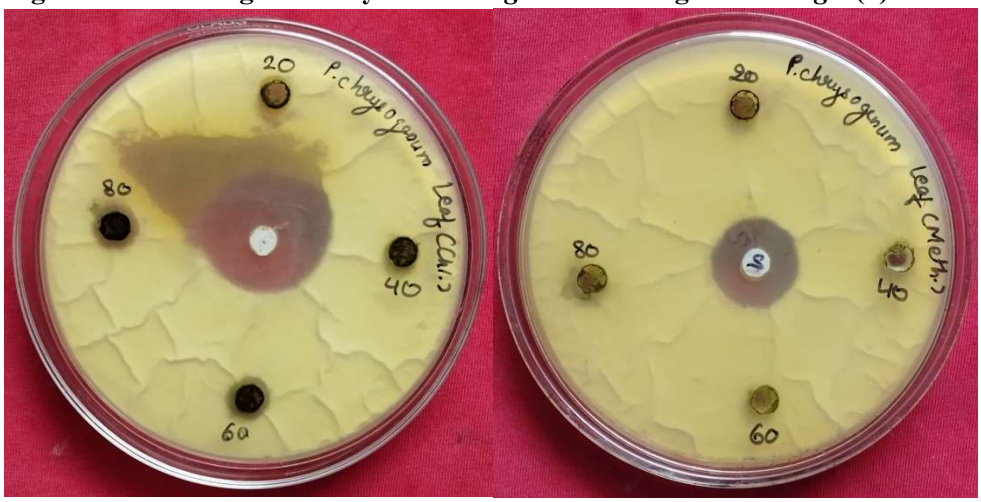
(a) **(b)**

Figure 12: Antifungal activity of *Costusigneus*leaves against *Candida albicans*(a) Chloroform extract (b) Methanol extract



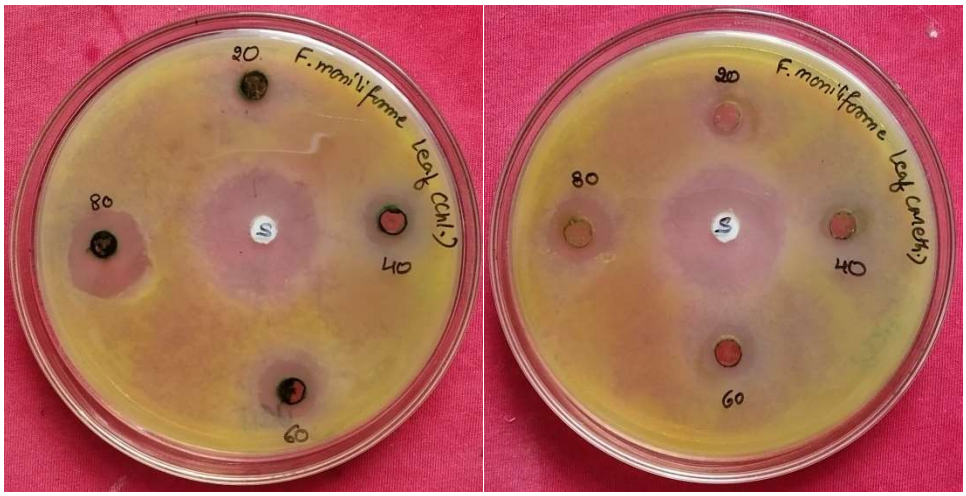
(a) **(b)**

Figure 13: Antifungal activity of *Costusigneus*leaves against *A. niger*(a) Chloroform extract (b) Methanol extract



(a) **(b)**

Figure 14: Antifungal activity of *Costusigneus*leaves against *P. chrysogenum*(a) Chloroform extract (b) Methanol extract

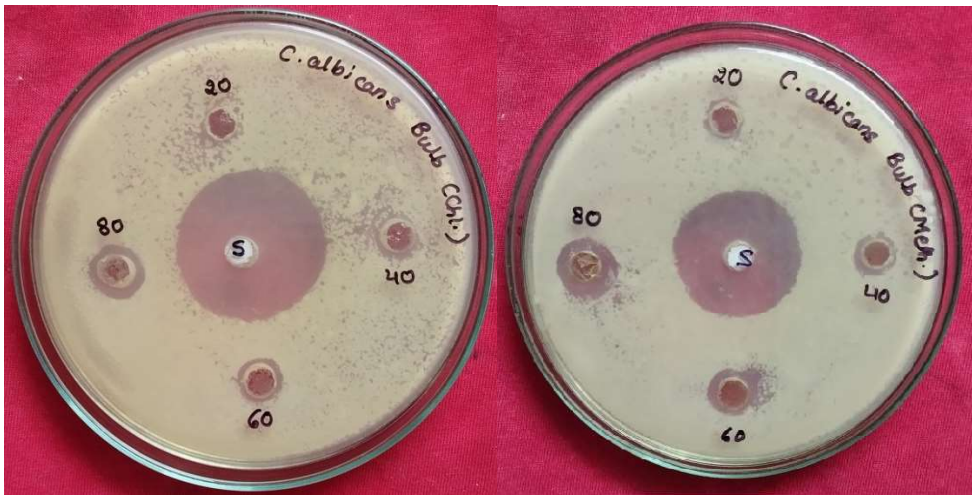


(a) (b)

