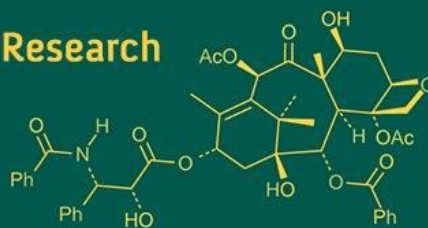
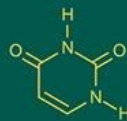
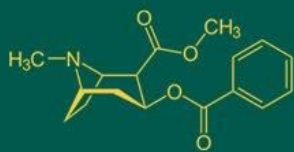


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Studies on the antioxidant and antibacterial activity of *Costus igneus* leaf powder- a natural preservative for food

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Abstract

Food products are rich in nutrients but are highly susceptible to microbial spoilage and oxidative degradation, necessitating the use of preservatives. Due to safety concerns associated with synthetic additives, natural plant-based bioactive compounds are gaining prominence. *Costus igneus*, traditionally used for diabetes management in Tamil Nadu, is reported to possess significant antioxidant and antimicrobial properties, making it a promising natural preservative source. The present study evaluated the phenolic content, antioxidant potential and antibacterial activity of ethanolic extracts of *C. igneus* leaf powder for potential application in food systems. The total phenolic content, determined using the Folin-Ciocalteu method, was found to be 96.43 ± 1.50 mg GAE/g, indicating a high concentration of polyphenolic antioxidants. The extract exhibited concentration-dependent DPPH radical scavenging activity, with inhibition values increasing from 37.89 ± 0.14 % at 25 μ g/ml to 88.20 ± 0.23 % at 400 μ g/ml and an IC_{50} value of 31.06 μ g/ml. Although the activity was lower than that of standard ascorbic acid, the extract demonstrated strong free radical scavenging potential. Antibacterial analysis showed that the extract inhibited *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*, with the highest activity observed at 500 μ g/ml. Gram-negative bacteria, particularly *P. aeruginosa* and *E. coli*, were more susceptible to the extract. Overall, the study demonstrates that *C. igneus* leaf powder possesses notable antioxidant and antibacterial properties, supporting its potential use as a natural preservative in functional foods and nutraceutical application.

Keywords: *Costus igneus*, Phenolic content, DPPH activity, Anti-microbial activity, Natural preservative

Introduction

Food products are highly nutritious and form an essential part of the human diet, providing proteins, carbohydrates, lipids, vitamins and minerals necessary for growth and health. However, their rich nutrient composition and high-water activity make them highly susceptible to microbial spoilage and oxidative deterioration, leading to reduced shelf life and compromised safety. Traditionally, synthetic preservatives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and nitrites have been employed to control spoilage and maintain product stability. Nonetheless, increasing awareness of their potential adverse health effects has driven interest toward natural alternatives. Natural preservatives derived from plants, animals and microorganisms have gained significant attention for their ability to enhance food safety, extend shelf life and preserve quality. Among these, plant-based bioactive compounds such as polyphenols, phenolics and flavonoids have demonstrated potent antioxidant and antimicrobial properties, making them promising candidates for food preservation. These compounds effectively inhibit lipid oxidation, microbial growth and quality degradation, while maintaining sensory and nutritional attributes. However, their incorporation into food systems may sometimes alter flavour, texture or consumer acceptability.

In the present scenario, life-threatening microbial infections continue to rise despite the availability of several synthetic antimicrobial agents, whose effectiveness is increasingly compromised by the rapid emergence of multidrug resistance.

This challenge underscores the need to explore plant-based alternatives for therapeutic development. *Costus igneus* leaves have been traditionally used by the tribal communities of the Kolli Hills in Namakkal district, Tamil Nadu, for the management of diabetes (Elavarasi and Saravanan, 2012) [4]. The phytochemicals present in *Costus igneus* are reported to contribute to a wide range of pharmacological activities, including antidiabetic, antiproliferative, antimicrobial, antiulcerogenic, anti-inflammatory, cognitive-enhancing, antioxidant, neuroprotective and hypolipidemic effects (Mathew and Varghese, 2019) [8]. The present study aimed to evaluate the leaf powder extracts of *Costus igneus* with a focus on their phenolic content, antioxidant and antibacterial properties, for better application in the food industry.

