The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(3): 120-125, 2025 www.thebioscan.com

PRELIMINARY ANTIMICROBIAL SCREENING OF LEAF EXTRACT OF *COSTUS IGNEUS* USING ETHANOL EXTRACT

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DOI: 10.63001/tbs.2025.v20.i03.pp120-125

KEYWORDS
Anti-microbial, ethanol extract, *Costus igneus*.

Received on:

02-06-2025

Accepted on:

07-07-2025

Published on:

14-07-2025

ABSTRACT

Costus igneus, also known as the "insulin plant," is a widely recognized medicinal plant in Indian traditional medicine, particularly for its role in managing diabetes and other metabolic disorders. This study explores the antimicrobial potential of Costus igneus, focusing on its ethanol extract derived from the leaves. The ethanol extract was tested for its antibacterial and antifungal activities using the agar well diffusion method against various microbial pathogens, including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus*. Results indicated significant antibacterial activity, particularly against Gram-positive bacteria, with zones of inhibition ranging from 10 mm to 25 mm. The antifungal activity showed an inhibition range of 10 mm to 14 mm, with *Aspergillus niger* being most susceptible to the extract. The study also compared the effectiveness of Costus igneus extracts to standard antibiotics (streptomycin and fluconazole), revealing comparable inhibitory effects. The findings suggest that Costus igneus, with its bioactive compounds, has considerable antimicrobial potential, making it a promising candidate for developing alternative antimicrobial agents. These results further validate its medicinal significance in folk medicine and open pathways for future research into plant-based antimicrobial agents.

INTRODUCTION

India, known for its rich biodiversity and cultural heritage, is one of the oldest repositories of medicinal plant knowledge. With more than 7,000 documented medicinal plant species, Indian traditional systems like Ayurveda, Siddha, and Unani have extensively utilized plants for the treatment of various ailments. According to Kulkarni *et al*. (2022), India is known as the Botanical Garden of the World since it is the world's greatest producer of therapeutic plants. *Costus igneus* is one of the well-known medicinal plants widely recognized for its traditional use in managing diabetes and other metabolic disorders. Commonly referred to as the "insulin plant," it has gained popularity in Indian folk medicine for its ability to regulate blood sugar levels. *Costus igneus* Nak. has been widely cultivated in tropical and subtropical regions due to its medicinal value. The various plant parts, such as leaf, stem, root, rhizome and whole plant, are shown to have important activities, such as leaves contributing to prominent hypoglycaemic potential. Leaves of *C. igneus* were one among the plants known to be effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district of Tamilnadu (Elavarasi *et al*, 2012). *Costus* is significant wellpring of diosogenin and is utilized in numerous humans and vetinary meds. It is severe astringent, cooling, stomach related, energjzer urd useful for the heart. It fixes kapha and pitta issue, dyspepsia, fever, hack and other respiratory maladies, diabetes, edema, blood illnesses, uncleanliness, und other skin sicknesses (Madhavan *et al.,* 2019). The rise of antibiotic-resistant pathogens has intensified the search for alternative antimicrobial agents, particularly those derived from natural sources. Medicinal plants are known to harbour bioactive compounds that can inhibit the growth of various microorganisms. In this context, *Costus igneus* has been explored for its inherent antimicrobial properties due to the presence of phytochemicals like flavonoids, terpenoids, and alkaloids. The potential for developing antimicrobials from higher plant appears rewarding as it will lead to the development of phytomedicines to act against microbes as a result; plants are one of the bedrocks for modern medicine to attain new principles (Sangavi *et al*., 2015).

Materials and Methods

The study plant, *Costus igneus*, was collected freshly around Pechiparai and Citraru dams located in Kanyakumari district and then processed by washing with distilled water for drying. The processed leaves were kept in a shed drying for 7 days. After drying, the leaves were ground into a fine powder and stored in a dry place for further compound extraction. About 5 grams of fine ground leaf powder was subjected to the best compound extraction system by employing 50 mL of ethanol extract. Antimicrobial activity was determined using the agar-well diffusion method as described by Perez *et al.* (1990). The organisms used in the present study was *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus*

Results and discussions

The ability of the crude ethanol extract to suppress microbial growth against bacterial and fungal pathogens was examined. Several bacterial pathogens including *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were shown to be susceptible to the antibacterial activity of *Costus igneus* leaves in the current investigation. The different samples utilized for the investigation include *Costus igneus* (E), positive control, and negative control. Based on the Zone of Inhibition, the antibacterial activity was measured.