Figure 15: Antifungal activity of *Costusigneus*leaves against *F. moniliforme*(a) Chloroform extract (b) Methanol extract

Table9: Antifungal activity of *Costusigneus*bulb against pathogenic fungus

Fungus	Extract	Inhibition zone (mm)				
		Standard	20 µL	40 µL	60 µL	80 µL
C. albicans	Chloroform	30	Nil	8	9	11
	Methanol	30	7	9	11	13
A. niger	Chloroform	20	8	9	10	13
	Methanol	20	Nil	Nil	9	10
P. chrysogenum	Chloroform	25	10	12	15	17
	Methanol	25	Nil	8	9	10
F. moniliforme	Chloroform	28	8	10	11	12
	Methanol	28	7	8	10	12

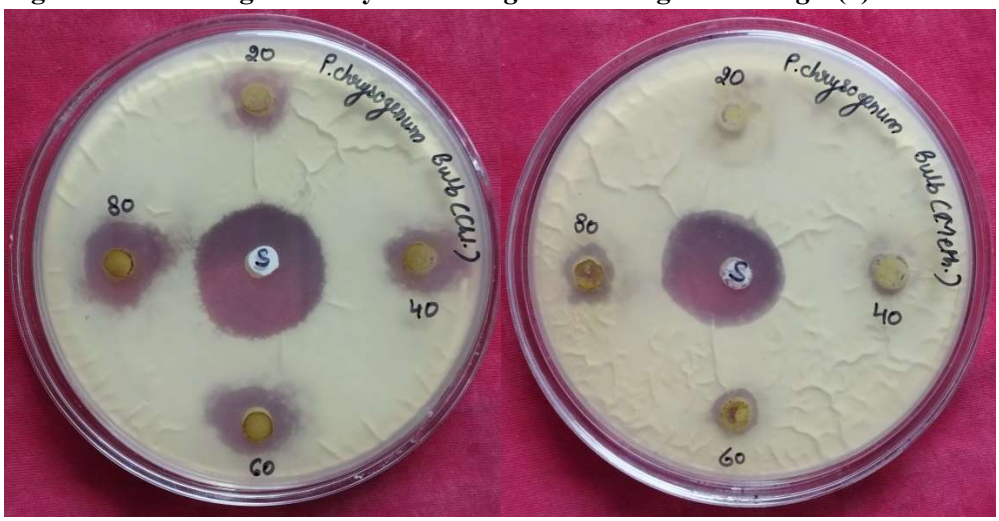


(a) (b)

Figure 16: Antifungal activity of *Costusigneus*bulb against *C. albicans*(a) Chloroform extract (b) Methanol extract

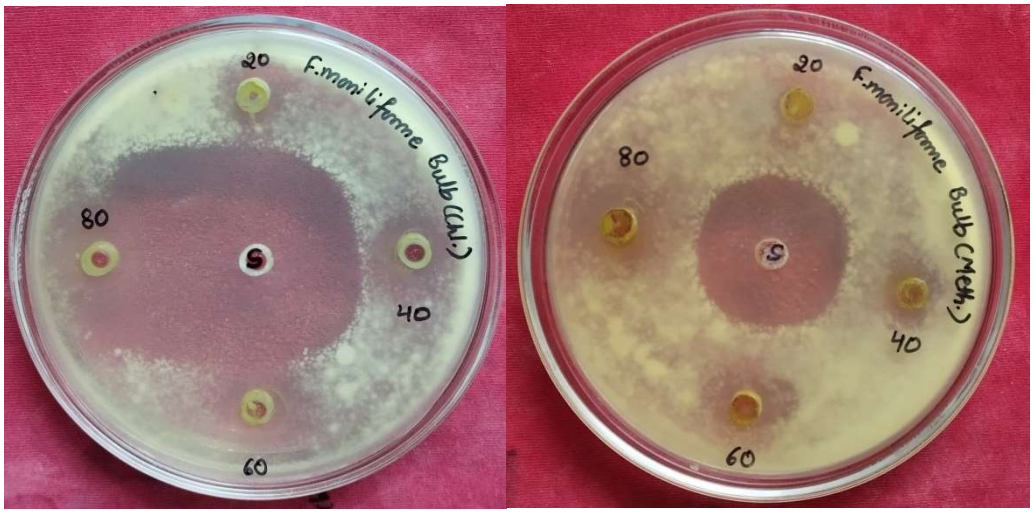
(a) **(b)**

Figure 17: Antifungal activity of *Costusigneus*bulb against *A. niger*(a) Chloroform extract (b) Methanol extract



(a) **(b)**

Figure 18: Antifungal activity of *Costusigneus*bulb against *P. chrysogenum*(a) Chloroform extract (b) Methanol extract



(a) **(b)**

Figure 19: Antifungal activity of *Costusigneus*leaves against *F. moniliforme*(a) Chloroform extract (b) Methanol extract

Conclusion

This study scientifically supports the usage of *Costusigneus* as a remedy for various bacterial and fungal infections in the field of medicine. The results of the phytochemical analysis indicated the presence of flavonoids, phytosterols and steroidal sapogenins in the leaf and bulb of the plant which represents that the antimicrobial activities of the plant parts were due to the presence of secondary metabolites.

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