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*Original Article*

# Phytochemical Analysis and *In Vitro* Antimicrobial Activity of *Costus speciosus* Extracts

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***Costus speciosus* (J. Koenig) Sm., commonly known as crepe ginger, is primarily used as an ornamental plant worldwide. The aim of this research was to study the phytoconstituents of locally collected *C. speciosus* plant samples and test their antimicrobial effect against selected bacterial strains. Phytochemical analysis showed the presence of alkaloids, flavonoids, steroids, phenolic compounds, tannins, glycosides, and cardiac glycosides in the extracts. The extracts displayed variable degrees of antibacterial activity against the microorganisms in a bioautography experiment based on thin-layer chromatography. All the extracts showed activity against *S. aureus*, and none showed any activity against *P. aeuroginosa*. The ethanol and ethyl acetate extracts from the plant leaf and stem showed efficacy against *S. typhi* and *B. subtilis*, while the ethyl acetate extracts prevented the growth of *S. pneumoniae*. Only the ethanol extract from plant leaves had a negligible effect on *K. pneumoniae*.**

**Keywords: *Costus speciosus*, Antimicrobial activity, Phytochemical composition**

## Introduction

Plants produce various chemicals with valuable pharmacological properties. The growing demand for plant-based products in medicine and industry has resulted in extensive investigations of various plants for potential therapeutic agents. Species mostly deemed as noxious weeds or ornamental garden plants have also become targets of systemic pharmacognostic research. *Costus speciosus* is an ornamental plant that has gained much popularity because of its potent anti-diabetic effect.

*Costus speciosus* (J. Koenig) Sm., also known as *Cheilocostus speciosus* (J. König) C. Specht, or *Hellenia speciosa* (J. Koenig), belongs to the family Costaceae, under the order Zingiberales[1]. The plant is indigenous to large parts of South-East Asia, and the species is mainly grown as an ornamental plant in Bangladesh.

*C. speciosus* demonstrates a variety of pharmacological activities, such as anti-diabetic, antibacterial, antifungal, antioxidant, anti-hyperglycemic, anti-inflammatory, anti-pyretic, anti-diuretic, anti-stress effects etc[2]. In some nations, the plant's rhizome is eaten as a vegetable, while its blooms and leaves are used to make drinks[3]. Its rhizome is administered to patients with diabetes, pneumonia, constipation, skin disorders, fever, asthma, bronchitis, inflammation, anemia, rheumatism, dropsy, cough, urinary diseases, jaundice, and several other illnesses in Ayurveda and traditional medicine of other nations[1-5].

Modern scientific research has confirmed many of these medicinal properties, which are attributable to various bioactive compounds in the plant. The rhizome of this plant is a rich source of diosgenin, a compound with proven anti-diabetic[6, 7] antimicrobial[8], anti-cancer[9,10], nephroprotective[11], and anti-hyperlipidemic[12] properties. Other medically important constituents of this plant include: tigogenin, costunolide, costusoside, eremanthin, dioscin, zingibernsis newsaponin, and gracillin[13].

Several studies have shown that the phytochemical composition and ultimately the bioactivities of the plant extracts depend on the plant part used (e.g. rhizome, roots, seeds, leaves, flowers)[14], extract preparation method[15,16], solvents used in extraction[17-19], soil nutrients[20], harvesting season[21], chemotypes[22], and genetic variations (cytotypes)[23].

Multiple ethnomedical studies have supported the use of *C. speciosus* in traditional Bangladeshi medicines[24], and numerous investigations have also been conducted on the plant extract's phytochemical makeup and biological effects. One study analyzed the polyphenol contents and examined the antioxidant, analgesic and diuretic properties of the methanol extract of *C. speciosus* rhizome[25]. Another study showed that the plant rhizome methanol extract possesses CNS depressant, anxiolytic and antidepressant-like activities[26]. In a study, ethanol extract of the plant rhizome was shown to have anti-inflammatory and analgesic activity in mice and rat models[27]. This study also verified that the extract contains steroids, flavonoids, tannins, and saponins. In addition, a research group found that the methanol extract of the rhizome of *C. speciosus* had effective free radical scavenging, nitric oxide scavenging and cytotoxic activity[28]. Another investigation revealed the analgesic and anti-inflammatory properties of the

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plant leaves' methanol extract[29]. In another study, the methanol extract of *C. speciosus* seeds was discovered to be an effective anti-inflammatory agent[30]. Likewise, the methanol extract of the plant flowers was found to exert anxiolytic activity on mouse models[31].

Based on the findings from the above studies, it can be seen that mostly the methanol extract of *C. speciosus*, especially the extract of the rhizome, was used for phytochemical and bioactivity-based research in Bangladesh. Moreover, the bioactivity-based studies were concentrated on the anti-inflammatory, antioxidant, analgesic and anti-pyretic effects of the extracts. Hence, the objective of the present study was to precisely evaluate the antimicrobial effects of ethanol and ethyl acetate-based extracts of *C. speciosus* leaf and stem of a locally grown variety.