The agar well diffusion technique was done to test the antibacterial activity of ethanol extracts of *C. igneus*. It was discovered that the plant extracts inhibited the tested microorganisms. When compared to Gram-negative bacteria, Gram-positive bacteria are shown to be more sensitive. Using the agar well diffusion technique, the ethanol extracts displayed the maximal inhibitory zone (Gram-positive bacteria had an inhibition zone of 17–25 mm, whereas Gram-negative bacteria had an inhibition zone of 16–22 mm against the positive control (Streptomycin).

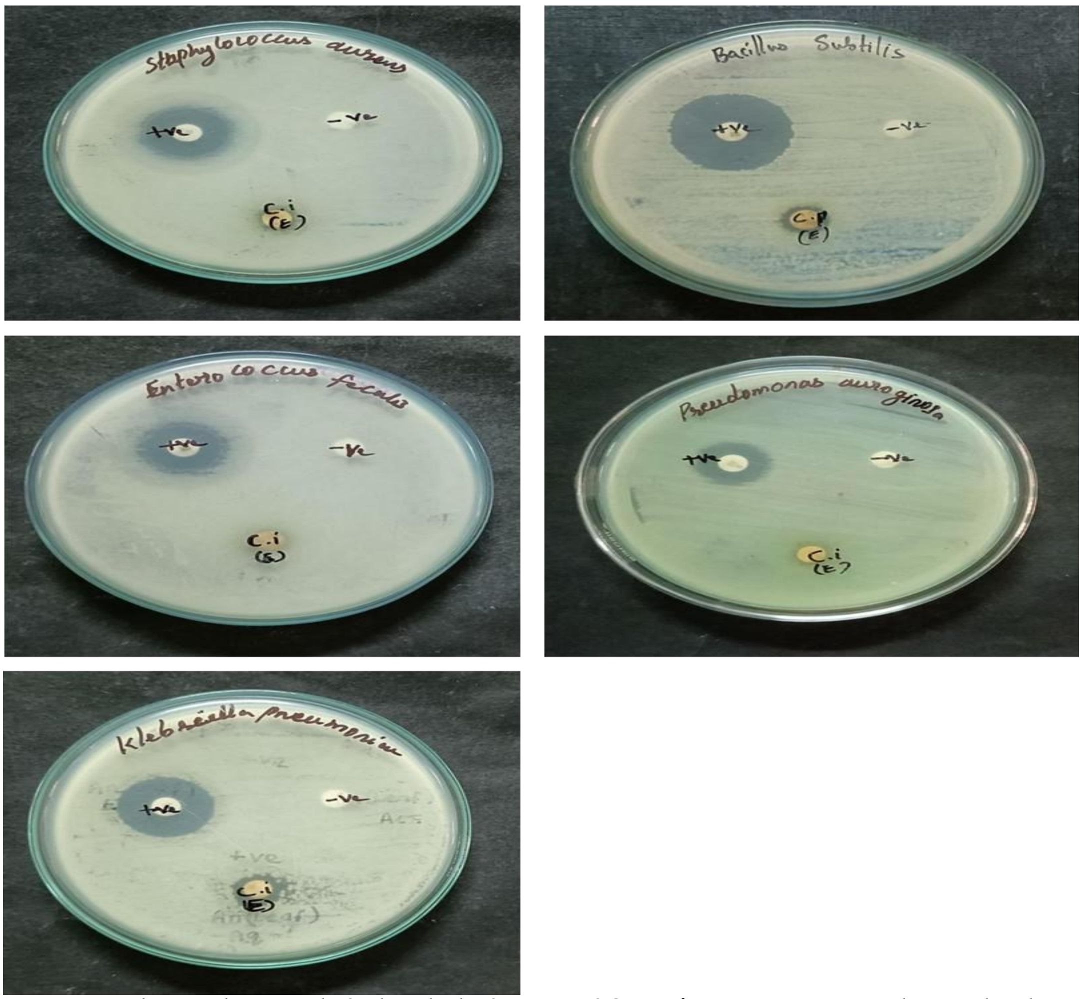

Figure 1: Antibacterial potential of Ethanolic leaf extract of *Costus igneus* against various bacterial pathogens

The positive control exhibited a notable zone of inhibition against *Pseudomonas aeroginosa* (16 mm), *Enterococcus feacalis* (17 mm), *Streptococcus aureus* (19 mm), and *Bacillus subtilis* (25 mm). *Pseudomonas aeroginosa*, a Gram- negative bacterium, had the lowest zone of inhibition (16 mm) whereas *Bacillus subtilis*, a Gram-positive bacterium showed the largest zone of inhibition (25 mm). *Klebsiella pnemoniae* cultures produce an inhibitory zone of 11 mm, followed by *Staphylococcus aureus* (10 mm) and *Bacillus subtilis* (10 mm). No zone of inhibition was observed for the bacterial strains *Enterococcus feacalis* and *Pseudomonas aeruginosa*. The significance < 0.05α (0.002032 p-value) of antibacterial activity between the extract and positive control suggested that the extract may be similar to a standard medicine, according to the bacterial growth control significance which was computed using one-tail ANOVA.

