



Phytochemistry and Pharmacology of Ethnopharmacologically Important Plant Growing in Borako (Bermo Subdivision), Jharkhand: *Costus Speciosus*

Debiprasad Mukherjee¹, Kalpana Prasad^{2*}

¹Department of Life Science, Binod Bihari Mahto Koyalanchal University, Dhanbad 826004 Jharkhand (INDIA)

²Associate Professor of Life Science, Binod Bihari Mahto Koyalanchal University, Dhanbad, Jharkhand (INDIA)

DOI : <https://doi.org/10.55248/gengpi.6.0225.0725>

ABSTRACT

Costus speciosus, a medicinal plant native to Bermo, Bokaro, Jharkhand, holds profound ethnopharmacological importance due to its diverse therapeutic applications. This study investigates the phytochemistry and pharmacology of its rhizome aqueous extract, focusing on cytotoxic and anti-inflammatory properties. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 15 bioactive compounds in the extract, with 2-Pentanone, 4-hydroxy-4-methyl- being the most abundant (64.08% relative abundance). These compounds are reported to exhibit significant pharmacological activities, including anti-inflammatory and cytotoxic effects.

The cytotoxicity of the extract was evaluated using the MTT assay on human cancer cell lines, revealing dose-dependent inhibition of cell viability. At a concentration of 120 mg/mL, the rhizome extract reduced cell viability to 38.7%, showcasing comparable efficacy to the reference drug doxorubicin (10% viability at 120 mg/mL). Additionally, the anti-inflammatory potential was assessed via the BSA denaturation assay, where the extract demonstrated 72.4% inhibition of protein denaturation at the highest concentration, close to the activity of indomethacin (90% inhibition).

These findings substantiate the traditional use of *C. speciosus* rhizomes in inflammation and cancer management. The diverse phytochemical profile, dominated by 2-Pentanone derivatives, underscores the therapeutic potential of the extract. This study bridges the gap between traditional knowledge and modern pharmacology, providing a scientific basis for the medicinal applications of *C. speciosus*. Future research should focus on isolating active compounds, exploring their mechanisms, and conducting in vivo studies to validate their therapeutic efficacy.

Keywords *Costus speciosus*, GC-MS, cytotoxicity, anti-inflammatory, ethnopharmacology, phytochemistry

Introduction

Costus speciosus (Koen.) Sm., commonly known as crepe ginger, is a perennial herbaceous plant native to tropical and subtropical regions. In India, it has been traditionally used in various folk medicine systems for treating ailments such as fever, diabetes, inflammation, and infections. Among its various parts, the rhizome is particularly noted for its rich phytochemical composition and wide array of therapeutic applications. This study focuses on investigating the rhizome aqueous extract of *C. speciosus*, collected from Bermo, Bokaro, Jharkhand, to evaluate its phytochemical and pharmacological properties. [1-3]

The rhizome of *C. speciosus* has been documented for its bioactive compounds, including steroids, saponins, alkaloids, flavonoids, and phenolic acids. These compounds contribute to its pharmacological activities, such as antioxidant, anti-inflammatory, antidiabetic, and antimicrobial effects. However, there is limited scientific evidence detailing the comprehensive chemical profile of its aqueous extract, particularly in the context of its pharmacological activities. [4-6]

Bermo, Bokaro, situated in the state of Jharkhand, offers a unique ecological niche, contributing to the distinctive phytochemical profiles of its medicinal plants. The traditional use of *C. speciosus* in this region underscores its ethnopharmacological significance. Furthermore, the plant's accessibility and potential as a sustainable resource highlight its importance in local healthcare systems. Previous studies on medicinal plants have shown that environmental factors, including soil composition and climate, significantly influence their phytochemical constitution, making regional studies vital for understanding plant bioactivity.

In addition to its traditional applications, recent pharmacological studies have begun to explore the potential of *C. speciosus* rhizomes in modern medicine. Reports suggest that its bioactive compounds exhibit promising activities against various diseases, including cancer and inflammatory conditions. However, these studies are fragmented, and a holistic investigation of its phytochemical profile and bioactivities remains unexplored. This

study addresses this gap by utilizing advanced techniques such as GC-MS for compound identification and biological assays for pharmacological evaluation. By focusing on the rhizome, this research aims to provide a detailed understanding of its chemical constituents and their potential applications in therapeutics. [7,8]

The outcomes of this research could contribute significantly to the growing body of evidence supporting the medicinal value of *C. speciosus*. Additionally, it may provide a scientific basis for its traditional uses, promoting its inclusion in evidence-based medicine and encouraging the development of plant-based drugs. This study also aims to highlight the importance of preserving ethnopharmacological knowledge and exploring regional medicinal plants for drug discovery.