## Materials and Methods

*Sample collection and processing*: Whole plant samples (leaves and stems) were collected from the Mir Mosharraf Hossain Hall premises of Jahangirnagar University. The total weight of the samples was about 10kg. Young leaves and stems were separated (about 7 kg). The samples were rinsed with cold tap water, followed by distilled water for 2-3 times. Then the samples were left to air-dry for 7 days. Leaves and stems were powdered and stored at 4°C.

*Extract preparation*: *C. speciosus* samples were divided into leaves and stems. Extracts from the dried leaves and stems of the samples were prepared in 95% ethanol and 95% ethyl acetate (50g of each in 500 ml solvent, in 2 phases; samples soaked in solvent for 72 hours in each phase). All steps are illustrated in figure 1, as described in our previous paper (Hossain *et al.,* 2019).

*Phytochemical assays:* Standard phytochemical assays (Wagner's test for alkaloids, Salkowaski's test for steroidal compounds, froth test for saponins, lead acetate test for tannins, test for flavonoids and phenolic compounds, Killer-Killani test for glycosides) were being carried out to determine the composition of the various extracts[32].

*Antimicrobial assay:* Antimicrobial effect of the extracts were tested using the standard bi-layer well diffusion assay, against the following Gram positive microorganisms; *Bacillus subtilis, Streptococcus pneumoniae, Staphylococcus aureus*, and also Gram negetive bacteria, e.g. *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeuroginosa* and compared with standard antibiotic Ciprofloxacin. Then, the extracts were tested against the microorganisms using thin-layer chromatography (T.L.C.)-based bioautography assay[33]. The assays were performed three times for each sample.

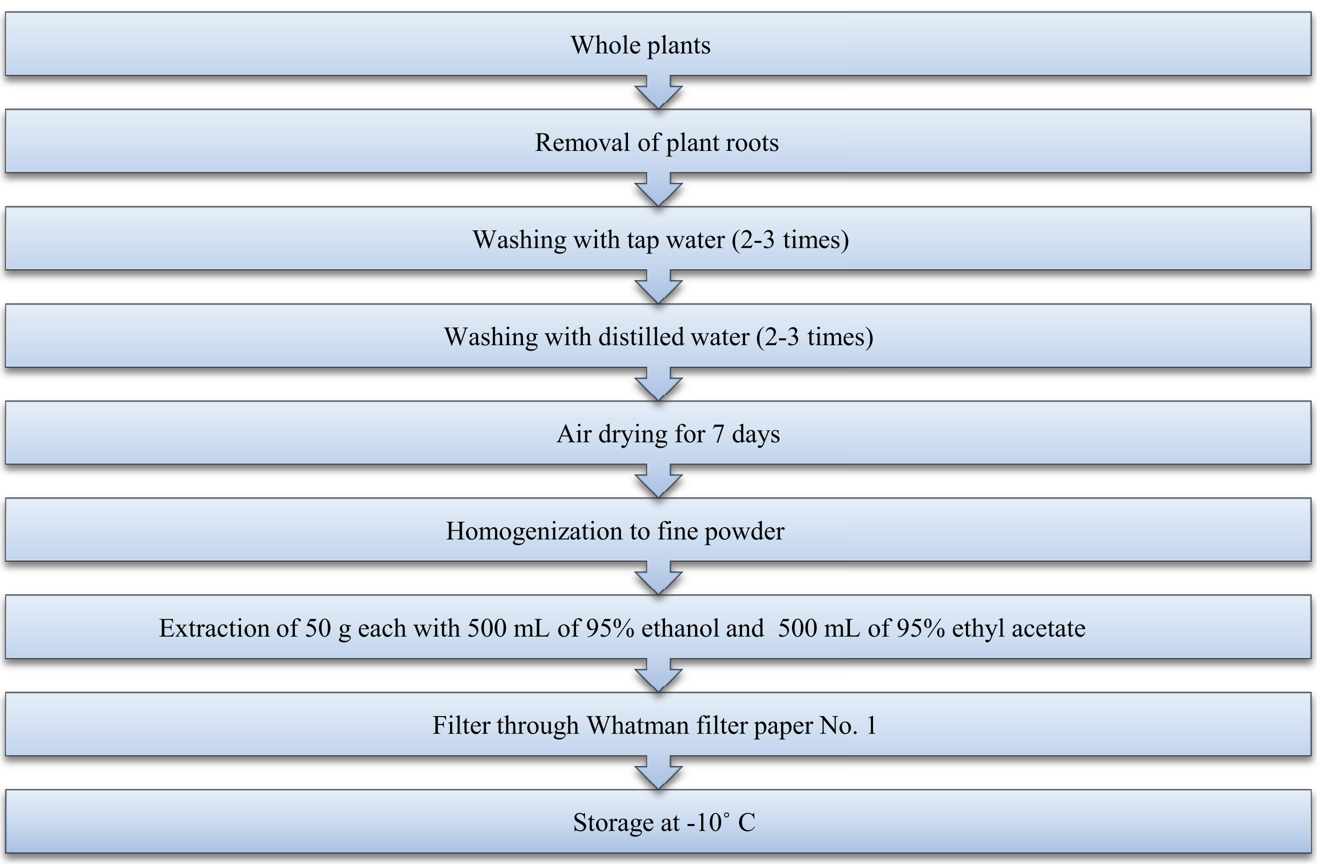


**Fig. 1:** *Plant sample preparation and extraction (adopted from Hossain et al., 2019)*

## Results

The weight of the final dried, powdered samples are listed below. The powdered samples were stored at 4ÚC while the ethanol and ethyl acetate extracts were stored at -10ÚC for further use.