Table 1: Antibacterial Screening of Ethanolic leaf Extract of *Costus igneus*

Bacteria Strain Name	Zone of inhibition (mm in diameter)		
	Costus igneus (E)	Positive control (Streptomycin 10mg)	Negative control
Staphylococcus aureus (G+)	10	19	-
Bacillus subtilis (G+)	10	25	-
Enterococcus faecalis (G+)	0	17	-
Pseudomonas aeruginosa (G-)	0	16	-
Klebsiella pneumoniae (G-)	11	22	-
ANOVA- One Tail at 0.05 α	*The One-tail ANOVA showed the significance (0.002032) of Antibacterial activity between the extract and positive control*		

***PC** Positive control (Streptomycin), **NC** Negative control, "-"No Zone, **G+** (Gram Positive bacteria), **G-** (Gram Negative bacteria)*

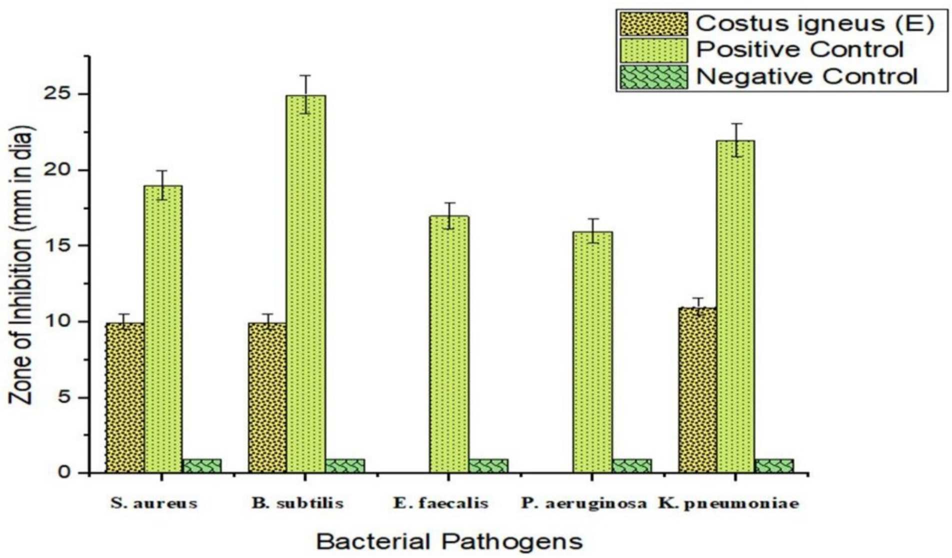

Figure 2: Graphical representation of Antibacterial potential of Ethanolic leaf extract of *Costus igneus* against various bacterial pathogens

The current investigation showed that the clinical isolates were not harmed by the negative control. Streptomycin exhibited exceptional antibacterial activity. The presence of strong phytoconstituents of the leaf extracts might be the cause of the observed action. This might be a hint that there is a good chance of separating more pure chemicals.

In this study, different concentrations of silver nanoparticles were used to check which concentration have the most antibacterial impact. The leaf extract was used to create silver nanoparticles, which turned out to be more effective. The electrostatic interaction between the positively charged nanoparticles and the negatively charged cell membrane of the microbe is most likely the source of the antibacterial activity. The green synthesis of silver nanoparticles promises an up-scalable and nontoxic method of producing a variety of metallic nanoparticles. It reflects that silver nanoparticles have an excellent antibacterial and antifungal effect.

A medicinal plants secondary metabolite may have several antibacterial activity mechanisms that might aid in preventing the development of resistance (Tadesse *et al.*, 2016). According to Taylor *et al.* (2001), extracts without antibacterial activity could possess antibacterial qualities against untested microorganisms. According to Farnsworth (1993), negative results do not always mean that the plant is inactive or that its bioactive components are absent. Some components may have antagonistic effects (This means the substances counteract or cancel each other out, rather than enhancing each other's effects) or counteract the beneficial effects of the bioactive compounds if the active principle is present in sufficient amounts (El-Sheekh *et al.*, 2014).

