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A Study on Antibacterial Properties of *Costus speciosus*

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Abstract

Antibiotic resistant bacteria have posed a serious threat to world health in recent years, forcing the investigation of alternate sources for antibacterials. Higher plants are a key source of medicinal compounds. The objective of this study was to evaluate the antibacterial properties of *C. speciosus* against gram-negative *E. coli* and gram-positive *Staphylococcus aureus*, to explore its potential as a natural alternative to conventional antibiotics. Leaves, stems, and rhizomes of *C. speciosus* were extracted using hexane, methanol, and water as solvents with reflux, sonication and maceratio. Antibacterial activity was assessed using the disk diffusion assay, with gentamycin as a positive control. The stem-methanolic extract showed greater inhibition against both organisms compared to hexane extracts. Stem-water extracts were effective only against *S. aureus*. The rhizome-methanolic extract inhibited *E. coli* more than *S. aureus*, while the hexane extract was more effective against *S. aureus.* The rhizome-water extract inhibited the growth of both bacterial strains. Leaf extracts demonstrated significant inhibition against *E. coli* but no activity against *S. aureus*. This study shows that *C. speciosus* has significant antibacterial properties, varying with plant part, solvent, and organism. Given the rise in antibiotic-resistant bacteria, *C. speciosus* is a promising natural source of antibacterial agents.

Keywords: Costus speciosus; Antibacterial Activity; Escherichia coli; Staphylococcus aureus; Natural Antibiotics

Introduction

Around 70-80% of the global population extensively utilizes herbal medications for primary healthcare due to their accessibility and affordability. Herbal plants' phytochemical components are well-tolerated by the human body and are popular for their economic value due to their use of renewable raw ingredients and eco-friendly techniques. The upsurge of bacteria that are resistant to antibiotics has posed a serious threat to world health in recent years, forcing the investigation of alternate sources for antibacterial medicines. *Costus speciosus* (family: Costaceae), often known as "Spiral Ginger" or "Crepe Ginger," has captured interest for its possible pharmacological effects. The antibacterial characteristics of *C. speciosus* are thoroughly examined in this research, revealing insight into the plant's potential as a source of novel antimicrobial agents. The previously unexamined stem was also tested to evaluate its potential antibacterial activity.

The plant is a rhizomatous herb having erect or spreading stems that can grow to a height of 2-3 meters. Crepe ginger is a frequent name for blossoms because they resemble crepe paper. *C. speciosus*, has 175 species (Ariharan*, 2012) and various common names in various languages, including Thembu, kemuka, Kushta, Kashmira, Shura, Katar katar, Pushpamoola, Kashmeeramu, Keukand, Keu, and Koshtam. *C. speciosus* is found in moist, wet, evergreen areas in Indo-Malaya and Sri Lanka (Pawar, 2012), Asia, Africa, and the Americas, and

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in mountain ranges in India. (Maji, 2020) Diosgenin, an antidiabetic compound used to treat Diabetes mellitus, is mostly found in the rhizome (Roy, 1977) Moreover, it contains various compounds such as flavonoids, alkaloids, cardio glycosides, saponins, sterols, tannins, arbutin, essential oils, phenols, carboxylic acids, valepotriates, steroids, and sterols. (saraf,2009) The rhizomes and roots are known for their antibacterial properties in Indian traditional medicine (Warrier PK, 1995).

The extract from the rhizome of *C. speciosus* showed antibacterial properties against Gram-positive bacteria like *B. subtilis* and *S. aureus* as well as Gram-negative bacteria like *K. pneumoniae*, the extract from the leaf hasn't shown any antibacterial activity. Another intriguing finding is the absence of any fungal, bacterial, viral, or insect disease incidents in the plant organs of either the natural population (in the natural locality) or the cultivated fields of *C. speciosus*. Plants exhibit built-in disease resistance, possibly due to the presence of phenolic and alkaloid compounds in their organs. The findings are promising for the potential application of *C. speciosus* rhizome aqueous extract as a bio-bactericide and herbal remedy like Zingiber officinale (Ariharan*, 2012).

The current study intends to look at the antibacterial abilities of *C.speciosus* against gram-positive bacteria such as *S. aureus* as well as gram-negative bacteria including *E.Coli*. The *C.speciosus* plant will be used in the study, and bioactive chemicals will be extracted from its leaves, stems, and rhizomes using three different solvents: Hexane (non-polar), Methanol (polar), and Water (polar). The disc diffusion assay will be used to measure the antibacterial effects of these extracts, along with Gentamycin serving as the reference antibiotic. Three major extraction methods were implemented for all three leaf, stem, and rhizome organs. After observing the results crude extraction was done only for the leaf organ because leaves didn't give any positive results for any of the above extraction methods.