**Table 1.** *Weight of samples obtained*

| Weight | Leaves | Stem |
|---|---|---|
| Wet weight | 4700g | 2000g |
| Dry weight | 365g | 128g |
| Powder weight | 297g | 114g |

*Phytochemicals assay results:* Numerous phytochemicals, which are typically found in plants, were examined in the extracts. The presence or absence of alkaloids, steroidal chemicals, phenolic compounds, flavonoids, saponins, tannins, glycosides, and cardiac glycosides was examined in the crude extracts. A summary of phytochemical test results is given in Table 2.

**Table 2:** *Summary of phytochemical analysis results for the extracts*

| Test | CSL-H | CSS-H | CSL-C | CSS-C |
|---|---|---|---|---|
| Hager's test (Alkaloids) | + | + | + | + |
| Wagner's test (Alkaloids) | + | + | + | + |
| Dragendraff's test (Alkaloids) | + | + | + | + |
| Salkowaski's test (Steroids) | + | + | + | - |
| Froth test (Saponins) | - | - | - | - |
| Flavonoids test | - | + | - | - |
| Lead acetate test (Tannins) | - | + | - | - |
| Phenolic compounds test | + | + | - | - |
| Glycosides test | + | + | - | - |
| Cardiac glycosides test | + | + | - | - |

Note*: (+) = Presence of Phytochemicals; (-) = Absence of Phytochemicals; CSL-H = C. speciosus leaves, Ethanol extract, CSS-H = C. speciosus stem, Ethanol extract, CSL-C = C. speciosus leaves, Ethyl acetate extract, CSS-C = C. speciosus stem, Ethyl acetate extract*

*Antimicrobial assay results*

The antimicrobial efficacy of the four extracts was tested against both Gram positive and Gram negative bacteria. The extracts showed no activity (no clear zone of inhibition) in the bi-layer well diffusion assay. Hence, the TLC-based bioautography assay results are summarized in the following table.

**Table 3.** *Summary of antimicrobial assay results*

| | CSL-H | CSS-H | CSL-C | CSS-C |
|---|---|---|---|---|
| *Bacillus subtilis* | + | - | + | + |
| *Streptococcus pneumoniae* | - | - | + | + |
| *Staphylococcus aureus* | + | + | + | + |
| *Klebsiella pneumoniae* | + | - | - | - |
| *Salmonella typhi* | + | + | + | - |
| *Pseudomonas aeuroginosa* | - | - | - | - |

Note: (+) = Presence of activity; (-) = Absence of activity; CSL-H = *C. speciosus* leaves, Ethanol extract, CSS-H = *C. speciosus* stem, Ethanol extract, CSL-C = *C. speciosus* leaves, Ethyl acetate extract, CSS-C = *C. speciosus* stem, Ethyl acetate extract

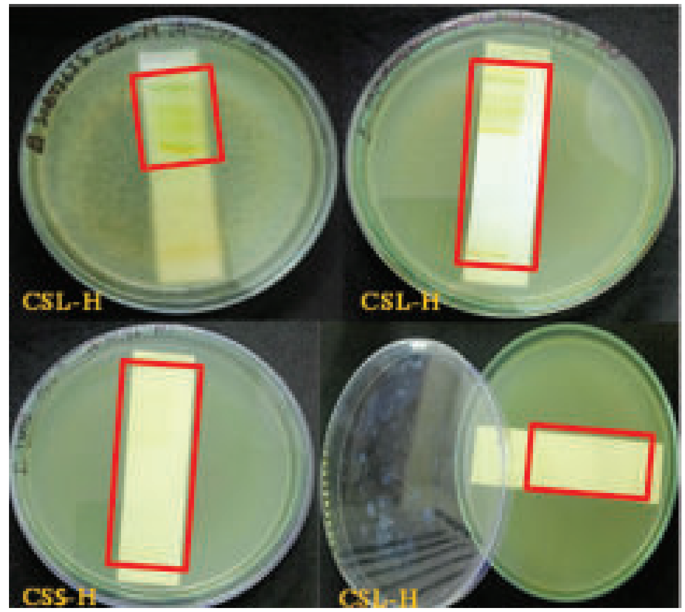


**Fig. 2:** *Antimicrobial activity of C. speciosus leaf and stem ethanol extracts, clockwise from top left corner to right; against B. subtilis for CSL-H, K. pneumoniae for CSL-H, and S. typhi for CSL-H, CSS-H(CSL-H = C. speciosus leaves, Ethanol extract, CSS-H = C. speciosus stem, Ethanol extract). The clear zones depicting active fractions of the extracts are marked in the red boxes.*

For bi-layer well diffusion assay, the ethanol and ethyl acetate extracts of *C. speciosus* leaf and stem were concentrated using a rotary evaporator at 40°C and reconstituted in $dH_2O$ and DMSO respectively. The antimicrobial efficacy of the extracts was tested against the following both Gram positive and negative bacteria. Standard antibiotic Ciprofloxacin (5 µg/disc) was used as positive control and ethanol and ethyl acetate (50 µl each) were used as negative controls. Different concentrations (50-150 µl) of the extracts were tested. However, none of the extracts showed any clear zone of inhibition in the bi-layer well diffusion assay. Hence, TLC-based bioautography assay was performed to confirm the antimicrobial activity of the extracts.