According to earlier research, ethanol was the most effective water miscible solvent, exhibiting a broad range of antimicrobial agents against both Gram positive and Gram-negative bacteria (Borkataky *et al.*, 2013). Because they can accomplish the same goal with fewer adverse effects than synthetic antimicrobials, plant-based antimicrobials offer huge therapeutic promise (Iwu *et al.*, 1999). Plant based antimicrobial chemicals are a huge unexplored medical resource (Gunalan *et al.*, 2011). The bioactivity of each solvent extract against the tested pathogens varied. This might be because different solvents have varying degrees of solubility for different phytoconstituents (Vasantha *et al.*, 2012). Similarly, Ethanol solvents are a reliable way to extract broad range antibacterial compounds from plants.

However, in order to evaluate the growth restriction capability of *Costus igneus* (Ethanol), antibiotic susceptibility tests were established against the fungal strains *Aspergillus flavus, Aspergillus niger* and *Aspergillus terreus*. Using a clear ruler, the inhibition zones surrounding the disc were measured in millimeters.The inhibitory zone measured using the agar disc diffusion method varied significantly in the case of fungus. In the disc diffusion technique, the ethanol extracts displayed the highest inhibitory zone. The disc diffusion methods zone of inhibition for fungal infections was 10–14 mm, whereas the zone of inhibition for the positive control (Fluconazole 25 mg) was 10–13 mm.

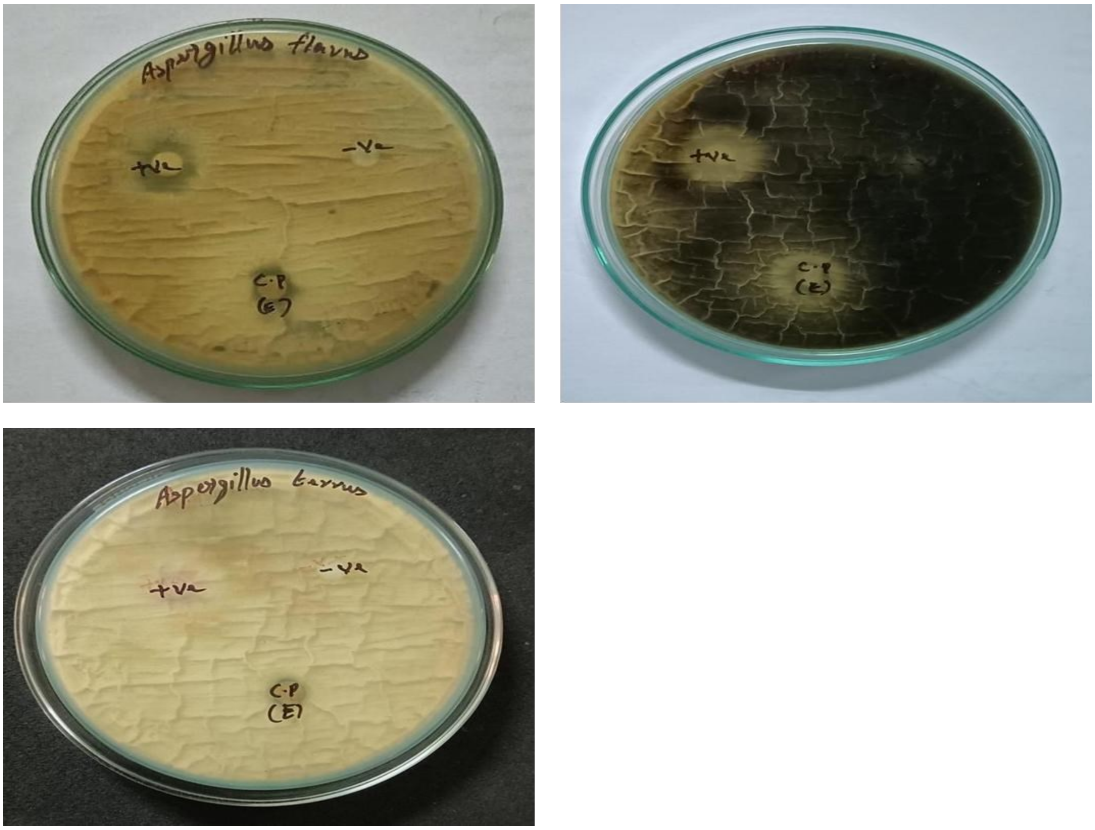

Figure 3: Antifungal Potential of Ethanolic leaf extract of *Costus igneus* against various bacterial pathogens

Costus igneus (Ethanol) had the maximum zone of inhibition of ethanolic extracts against *Aspergillus niger* (14 mm), *Aspergillus flavus* (11 mm) and *Aspergillus terreus* (10 mm). In contrast, *Aspergillus niger* (13 mm) had the largest zone of inhibition for fluconazole (positive control) followed by *Aspergillus flavus* (12 mm) and *Aspergillus terreus* (10 mm). Therefore, a significance < 0.05α (0.02054 p-value) was found in the comparative antifungal activity of *Costus igneus* (E) and fluconazole respectively. *Costus igneus* (E) was shown to be effective with both positive and negative controls.