Materials and methods

Collection of plant materials

Fresh *Costus igneus* leaves were collected from the herbal garden of the Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute (VCRI), Namakkal, Tamil Nadu, India. The collected leaves were then placed in sterile polyethylene bags and transported to the Department of Livestock Products Technology (Meat Science), VCRI, Namakkal. The plant was authenticated by Plant Taxonomist, Research Centre in Botany, Arignar Anna Government Arts College, Namakkal - 637 002, under authentication number AAGA/BOT/001/2025.

Preparation of *Costus igneus* leaf powder

The collected plant leaves were initially washed under running tap water to remove dirt, sand and other impurities, followed by rinsing with distilled water to ensure complete cleanliness and sterility. The plant leaves were shade-dried for 60 days, ground into a fine powder using a mixer, sieved through muslin cloth to obtain uniform particle size. The powders were packed in sterile polyethylene pouches and stored at 4±1°C until further use.

Preparation of ethanolic extract of *Costus igneus* leaf powder

Preparation of Cold extraction from the sample

The cold maceration method involves soaking plant materials in a suitable solvent at low or ambient temperatures to extract bioactive compounds, making it an effective technique for preparing plant extracts. As per Pangi *et al.* (2022) [10], with slight modifications, the *Costus igneus* leaf extract was prepared as follows: 10 g of CP sample was dissolved in 100 mL of ethanol and incubated at 4°C for 24 hours in a refrigerator. The ethanol-immersed sample was then separated and filtered sequentially through muslin cloth, filter paper and Whatman No. 1 paper to obtain the extract. The filtrate was subsequently concentrated using a heating mantle at 50°C.

Determination of total phenol content

Total phenolic content was estimated by Folin Ciocalteu's method. 1 ml of aliquots and standard gallic acid (0.007 mg/ml to 1mg/ml) was positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. It was allowed to incubate for 2 hours at

room temperature. Intense blue colour was developed. After incubation, absorbance was measured at 750 nm spectrophotometer using UV visible spectrophotometer instrument. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of sample were expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass (Bhalodia *et al.*, 2011; Patel *et al.*, 2010) [1, 11].

In vitro antioxidant activity (DPPH method)

The antioxidant activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, which measures the free radical scavenging capacity of the extract. Ascorbic acid was used as the standard reference. The decrease in absorbance was measured at 517 nm using a UV-Visible spectrophotometer, with ethanol serving as the blank (Williams *et al.*, 1995) [17].

The percentage of DPPH radical inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The IC₅₀ value, representing the concentration required to scavenge 50% of DPPH radicals, was determined from a linear regression curve plotted between percentage inhibition and sample concentration.

In vitro Antibacterial Activity (Agar Well Diffusion Method)

Crude ethanolic extracts of *Costus igneus* leaf powder (500, 250, 100 and 50 µg/ml) and the standard antibiotic gentamicin were tested for antibacterial activity using the agar well diffusion method followed by Thiruvassagam (2022) [16] with slight modification. Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard. Wells were cut and concentration of *Costus igneus* leaf powder (500, 250, 100 and 50 µg/ml) was added on nutrient agar plates inoculated with four bacterial strains (*S. aureus*-902, *B. cereus*-430, *E. coli*-443, *P. aeruginosa*-424) under aseptic conditions in a laminar airflow chamber. The plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition (measured in millimeters) were recorded and compared with the standard to evaluate antibacterial efficacy.

Statistical analysis

The data obtained from this study were pooled and statistically analysed following the procedure described by Snedecor and Cochran (1994) [15].