For TLC, the extracts were concentrated using a rotary evaporator at 40°C and reconstituted in $dH_2O$ and ethyl acetate respectively. Here, DMSO was not used to reconstitute the ethyl acetate extract, as DMSO caused smearing in previous TLC experiments. After the TLC run, the TLC plates were thoroughly dried in the oven at 40°C for 45 minutes to remove the solvents. Then the plates were used in the consecutive steps of the bioautography assay. After 24 hours of incubation, there were distinct clear zones of inhibition. The results are shown in figures 2 and 3, where the inhibition zones are marked in red. Active portions of the TLC plates were matched with reference plates saved earlier and marked for future work (further purification). The results show that the extracts were most active against *S. aureus* and *S. typhi*. Also, the ethyl acetate-based extracts had wider and clearer inhibition zones than the ethanol-based extracts.

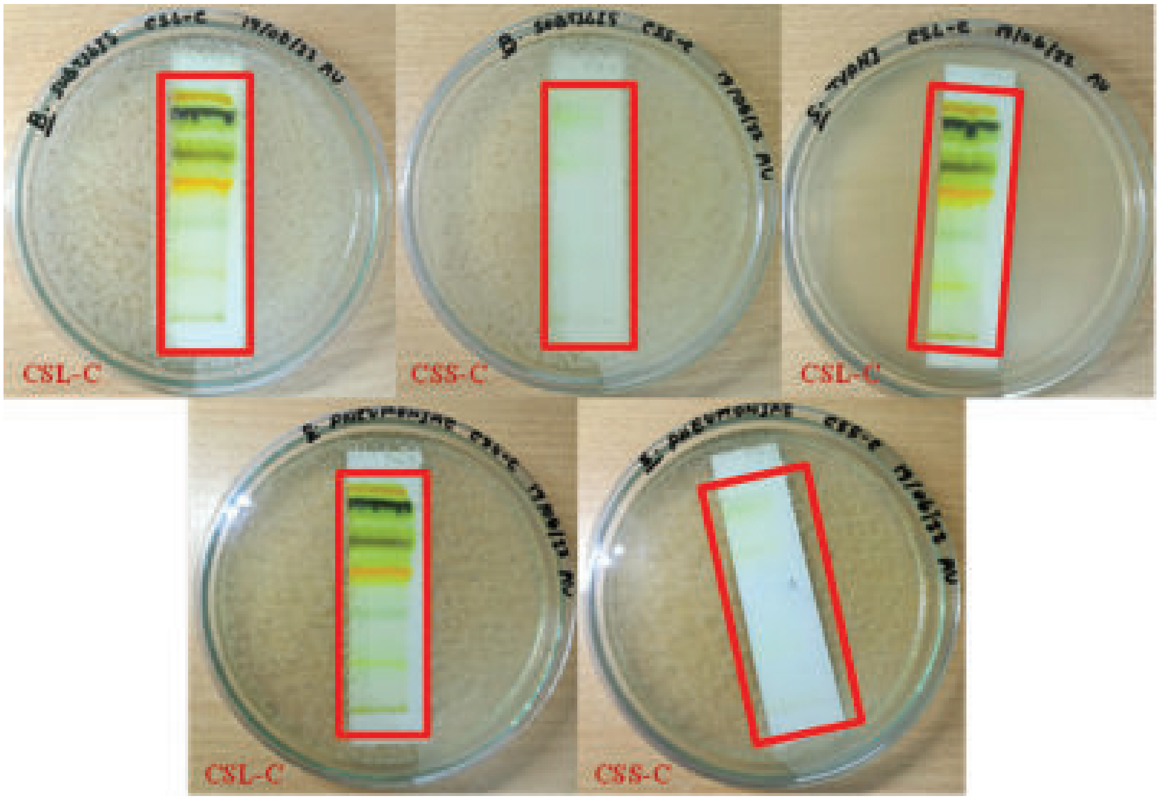


**Fig. 3:** *Antimicrobial activity of C. speciosus leaf and stem ethyl acetate extracts, clockwise from top left corner to right; against B. subtilis for CSL-C, CSS-C, S. typhi for CSL-C, and S. pneumoniae for CSL-C, CSS-C (CSL-C = C. speciosus leaves, Ethyl acetate extract, CSS-C = C. speciosus stem, Ethyl acetate extract). The clear zones depicting active fractions of the extracts are marked in the red boxes.*

## Discussion

Phytochemical analysis in this study has shown the presence of alkaloids, steroids, flavonoids, tannins, phenolic compounds, glycosides and cardiac glycosides but no saponins in ethanol extracts of *C. speciosus* leaf and stem. Similarly, alkaloids and steroids were found in the plant leaf and stem ethyl acetate extracts, but no flavonoids, saponins, tannins, phenolic compounds, glycosides or cardiac glycosides were detected.