Materials and Methods

1.Collection and preparation of plant materials

Fresh samples of Costus speciosus stem, rhizome, and leaf were collected from Karapitiya, Southern Province, Sri Lanka. They were thoroughly washed under running tap water. The mud was wiped off the rhizomes' surface using a brush. Tissue papers were used to wipe away excess water. After being air-dried, they were sliced into 0.5 mm-thick slices using a scalpel. The fresh weight was measured and recorded, and the pieces were dried in a hot oven at 60^{o}C for 4 hours at a time until completely dried. The dry weight was recorded. Plant pieces were allowed to cool and powdered in a grinder to obtain a fine powder. The powders were stored separately in air-tight containers and kept in a dry, cool place.

2.Extraction techniques

Four Extraction techniques were used as seen in Table 1.

3.Preparation of extracts

The extracts were filtered through a funnel using Whatman no.1 filter paper. In a previously weighed evaporating dish, the filtrate was collected. The weight was recorded following collection. It was kept in a water bath and allowed to evaporate. When the solvent had entirely evaporated, the evaporating dish was weighed once more, and the weight of the extracts was calculated. The leftover residue (extracts) was scraped out with a clean spatula and inserted into vials and stored at 4^{o}C.

4. Stock solution preparation

The stock solutions were prepared right before the antibacterial testing. To reach a concentration of 100 mg/ml for each, aqueous extracts were dissolved in distilled water, methanol extracts in DMSO, and hexane extracts in hexane, all following the weight of the extracts. Subsequently, a 50 mg/ml solution was created by diluting the 100mg/ml stock solution.

5. Bacterial inoculum preparation

All bacterial strains' pure cultures were chosen. To create isolated colonies, the stock culture was streaked on Luria-Bertani (LB agar) media. Following 16 hours of incubation at 37 ^{0}C, a pure colony was chosen using an inoculating loop and moved into a 500 mL Erlenmeyer flask containing 100 mL of sterile LB broth. Bacterial cultures were then incubated at 370C for 16 hours. By obtaining readings for 600 nm on a spectrophotometer, the concentrations of microorganisms were ascertained. According to Zein Sima et al. (2020) and Duraipandiyan et al. (2012), these cell suspensions were diluted using sterile LB to produce final cell counts of roughly 10^7 CFU/ml.

6. Media and Agar plate preparation

A quantity of 38 g of Muller Hinton Agar was dissolved in 1000 mL of purified water. It was autoclaved at 121°C for 15 minutes and afterwards cooled to around 45°C. Following that the agar was transferred into petri dishes and allowed to set. (Arunprasath & Gomathinayagam et al, 2014).

***Table 1*.** Extraction Techniques Used

Extraction Technique	Plant material	Weight (g)	Solvent	Volume (ml)	W:V	Temperature (C^o)	Time (h)
Reflux	L, S, R	4	M, H,W	40	1:10	45-50	6
Sonication	L, S, R	4	M, H	20 +20	1:5	40	6
Maceration	S, R	2	M, H, W	200	1:100	RT	3 days
Crude	FL	3	DW	-	-	RT	-

7. Disc diffusion assay

Cotton swabs, pipette tips, and pre-autoclaved petri plates were utilized. Using a sterile and previously sterilized cotton swab, each bacterial sample was applied across sterile agar plates. Approximately 6 mm in diameter filter paper disks were impregnated with 10 µL of the stock solutions of plant extracts at 50 mg/ml and 100 mg/ml concentrations. The positive control (P.C) was a 10mg/ml gentamycin solution and the negative control (N.C) was the solution used to dissolve the extracts. (distilled water, DMSO, and hexane). P.C and N.C disks were impregnated with 10 µL of these solutions respectively. The plates were incubated for 16h at 37^{O}C. The zone of inhibition was recorded in millimeters and each test was repeated three times. (Duraipandiyan et al, 2012).

Results and Discussion

Reflux and sonication extraction were performed on all three plant organs. The stem and rhizome yielded effective results, whereas the leaves did not. Consequently, antibacterial activity was further analysed using maceration for the stem and rhizome, and crude extraction for the leaves. Residues stored directly before stock preparation demonstrated strong antibacterial properties, with storage temperatures showing no significant effect. Inhibition zones, measured in millimeters (mm), were recorded for two bacterial strains. Table 2 presents the results of the reflux extraction. Table 3 presents the inhibitory results of extracts obtained via sonication. Table 3 presents the inhibitory results of extracts obtained via sonication. Table 4 presents the results of Maceration and Table 5 presents the results of crude extraction.