Experimental

Plant Material Collection and Preparation

Fresh rhizomes of *Costus speciosus* were collected from Bermo, Bokaro, Jharkhand. The plant was authenticated by a qualified botanist, and a voucher specimen was deposited in the herbarium. The rhizomes were washed thoroughly, shade-dried, and ground into a fine powder. Aqueous extraction was performed by soaking 100 g of the powdered rhizome in 1 L of distilled water for 72 hours with intermittent shaking. The mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The concentrated extract was stored at 4°C for further analysis.

Phytochemical Analysis by GC-MS

The rhizome aqueous extract was subjected to GC-MS analysis to identify its chemical constituents. The analysis was carried out using a gas chromatograph equipped with a mass spectrometer and a capillary column (30 m × 0.25 mm × 0.25 μm). The injector and detector temperatures were set at 250°C and 280°C, respectively. The oven temperature was programmed to increase from 50°C to 300°C at a rate of 10°C per minute. Helium was used as the carrier gas at a flow rate of 1 mL/min. The compounds were identified by comparing their mass spectra with those in the NIST library.

MTT Assay for Cytotoxicity

The cytotoxic activity of the rhizome aqueous extract was evaluated using the MTT assay. Human cancer cell lines (HepG2) were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight. The cells were treated with the extract at concentrations of 30, 60, and 120 mg/mL for 24 hours. Following treatment, 20 μL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a microplate reader. Doxorubicin was used as the reference drug.

BSA Denaturation Assay for Anti-inflammatory Activity

The anti-inflammatory potential of the rhizome aqueous extract was assessed using the BSA denaturation assay. A reaction mixture containing 1% BSA solution and varying concentrations of the extract (30, 60, and 120 mg/mL) was incubated at 37°C for 20 minutes. Denaturation was induced by heating the mixture at 70°C for 5 minutes. After cooling, the turbidity was measured at 660 nm using a spectrophotometer. Indomethacin was used as the reference standard, and the percentage inhibition of denaturation was calculated.

Results

GC-MS Analysis for plant phytochemistry

The GC-MS analysis of the rhizome aqueous extract identified 15 bioactive compounds (Table 1 and Figure 1). The most abundant compound was 2-Pentanone, 4-hydroxy-4-methyl-, with a relative abundance of 64.08%. Other notable compounds included 2-Pentanone, 4-hydroxy- (17.77%) and Phosphoric triamide, N,N',N"-tris(dimethylaminocarbonyl)- (2.17%). These compounds are known for their diverse pharmacological properties, including anti-inflammatory, antimicrobial, and cytotoxic activities.

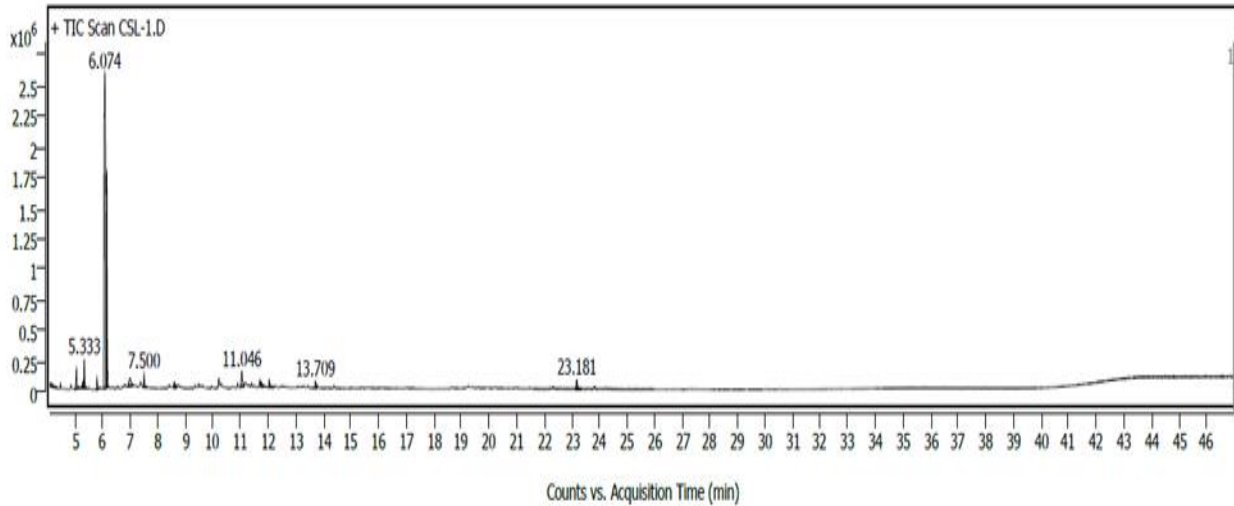


Figure 1: GC-MS chromatograph for the aqueous extract of *C. speciosus*.

Table 1: GC-MS Identification of Compounds.