Earlier studies have reported the presence of many compounds in different solvent-based extracts of *C. speciosus* plant parts. One study reported the presence of flavonoids, alkaloids, glycosides, steroids, phenols, tannins, saponins, and a resin in the methanol extract of the plant leaves[34]. Another study reported that the plant rhizome ethyl acetate extract contained quinone, glycoside, cardiac glycoside, terpenoids, but no alkaloids, steroids, flavonoids, phenolic compounds, saponins and tannins[35]. Presence of alkaloids, flavonoids, cardiac glycosides, saponins, sterols, tannins and anthroquinone glycosides in plant rhizome were reported in another research[36].

In the present study, none of the extracts showed any clear zone of inhibition in the bi-layer well diffusion assay. This could be due to the improper diffusion of non-polar active compounds into the agar media. Hence, thin-layer chromatography was done for the extracts and then a direct bioautography assay was performed. Interestingly, the extracts showed significant clear zones of inhibition in this assay. The reason for the extracts showing no activity in bi-layer well diffusion assay, but having potent activity in TLC-based bioautography assay could also be explained by the phenomenon of inhibition. In bi-layer well diffusion assay, some of the components in the extracts might have inhibited the antimicrobial activity of the other components. But this inhibition was negated in bioautography assay, as the components were first separated in TLC. In TLC-based bioautography assay, ethanol extract of the plant leaves showed activity against *B. subtilis*, *S. aureus*, *K. pneumoniae*, and *S. typhi*. Ethyl acetate extract of the plant leaves inhibited the growth of *B. subtilis*, *S. pneumoniae*, *S. aureus*, and *S. typhi*. These results are supported by the findings of Mahendranathana and Abhayarathne 2021[16]. Ethanol extract of the plant stem showed activity against *S. aureus* and *S. typhi*. Ethyl acetate extract of plant stem showed a clear zone of inhibition against *B. subtilis*, *S. pneumoniae*, and *S. aureus*. From the results, it can be seen that the extracts showed the most activity against *S. aureus* and strong activity against *B. subtilis* and *S. typhi*.

Ethanol-based extracts showed greater activity (clearer zones) near the upper regions on the TLC plates. Ethyl acetate-based extracts showed clear zones spread uniformly along the length of the TLC plates. As ethyl acetate is less polar than ethanol, ethanol-based plant extracts would contain more polar compounds than ethyl acetate-based extracts. The TLC solvent was an equal mixture of ethyl acetate (low polarity) and hexane (non-polar). It can be presumed that, for ethanol-based extracts, the antimicrobial compounds are less polar in nature, as they travelled further than the inactive fractions. It can also be deduced that the antimicrobial components in ethyl acetate-based extracts are less polar or non-polar. Moreover, the ethyl acetate-based extracts showed greater activity than the ethanol-based extracts in multiple replicates of the TLC-based bioautography assay. Hence, it can be hypothesized that the plant extracts' antimicrobial components are mostly non-polar.

A literature review shows that the extracts of *C. speciosus* have antimicrobial activity against several species. One study tested the antibacterial activity of the acetone, ethanol and aqueous extracts of the leaves of *C. speciosus* against *Escherichia coli* and *S. aureus*. In the agar well diffusion method, the acetone-based extracts showed the most potent inhibitory actions, while the ethanol extracts showed the lowest level of inhibition[16]. Methanol, petroleum ether, chloroform, and acetone extracts of *C. speciosus* leaves were reported previously to show activity against *S. aureus*, *B. subtilis*, *S. typhi*, *E. coli*, *Candida albicans*, and *Aspergillus oryzae*[34]. The n-hexane partition of the methanol extract of plant stems and flowers was shown to have potent inhibitory activity against *Mycobacterium tuberculosis* H37Rv. The extract's effect on the bacterial cellular structure mediated the anti-TB activity. GC-MS phytochemical analysis of these extracts confirmed that most of the active compounds were lipophilic fatty acids[37]. However, another study reported that *C. speciosus* leaf hexane, methanol and aqueous extracts showed no activity against *B. subtilis*, *E. coli*, *K. pneumoniae*, and *S. aureus*[17].

Most of the previous studies on the antibacterial activity of *C. speciosus* focused on rhizome extracts. One such study found that the aqueous extract of the plant rhizome was effective against *S. aureus*, *Staphylococcus epidermis*, *E. coli*, *P. aeruginosa*, and *S. typhimurium*[38]. Another study showed that the plant rhizome aqueous, methanol, hexane and ethyl acetate extracts showed insignificant activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli* and *S. typhi*[35]. Hexane, chloroform, ethyl acetate, and methanol extracts of the plant rhizome were reported to have antifungal activity in low concentration[15]. The hexane extract showed the maximum activity against *Trichophyton mentagrophytes, Trichophyton rubrum*, *Epidermophyton floccosum*, and *Curvulari lunata.* Another research group reported that the chloroform:ethyl acetate (1:1), ethyl acetate and total methanol extracts of plant rhizome significantly inhibited the growth of *Bacillus cereus.* The chloroform fraction showed potent activity against *S. aureus* and *Bacillus cereus.* The total methanol extract and chloroform fractions had strong activity against *C. albicans*[39]. *C. speciosus* rhizome methanol extract has antifungal activity against *A. niger, Rhizopus oryzae, Aspergillus terreus, Cladosporium species, Colletotricum crassipes, Collectotricum capsici, Armillaria mellea,* and *C. albicans*[40]. Another study tested the antibacterial activity of petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, and aqueous extracts of the plant rhizome. Results showed that the extracts had activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *Bacillus pumalis*, *C. albicans*, and *E. coli*[41]. A bioactive principle in ethanol extract of plant rhizome was found to inhibit *B. subtilis*, *S. aureus*, *Proteus mirabilis*, *B. cereus*, *E. coli*, *S. typhi*, *P. aeruginosa*, *Staphylococcus epidermidis*, and *C. albicans*[42].