Table 2: Antifungal screening of Ethanolic leaf extract of *Costus igneus*

Fungal pathogens	Zone of inhibition (mm in diameter)		
	Costus igneus (E)	Positive control (Fluconazole 25mg)	Negative control
Aspergillus flavus	11	12	-
Aspergillus niger	14	13	-
Aspergillus terreus	10	10	-
ANOVA- One Tail at 0.05 α	*The One-tail ANOVA showed the significance (0.02054) of Antifungal activity between the extract and positive control*		

PC *Positive control ((fluconazole),* ***NC*** *Negative control (ethanol), "-" No Zone,* ***mm*** *(Millimetre).*

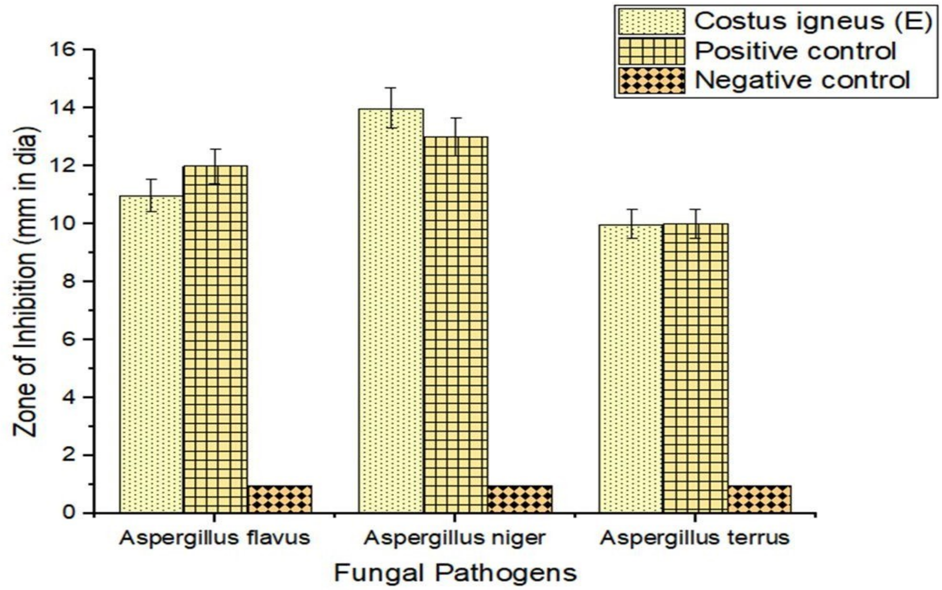

Figure 4: Graphical representation of Antifungal potential of Ethanolic leaf extract of *Costus igneus* against various pathogens

Saraf (2010) showed the antibacterial inactivity of *C. speciosus* rhizome methanol and aqueous extracts against *P. aeruginosa, S. aureus, K. pneumonia* and *E. coli*. *C. speciosus rhizome* hexane extract when tested at 12.5 mg/mL showed the highest antimicrobial property against *B. subtilis* (12 mm) and *S. aureus* (15 mm). According to Duraipandiyan *et al*., 2012. Duraipandiyan and Ignacimuthu (2011) also reported the promising antifungal property of *Costus speciosus* rhizome ethyl acetate fraction against *Aspergillus niger* and *Candida albicans*. According to Anand and Gokulakrishnan (2012), the plant extract includes flavonoids and alkaloids that have biological activity. The antimicrobial action may be attributed to these flavonoids and alkaloids. Arumugam *et al*. (2012) conducted antifungal studies on *Candida albicans* and *Aspergillus niger* with improved outcomes. The present investigation are consistent with those mentioned above.

According to Taylor *et al*. (2001), the inactivity of the aqueous extracts of the inflorescence, rhizome and stem against all tested fungi may be due to the active ingredients being present in insufficient amounts in the extract concentrations to demonstrate action. The extracts included certain phytoconstituents, which may be the cause of the observed antibacterial activity, according to the main phytochemical study. It is known that these bioactive substances have antibacterial properties and work through several mechanisms (Rao *et al*., 2011).

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