Results

Total phenol content

Polyphenols are a diverse group of naturally occurring compounds found in a variety of plants and fungi. These compounds are characterized by the presence of multiple phenol units and are well-known for their wide range of biological activities, including antioxidant, anti-inflammatory and antimicrobial properties (Singla *et al.*, 2019) [14]. The total phenolic content was determined using the Folin-Ciocalteu reagent, which reacts with reducing

polyphenolic compounds. The phenolic constituents present in the plant samples react with the phosphomolybdic-phosphotungstic acid complexes of the Folin-Ciocalteu reagent under alkaline conditions to form a blue-coloured complex (molybdenum blue). The intensity of this colour was measured spectrophotometrically and the total phenolic content was quantified accordingly (Kale *et al.*, 2010) ^[7]. The total phenolic content was calculated from the extrapolation of a calibration curve prepared using gallic acid solutions. The estimation of phenolic compounds was

carried out in triplicate and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample.

The calibration curve was constructed using gallic acid concentrations ranging from 0.039 to 5 mg/ml and absorbance was measured at 750 nm. The standard curve exhibited a good linear relationship between absorbance and concentration, confirming the reliability of the assay (Fig. 1).

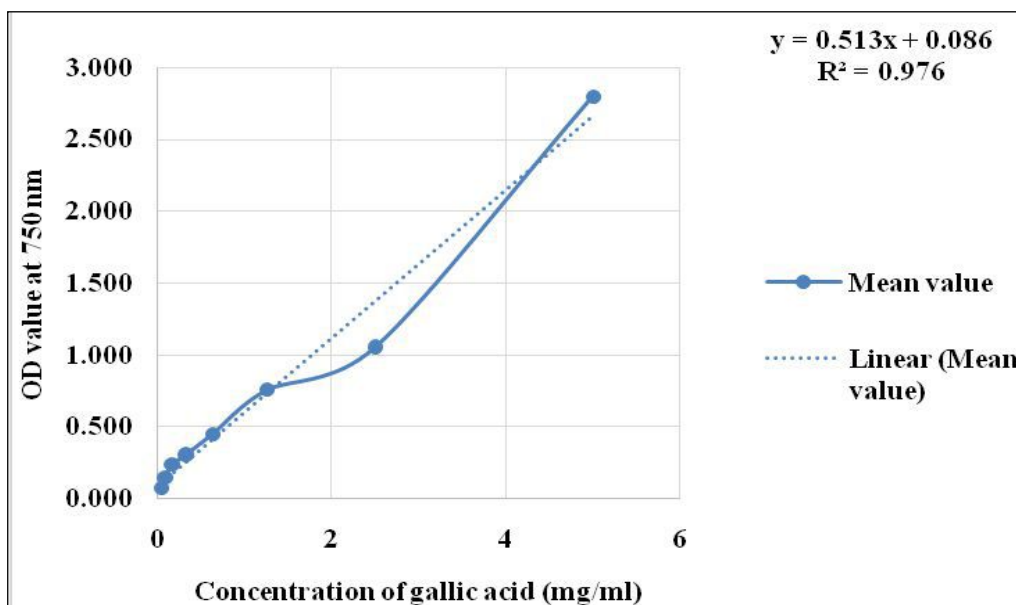


Fig 1: Standard Curve for Total Phenol using Gallic Acid

The mean total phenolic content of the leaf powder extract was found to be 96.43 ± 1.50 mg GAE/g, indicating its strong antioxidant potential. The present results are comparable to those reported by Chacko *et al.* (2018) ^[2] (78.89 ± 3.94 mg/g) but higher than those obtained by Muthukumar *et al.* (2019) (25.3 ± 0.0027 mg/g) and Rasool *et al.*, 2011 ^[13] reported that highest antioxidant activity was exhibited by ethyl extract of *C. rosea*.