One study showed that the antibacterial activity of rhizome extracts obtained through different extraction procedures. It was found that when the cold percolation method was used to obtain the chloroform, acetone, ethanol and aqueous extracts of plant rhizome, the extracts had no activity against *K. pneumoniae*, *S. aureus*, *Pseudomonas vulgaris*, *P. aeruginosa*, *C. albicans*, and *Aspergillus niger*. When the Soxhlet method was used to prepare the aqueous extracts of plant rhizome, the extracts showed activity against *K. pneumoniae* only[43]. Another study reported that the plant rhizome's hexane and methanol extracts showed activity against *B. subtilis*, *E. coli*, *K. pneumoniae*, and *Staphylococcus aureus*[17]. However, the aqueous extract of plant rhizome showed no such activity. Another study reported that the methanol extract of plant rhizome failed to inhibit the growth of *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*, while the aqueous extract had antibacterial activity against *S. aureus*[36].

It can be seen from the above studies that there was no research done on the antibacterial activities of the ethyl acetate extracts of *C. speciosus* leaves and ethanol and ethyl acetate extracts of the plant stem, to the best of the researchers' knowledge. Hence, the present study tested the antimicrobial activity of the plant leaves and stems' ethanol and ethyl acetate extracts.

In the current study, the ethyl acetate-based extracts showed greater activity than the ethanol-based extracts (large and distinct zone of inhibition). Further research is needed to identify the specific compounds responsible for antimicrobial activity in these extracts.

Few studies reported the association of various endophytic bacteria and fungi with different parts of *C. speciosus* plant[44-49]. These research works also showed that the endophytes had effect on plant growth[44-47]. Some studies showed that the endophytic microorganisms produced pharmacologically active substances, such as antifungal and anti-cancer compounds[48, 49]. From these studies on endophytes associated with *C. speciosus*, it can be presumed that some of the bioactivities of *C. speciosus* plant extracts could be due to the presence of these endophytes. As traditional medicine practitioners mostly use aqueous extracts of this plant to treat diseases, the components secreted by endophytes would likely be present in the prepared decoctions. One of the future objectives of this current study would be to study the endophytes associated with locally grown *C. speciosus* plants. Again, *C. speciosus* is chemically divergent, as it can have many chemotypes and cytotypes[20, 21]. Hence, another objective of this study would be to identify the chemotypes and cytotypes of *C. speciosus* plants in Bangladesh.

The primary aim of this current study was to do preliminary testing of the antimicrobial effects of *C. speciosus* leaf and stem extracts against selected pathogens. Future works would include testing out the efficacy of these extracts against other pathogens, identifying the specific antimicrobial lead compounds through chromatographic techniques, testing the antioxidant properties of the isolated lead compounds, and determining their molecular structures.

## Conclusion

*C. speciosus* plant extracts have been studied extensively in many South and South-East Asia countries. However, this species is yet to be thoroughly investigated here in Bangladesh. The current study analyzed the phytochemical constituents and antimicrobial activity of ethanol and ethyl acetate-based extracts of *C. speciosus* leaf and stem. Future research focusing on the locally grown *C. speciosus* will help understand their chemical constituents and potential applications. This work aims to continue exploring native *C. speciosus* and other species of the genus *Costus* as sources of antimicrobial lead molecules.