1. Leaf Extracts

In Table 2, 3 leaf extracts show no inhibition zones against both E. Coli and S. aureus. Table 4 shows the outcomes of an antibacterial activity test conducted on crude leaf extracts of Costus speciosus against two strains of bacteria: S. aureus and E. coli. It indicates that the leaf extract exhibits an inhibitory zone of 10 ± 0 mm against E. coli (figure: 1 A). This implies that the leaf's constituent parts include bioactive substances with antimicrobial properties. The leaf extract does not inhibit S. aureus growth. Key considerations for

extraction include solvent selection, avoiding heat, performing at room temperature, not adding large solvent volumes, and avoiding lengthy steps. Crude extraction involves directly applying the squeezed liquid from fresh, finely crushed leaves to the disc. For sonication and reflux, leaf powder is used. Fresh leaves are oven-dried for 12 hours at 60°C and then ground into powder, which may contribute to the absence of antibacterial bioactive compounds in leaves. Since plant leaves are less robust than stems or rhizomes, it is best to avoid higher temperatures and complex procedures.

2. Stem Extracts

According to Table 2, methanol extracts of the stem (figure: 1 B) showed greater inhibition (12 ± 0 and 14.3 ± 1 mm) against both bacterial strains compared to hexane extracts (10 ± 0.81 and 10 ± 0.40 mm), with water extracts showing no inhibition. In Table 3, stem extracts showed antibacterial activity in both solvents, with methanol extracts being more effective against E. coli (9 mm) and hexane extracts being effective against S. aureus (8 ± 1.24 mm). Table 4 indicates that stem hexane and methanol extracts suppress both bacteria, with methanol being more effective (11 mm for E. coli and 9 mm for S. aureus). Stem water extracts showed no antibacterial action against E. coli but produced good results for S. aureus (8 mm). Reflux, sonication, or maceration can be utilized for stem extractions with methanol or hexane as a solvent.

3. Rhizome Extracts

In Table 2, rhizome extracts show moderate antibacterial activity, particularly in hexane (7 ± 1 mm and 8 ± 0.40 mm) and water extracts (7 ± 1.5 mm and 7 ± 0.84 mm), with no activity in methanol extracts. Table 3 demonstrates that E. coli (15 ± 1.24 mm) is more susceptible to rhizome methanol extract than S. aureus (7 mm figure: 1C). Conversely, rhizome hexane extract is more effective against S. aureus (8.5 ± 1.5 mm) than E. coli (6 mm). Table 7 shows that rhizome methanol extracts do not exhibit antibacterial action, similar to reflux results. However, comparing rhizome hexane and water extracts reveals that rhizome water extracts (10 ± 2 mm and 11 ± 1.5 mm) exhibit substantial antibacterial activity (figure: 1 D), comparable to reflux findings. Rhizome hexane extracts show moderate antibacterial activity (7 mm and 8 ± 0.30 mm), similar to sonication and reflux outcomes. Reflux and maceration techniques with hexane or water as a solvent, or sonication with methanol or hexane as a solvent, are effective combinations for optimal results.

Rhizome methanol extracts from Reflux and Maceration did not show antibacterial properties while rhizome methanol extracts from Sonication produced good inhibition zones. The longer extraction time in Reflux and Maceration might lead to the extraction of non-polar compounds or degradation of sensitive antibacterial compounds, reducing their activity. The higher solvent volume may lead to a lower concentration of active compounds in the extract, potentially affecting antibacterial activity. Furthermore, in Ultrasound Sonication, replacing 20 ml of methanol after the first 3h may have refreshed the solvent, further enhancing the extraction of antibacterial compounds. Further analysis of the chemical composition of the extracts and their components would be necessary.