In vitro antioxidant activity (DPPH method)

The antioxidant activity of *C. igneus* leaf powder extract was evaluated using the DPPH free radical scavenging assay and the results were compared with ascorbic acid as the standard antioxidant. Standard graph of Ascorbic acid was shown in fig 2&3. The percentage of inhibition increased with increasing concentration for both the sample and the standard, indicating a concentration-dependent free radical scavenging activity (Table 1).

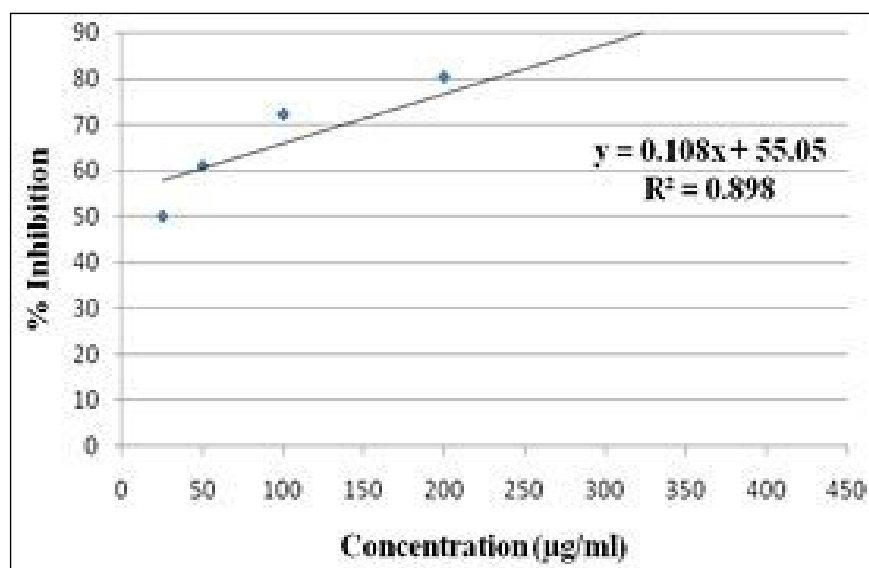


Fig 2: Standard Curve for Ascorbic acid

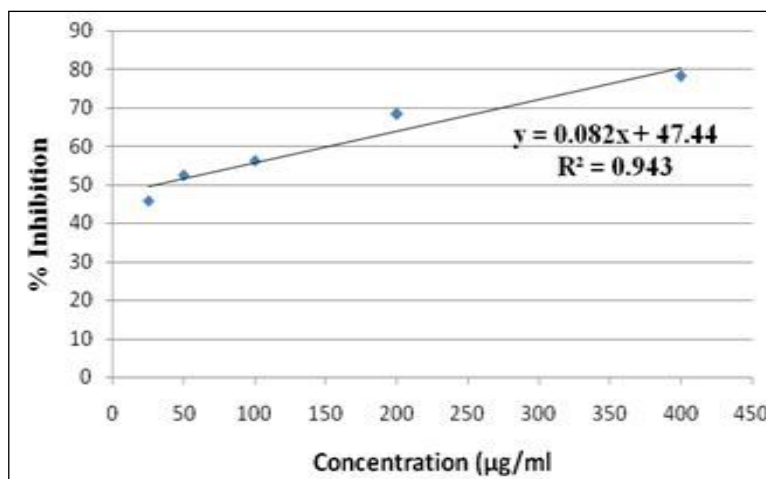


Fig 3: % inhibition of *C. igneus* leaf powder extract

At a concentration of 25 µg/ml, *C. igneus* leaf powder extract exhibited 37.89±0.14 % inhibition, which progressively increased to 88.20±0.23 % at 400 µg/ml. In comparison, the ascorbic acid standard showed higher scavenging activity, ranging from 50.12±0.27 % at 25 µg/ml to 94.34±0.40 % at 400 µg/ml. Statistical analysis revealed

significant differences ($p < 0.05$) between concentrations (A-E) and between the extract and the standard (a, b), indicating that both concentration and sample type significantly influenced DPPH free radical scavenging activity.