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## References


1. Costus speciosus (J. Koenig) Sm. | Species. (n.d.). India Biodiversity Portal. Retrieved October 17, 2022, from https://indiabiodiversity.org/species/show/229300
2. Srivastava S, Singh P, Mishra G, Jha KK and Khosa RL. 2011. Costus speciosus (Keukand) : a review. *Der Pharm. Sin.* 2 (1), 118–128.
3. Van Wyk BE and Wink M. 2004. In: Medicinal Plants of the World. 1st ed. Pretoria, Briza
4. Shobana S and Akhilender Naidu K. 2000. Antioxidant activity of selected Indian spices. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 62, 107–110
5. Jain SK. 1991. In: Dictionary of Indian Folk Medicine and Ethno botany. Deep Publications, New Delhi
6. Kim DY and Choi BY. 2019. Costunolide-A bioactive sesquiterpene lactone with diverse therapeutic potential. *Int. J. Mol. Sci*. 20, 2926.
7. Sohrab S, Mishra P and Mishra SK. 2021. Phytochemical competence and pharmacological perspectives of an endangered boon-Costus speciosus (Koen.) Sm.: a comprehensive review. Bull. Natl. Res. Cent. 45, 209.
8. Oliver BO. 1980. Hypoglycemic plants in West Africa. *Journal of Ethnopharmacology*. 2, 119–128.
9. Selim S and Al Jaouni S. 2015. Anti-inflammatory, antioxidant and antiangiogenic activities of diosgenin isolated from traditional medicinal plant, Costus speciosus (Koen ex.Retz.) Sm. *Natural Product Research*, 30(16), 1830–1833.
10. Liu M J, Wang Z, Ju Y, Wong RNS and Wu QY. 2004. Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca2+ homeostasis. *Cancer Chemotherapy and Pharmacology,* 55(1), 79–90.
11. Mishra P, Mandlik D, Arulmozhi S and Mahadik K. 2021. Nephroprotective role of diosgenin in gentamicin-induced renal toxicity: biochemical, antioxidant, immunological and histopathological approach. *Future Journal of Pharmaceutical Sciences*, 7(1).
12. Gong G, Qin Y, Huang W, Zhou S, Wu X, Yang X, Zhao Y and Li D. 2010. Protective effects of diosgenin in the hyperlipidemic rat model and in human vascular endothelial cells against hydrogen peroxide-induced apoptosis. *Chemico-Biological Interactions*, 184(3), 366–375.
13. Akbar S. 2020. Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications (1st ed. 2020). Springer.
14. Gupta MM, Farooqui SU and Lal RN. 1981. Distribution and Variation of Diosgenin in Different Parts of Costus speciosus. *Journal of Natural Products*, 44(4), 486–489.
15. Abd M, Majeed A, Ahmad HS, Hasan AS, Al-Dulaimi FKY and Shihab H. 2019. Effect of Costus Specious Extract on Some Types of Pathogenic Bacteria. *J. Global Pharma. Techno.* 11(2), 481-485
16. Duraipandiyan V, Al-Harbi NA, Ignacimuthu S and Muthukumar C. (2012). Antimicrobial activity of sesquiterpene lactones isolated from traditional medicinal plant, Costus speciosus (Koen ex.Retz.) Sm. *BMC. Complement. Altern. Med.* 12(1), 13.
17. Mahendranathan C and Abhayarathne A. 2021. Efficacy of different solvent extracts from the aerial parts of Costus speciosus (Koen.) for the potential antibacterial activity against selected human pathogenic bacteria. *Int. J. Res. Publi.* 78(1).
18. Malabadi RB. 2005. Antibacterial activity in the Rhizome extracts of Costus speciosus (Koen). *J. Phyto. Res.* 18(1), 83–85.
19. Yadav RL, Anwar M, Singh R and Singh DV. 1983. Response of Costus speciosus Koen. to nitrogen, phosphorus and potassium fertilizer. *The J. of Agri. Sci.* 101(3), 755–756.
20. Rawat P, Kumar M, Srivastava A, Kumar B, Misra A, Pratap Singh S amd Srivastava S. 2021. Influence of Soil Variation on Diosgenin Content Profile in Costus speciosus from Indo Gangetic Plains. *Chemistry and Biodiversity*, 18(6).
21. Mitra P, Ghosh T and Mitra PK. 2019. Seasonal Effect on In Vitro Antioxidant Activity of Costus speciosus Leaves. *Haya: The Saudi Journal of Life Sciences*. 4(4), 164–168.
22. Srivastava S, Srivastava A, Kumar M, Misra A, Shukla P and Agrawal P. 2019. Evaluation of diosgenin content in Costus speciosus germplasm collected from Eastern Ghats of India and identification of elite chemotypes. *Pharmacognosy Magazine*. 15(66), 462.
23. Lodh D and Basu S. 2013. Karyomorphological analysis and cytotypic diversity in natural populations of Costus speciosus Koen. ex Retz. *The Nucleus*. 56(3), 155–162.
24. Hossain S, Rahman S, Morshed MT, Haque M, Jahan S, Jahan R and Rahmatullah M. 2013. Tribal Cross-Talk as an effective way for ethnobotanical knowledge transfer - inference from Costus specious as a case study. *Ameri-Eur. J. Sust. Agri*, 7(5), 373–390.
25. Ershad Naznin N, Mazumder T, Sharif Reza M, Jafrin S, M. Alshahrani S, M Alqahtani A, Alqahtani T and FM Shahid Ud Daula A. 2022. Molecular docking supported investigation of antioxidant, analgesic and diuretic effects of Costus speciosus rhizome. *Bull of Chem. Soc. of Eth.* 36(3), 627–640.

**26.** Banik S, Chen U, Hussain M, Mazumder T and Naim Uddin S 2019. Neuropharmacological evaluation of methanolic extract of Costus speciosus Linn. rhizome in Swiss albino mice. *Asian Pacific Journal of Tropical Biomedicine*, 9(5), 217.

**27.** Alam A, Subhan N, Awal A, Alam S and Akramudau K. 2008. Anti-inflammatory and anti-noceceptive action of the crude extracts of Costus specious on rodents. Oriental Pharmacy and Experimental Medicine, 8(3), 243–251.