Table 2. **Antibacterial Activity of Reflux Extracts**

Organism	Plant part	Diameter of zone of inhibition (mm)											
		hexane				methanol				Water			
		PC	50	100	NC	PC	50	100	NC	PC	50	100	NC

E.coli	Leaf	22 ± 0.66	0	0	0	24 ± 0.47	0	0	0	23 ± 0.81	0	0	0
	Stem	24 ± 0.62	9 ± 0.81	10 ± 0.81	0	22.8 ± 0.94	11 ± 1	12 ± 0	0	21 ± 0.81	0	0	0
	Rhi-zome	22 ± 0.47	6.5 ± 1	7 ± 1	0	23.5 ± 0.62	0	0	0	25 ± 0.62	6.5 ± 1	7 ± 1.5	0
S.aureus	Leaf	23 ± 0.81	0	0	0	23 ± 0	0	0	0	22 ± 0.23	0	0	0
	Stem	23 ± 0.47	7 ± 0.40	10 ± 0.40	0	26.5 ± 0	12 ± 2.05	14.3 ± 1	0	21 ± 0.47	0	0	0
	Rhi-zome	23 ± 0.81	7.5 ± 0.81	8 ± 0.40	0	22.3 ± 0.23	0	0	0	25 ± 0.47	7 ± 1.24	7 ± 0.84	0

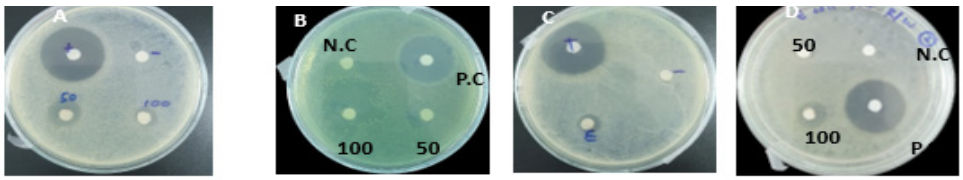

Figure 1. Inhibition zones . A) crude extract of leaves - ***E.coli*** B) stem methanol extract (reflux) – **S.aureus** C) rhizome methanol extract (sonication) – **E.coli** D) rhizome water extract (maceration) – **E.coli**

Table 3. Antibacterial activity of Sonication Extracts

Organism	Plant part	Diameter of zone of inhibition (mm)							
		hexane				methanol			
		PC	50	100	NC	PC	50	100	NC
E.coli	Leaf	24 ± 0	0	0	0	22 ± 0.25	0	0	0
	Stem	25 ± 2	7 ± 0.41	9 ± 1	0	24 ± 0.47	8 ± 1.24	9 ± 0	0
	rhizome	22 ± 0	6.5 ± 0.5	6 ± 0	0	23.5 ± 1.5	8.3 ± 1.24	15 ± 1.24	0
S. aureus	Leaf	24 ± 1	0	0	0	23 ± 0.47	0	0	0
	Stem	25 ± 0	7 ± 0.81	8 ± 1.24	0	24 ± 0	6 ± 0 .23	7 ± 0.34	0
	rhizome	23 ± 0	7.6 ± 1.24	8.5 ± 1.5	0	22.3 ± 0.94	6.5 ± 0.5	7 ± 0	0

Table 4. Antibacterial activity of Maceration

Organism	Plant part	Diameter of zone of inhibition (mm)											
		Hexane				methanol				Water			
		PC	50	100	NC	PC	50	100	NC	PC	50	100	NC
E.coli	Stem	25 ± 0	6.26 ± 0.30	7.3 ± 0.26	0	25 ± 2.82	8 ± 0	11 ± 0	0	25.3 ± 1.15	0	0	0
	rhi-zome	22 ± 0.81	6.5 ± 0	7 ± 0	0	26 ± 1.41	0	0	0	25.6 ± 1.15	10 ± 2	9.3 ± 1.3	0
S.aureus	Stem	26 ± 0	6.5 ± 0.30	7.25 ± 0.35	0	35 ± 0	8.5 ± 0.70	9 ± 0	0	26.6 ± 0.57	7 ± 0	8 ± 0	0
	rhi-zome	23 ± 1	7.5 ± 0	8 ± 0.30	0	25 ± 0	0	0	0	29 ± 1	9.5 ± 2	11 ± 1.5	0

Table 5. Antibacterial activity of Crude extracts

Plant parts used	Bacteria used					
	E Coli			S aureus		
	Inhibition zone measurements in mm					
	Gentamycin	Extract	N.C	Gentamycin	Extract	N.C
Fresh leaves	27 ± 1.73	10 ± 0	0	29.5 ± 0.70	0	0

Conclusion

The investigation unveiled the antibacterial properties of leaves, stems, and rhizome of *C. speciosus* in relation to bacterial growth. The selection of an extraction procedure should be attuned to the delicate nature of leaves to prevent damage. The detection of antibacterial activity in the stem of the *C.speciosus* plant represents a novel discovery; the study data unequivocally supports the idea that stems possess a reservoir of bioactive compounds suitable for further exploration as antibiotic agents. Well-defined inhibition zones were observed for rhizome extracts against both bacterial strains.

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