Table 1: Mean±SE of In-vitro Antioxidant activity of ethanolic extracts of *Costus igneus* leaf powder by DPPH free radical scavenging activity (%) and its IC₅₀ (mg/ml) (n=3)

S. No	Sample concentration (µg/ml)	Percentage of inhibition (%)		t- value	P-value
		<i>C. igneus</i>	Ascorbic acid		
1	25 µg/ml	37.89±0.14 ^{aA}	50.15±0.29 ^{bA}	4.981	<0.001
2	50 µg/ml	52.47±0.16 ^{aB}	61.12±0.32 ^{bB}	9.984	<0.001
3	100 µg/ml	66.24±0.25 ^{aC}	72.35±0.26 ^{bC}	19.982	<0.001
4	200 µg/ml	78.26±0.11 ^{aD}	80.32±0.26 ^{bD}	22.833	<0.001
5	400 µg/ml	88.20±0.23 ^{aE}	95.15±0.20 ^{bE}	292.651	<0.001
IC ₅₀ (mg/ml)					
		31.06	25.00		

The results of the present study are lower than those reported by Chacko *et al.* (2018) [2], who found that the plant extract exhibited 71.85 % DPPH free radical scavenging activity, while ascorbic acid showed 84.47 % inhibition at a concentration of 160 µg/ml. However, Hajam *et al.* (2022) reported that *C. igneus* Nak leaf extract showed 91.08±1.47 % inhibition at a concentration of 100 µM/ml. In another study, Thiruvassagam (2022) [16] observed that at a concentration of 20 mg/ml, the ethanolic extract of *C. igneus* exhibited 65.90 % scavenging activity, compared to 77.27 % for the standard ascorbic acid.

This variation may be attributed to several factors such as differences in extraction solvent, plant part used, geographical origin and phytochemical composition of the *C. igneus* samples. The polarity of the extraction solvent greatly influences the yield and type of antioxidant compounds extracted; for instance, methanolic or ethanolic extracts often exhibit higher scavenging activity compared to aqueous extracts due to better solubility of phenolic and flavonoid constituents. Additionally, environmental factors such as soil composition, climatic conditions and stage of leaf maturity at the time of harvest can affect the concentration of bioactive compounds. The analytical conditions including incubation time, DPPH concentration and reaction temperature may also contribute to the observed differences.

The antioxidant property of *C. igneus* may be attributed to the presence of phenolic compounds, flavonoids and other phytoconstituents known for their electron-donating ability. These bioactive compounds can effectively scavenge free radicals, thereby reduce oxidative stress and prevent cellular damage. Similar concentration-dependent antioxidant effects have been reported in other medicinal plants rich in phenolic constituents. Overall, the findings indicate that *C. igneus* leaf powder extract exhibits promising antioxidant activity, suggesting its potential use as a natural source of antioxidants in nutraceuticals and functional food applications. Further studies involving identification and quantification of active compounds, as well as in vivo antioxidant evaluations, would provide deeper insights into its mechanisms and potential health benefits.

The IC₅₀ (the microgram of extract to scavenge 50 % of the radicals) value was used for calculation using the linear regression analysis. Decreased value indicated the higher antioxidant activity. The IC₅₀ value of the *C. igneus* ethanolic extract was determined to be 31.06 µg/ml, confirming its free radical scavenging potential.

In vitro Antibacterial Activity

The antibacterial activity of the ethanolic extract of *Costus igneus* leaf powder was evaluated against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* using the disc diffusion method at

concentrations of 50, 100, 250 and 500 µg/ml. Ceftriaxone sodium served as the standard control and the mean zones of

inhibition (mm) are presented in Table 2 and Figure 3.