**28.** Jha MK, Alam MB, Hossain MS and Islam A. 2010. In vitro antioxidant and cytotoxic potential of costus speciosus (koen.) smith rhizome. *International J of Pharma. Sci. and Res.* Vol. 1 (10), 138-144

**29.** Azam S, Ansari P, Jalil S, Ibrahim AH, Sultana N, Hossain MM, Naveed JM and Hossain MF. 2016. Antinociceptive activity investigation of the methanolic crude extract of Costus speciosus in Mice. *Progress in Nutrition*. 18(4), 436–442.

**30.** International Journal of Pharmacognosy and Phytochemical Research 2016; 8(3); 524-530

**31.** Chen U, Bhuiyan FR and Yeasmin F. 2019. Anxiolytic activity of methanolic extract of Costus speciosus flower on swiss albino mice. J.of Pharma. and Phyto. 8(4), 3449-3451.

**32.** Harborne JB. 1984. Phytochemical Methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York.

**33.** Choma IM and Grzelak EM. 2011. Bioautography detection in thin-layer chromatography. *J. of chromatography*. 1218(19), 2684–2691.

**34.** Arunprasath A and Gomathinayagam M. 2014. Qualitative study of Costus speciosus (Koen ex. Retz.) Sm. and its potentiality against human pathogenic microbes. *Int. J. Pharmaceut. Biol. Arch.* 5, 93–98.

**35.** Shaikh SS, Bawazir AS and Yahya BA. 2022. Phytochemical, Histochemical and In Vitro Antimicrobial Study of Various Solvent Extracts of Costus speciosus (J. Koenig) Sm. and Costus pictus D. *Don. Turkish. J. Pharma. Sci.* 19(2), 145–152.

**36.** Saraf A. 2010. Phytochemical and Antimicrobial Studies of Medicinal Plant Costus Speciosus(Koen.). *E-Journal of Chemistry*. 7(s1), S405–S413.

**37.** Mohamad S, Ismail NN, Parumasivam T, Ibrahim P, Osman H and A Wahab H. 2018. Antituberculosis activity, phytochemical identification of Costus speciosus (J. Koenig) Sm., Cymbopogon citratus (DC. Ex Nees) Stapf., and Tabernaemontana coronaria (L.) Willd. and their effects on the growth kinetics and cellular integrity of *Mycobacterium tuberculosis* H37Rv. *BMC Comp. and Alter. Med.* 18(1).

**38.** Ariharan VN, Devi VNM, Rajakokhila M and Prasad PN. 2012. Antibacterial activity of Costus speciosus rhizome extract on some pathogenic bacteria. *Int. J. Adv. Life Sci.* 4, 24-27.

**39.** Ibrahim SRM, El-Shaer NS ADA, Asfour HZ, Elshali KZ, Shaaban MIA, Al-Attas AAM and Mohamed GAA. 2019. Antimicrobial, antiquorum sensing and antiproliferative activities of sesquiterpenes from Costus speciosus rhizomes. *Pak. Jo. Pharm. Sci.* 32(1), 109–115.

**40.** Sulakshana G and Sabitha Rani A. 2015. In vitro evaluation of antifungal activity in three different species of Costus. *World J. Pharm. Res.* 4(9), 1139–1144.

**41.** Mar O. 2020. Study on some heavy metal contents, nutritional values and antimicrobial activity of rhizome of Costus speciosus (Koen.) Sm. *J. Myan. Aca. Arts and Sci*. 18(4A).

**42.** Borkataky M, Kakoti BB and Saikia LR. 2017. Isolation of Antimicrobial Principle from Costus speciosus (Koen Ex. Retz.) Sm. *Nat. Aca. Sci. Letters*. 40(5), 383–387.

**43.** Kurdekar RR, Hegde GR, Hegde, Gurumurthi and Hebbar SS. 2012. Antimicrobial screening of Medicinal plants against human Pathogens- A Comparative account of two different methods of Extraction. *Int. J. Drug Develop. Res.* 4(1): 82-89.

**44.** Barman D and Dkhar MS. 2018. Plant Growth-Promoting Potential of Endophytic Bacteria Isolated from Costus speciosus in Tropical Deciduous Forest of Eastern Himalaya. In: Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 89(3), 841–852.

**45.** Momin MD and Tripathi SK. 2018. Studies of Endophytic Actinomycetes Associated with Medicinal Plants of Mizoram, Northeast, India. *Int. J. Current Microbiol. and Appl. Sci.* 7(12), 1398–1407.

**46.** Barman D and Dkhar MS. 2020. Seasonal Variation Influence Endophytic Actinobacterial Communities of Medicinal Plants from Tropical Deciduous Forest of Meghalaya and Characterization of Their Plant Growth-Promoting Potentials. *Current Microbiol*. 77(8), 1689–1698.

**47.** Ding T and Melcher U. 2016. Influences of plant species, season and location on leaf endophytic bacterial communities of non-cultivated plants. *PLoS ONE*. 11(3):e0150895

**48.** Qader MM, Kumar NS, Jayasinghe L, Araya H and Fujimoto Y. 2017. Bioactive sesquiterpenes from an endophytic fungus Bipolaris sorokiniana isolated from a popular medicinal plant Costus speciosus. Mycology, 8(1), 17–20.

**49.** Hazalin NA, Ramasamy K, Lim SSM, Wahab IA, Cole AL and Abdul Majeed AB. 2009. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. BMC Complementary and Alternative Medicine, 9(1).