Table 2: Means±SD of zone of inhibition obtained by sample CP against Test organisms

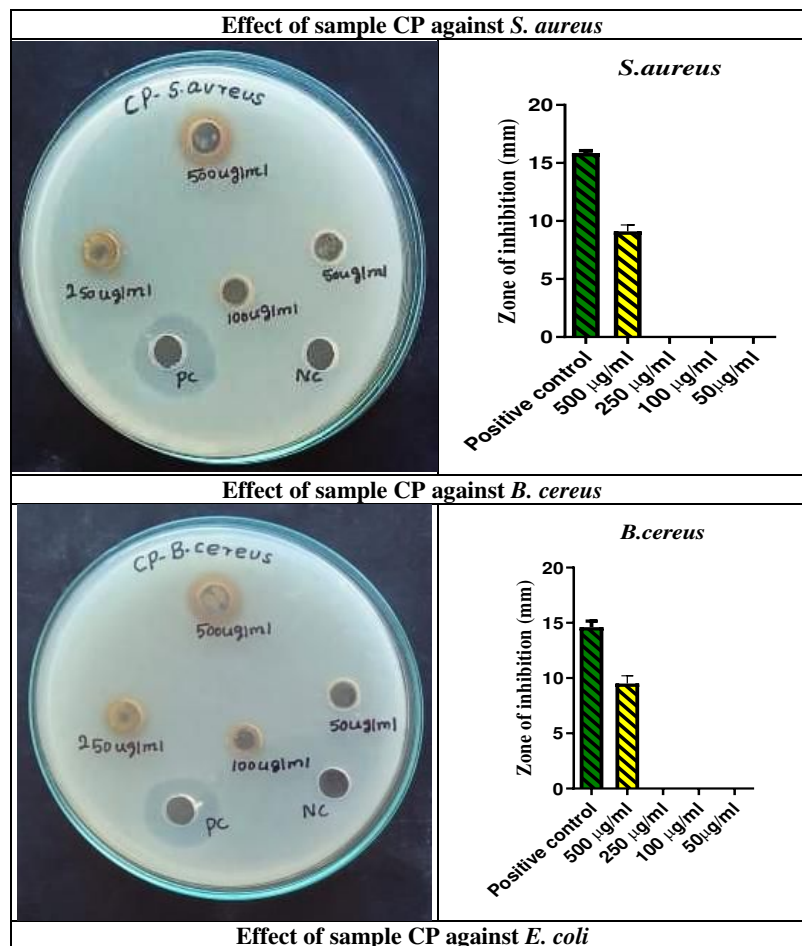
S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm)				
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	Standard control
1.	<i>S. aureus</i>	<i>Costus igneus</i>	9.1±0.56	0	0	0	15.85±0.21
2.	<i>B. cereus</i>		9.5±0.70	0	0	0	14.6±0.56
3.	<i>E. coli</i>		12.1±0.14	7.35±0.21	0	0	15.2±0.70
4.	<i>P. aeruginosa</i>		12.85±0.21	7.1±0.14	0	0	16.35±0.49

At 500 µg/ml, the extract exhibited noticeable antibacterial activity against all tested organisms, showing inhibition zones of 9.1±0.56 mm for *S. aureus*, 9.5±0.70 mm for *B. cereus*, 12.1±0.14 mm for *E. coli* and 12.85±0.21 mm for *P. aeruginosa*. Inhibition was observed at 250 µg/ml against *E. coli* (7.35±0.21 mm) and *P. aeruginosa* (7.1±0.14 mm), while no inhibition was detected at 100 µg/ml and 50 µg/ml concentrations for any organism.

Among the tested bacteria, *P. aeruginosa* exhibited the highest susceptibility, followed closely by *E. coli*, whereas *S. aureus* and *B. cereus* were less sensitive to the extract. Although the antibacterial activity of *C. igneus* leaf powder extract was lower than that of the standard ceftriaxone sodium, the results indicate that the ethanolic extract possesses concentration-dependent antibacterial potential, particularly against Gram-negative bacteria.

The present results are lower than those reported by Rao *et al.* (2016) [12], who observed that *Costus igneus* leaf extract exhibited high antimicrobial activity against *Bacillus subtilis* and *Candida parapsilosis* even at a low concentration of 2.5 µg/ml. Furthermore, they reported zones of inhibition

measuring 1.1 cm for *Escherichia coli* (10 µg/ml), 1.3 cm for *Staphylococcus aureus* (10 µg/ml) and 1.4 cm for *Bacillus subtilis* (10 µg/ml). Similarly, Damam *et al.* (2017) [3] reported that the methanolic leaf extract showed high inhibition zones against *Pseudomonas aeruginosa* (28 mm), followed by *Klebsiella pneumoniae* (22 mm), *Proteus vulgaris* (20 mm), *Staphylococcus aureus* (12 mm), *Escherichia coli* (12 mm) and *Bacillus subtilis* (11 mm), indicating comparatively lower sensitivity of *B. subtilis*. The leaf extracts were also reported to be more inhibitory towards fungi than bacteria. Thiruvassagam (2022) [16] also reported a comparable trend in the antibacterial activity of *Costus igneus* leaf powder extract, supporting the present observations. Jain *et al.*, (2024) [6] reported 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 of methanolic extract gave better results against all studied bacteria.



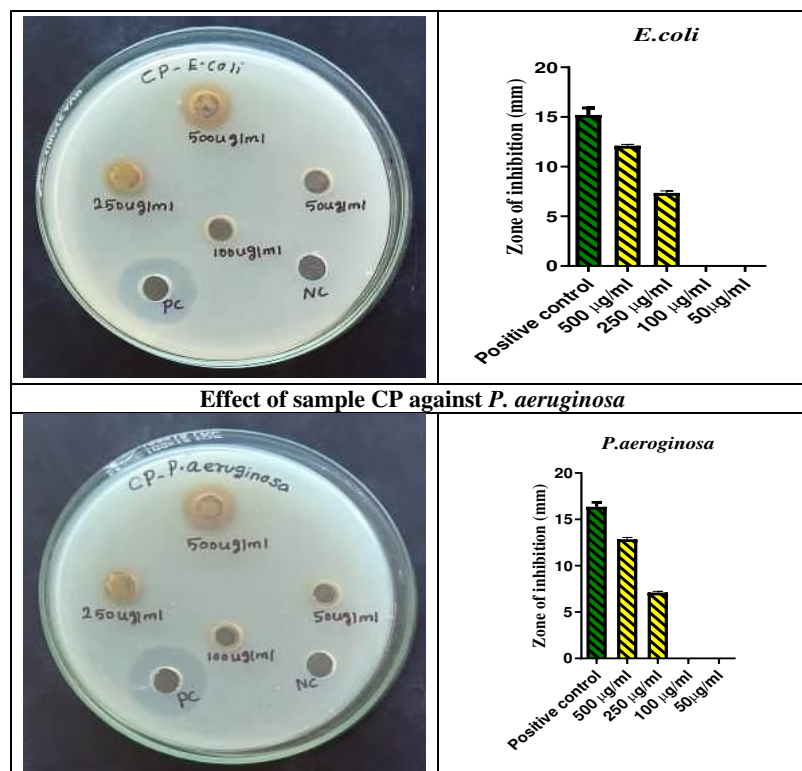


Fig 4: In vitro Antibacterial Activity of *Costus igneus* leaf powder extract

Conclusion

The present study demonstrates that *Costus igneus* leaf powder extract possesses strong bioactive potential, as evidenced by its high phenolic content, notable DPPH radical scavenging capacity and antibacterial activity. The extract showed concentration-dependent effects, with greater efficacy against Gram-negative bacteria. Although variations from earlier studies may arise from differences in extraction methods and plant characteristics, the overall findings support the use of *C. igneus* as a natural antioxidant and antimicrobial source. These results indicate its potential application in enhancing the shelf life and quality of food products. Further detailed studies are recommended to characterize active compounds and validate its effectiveness in real food systems.

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