S.No.	Common Name	RT Value	Area
1.	2-Pyridinamide, 1-oxide	11.756	0.71
2.	Tetrazole, 1-(3,4-dimethoxybenzylideneamino)-	15.028	0.79
3.	Acetic acid, 2-(N-methyl-N-phosphonomethyl)amino-	8.601	0.84
4.	2-Pentanone, 4-hydroxy-	5.792	0.92
5.	Catecholborane	12.035	0.96
6.	1,1,3,3-Tetra-tert-butyl-2-phenylsulfonylthiourea	4.126	1.05
7.	Propanoic acid, 2-oxo-, methyl ester	5.303	1.05
8.	1H-Tetrazaborole, 4,5-dihydro-1,4-dimethyl-	7.500	1.23
9.	Loliolide	23.181	1.31
10.	Phosphoric triamide, N,N',N''-tris(dimethylaminocarbonyl)-	6.991	2.17
11.	4-Amino-3-hydroxytetrahydrothiophene 1,1-dioxide	5.051	2.23
12.	1,2,4,5-Tetrazine-3,6-diamine, 1,4-dioxide	11.046	2.38
13.	3-Hexen-2-one	5.333	2.51
14.	2-Pentanone, 4-hydroxy-4-methyl-	6.151	17.77
15.	2-Pentanone, 4-hydroxy-4-methyl-	6.074	64.08

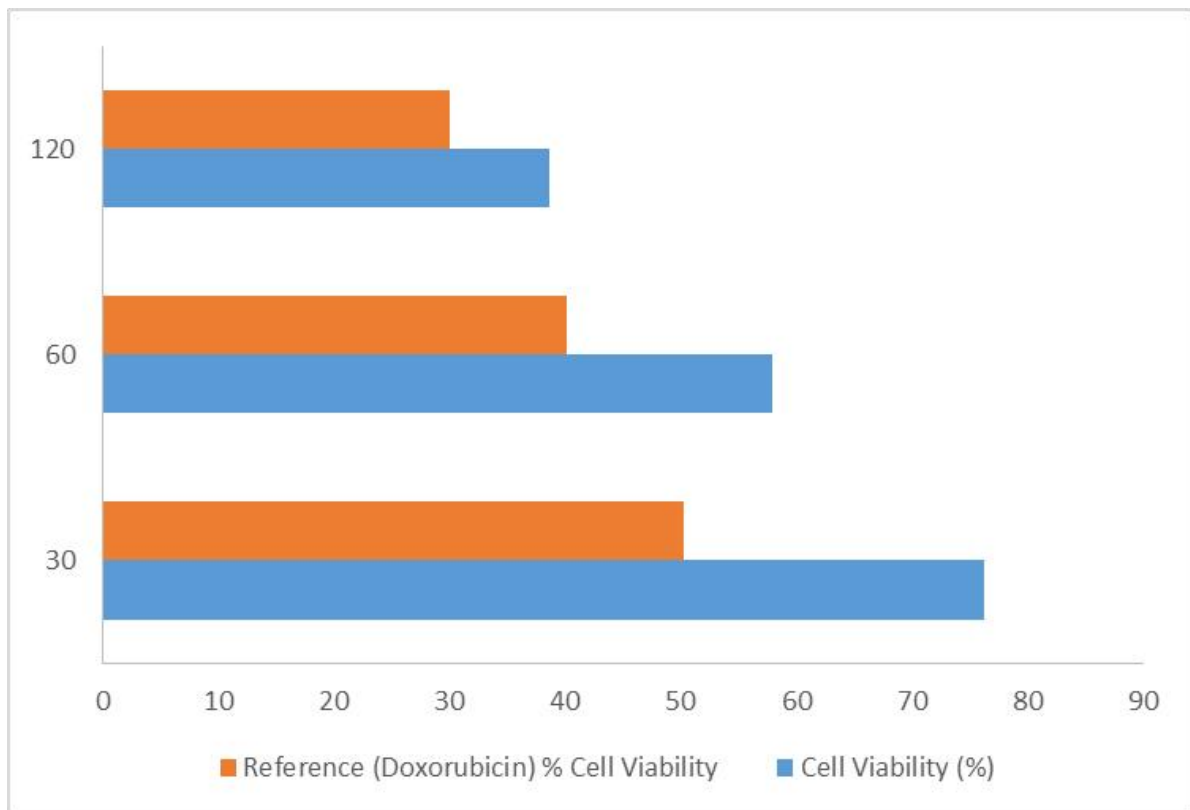
Cytotoxicity of the extract

The cytotoxicity results revealed dose-dependent inhibition of cell viability (Table 4.10). The rhizome extract exhibited the highest cytotoxic activity at 120 mg/mL, reducing cell viability to 38.7%. This was comparable to the reference drug doxorubicin, which reduced viability to 10% at the same concentration.

Table 4.10: Cytotoxic Assay – MTT Assay (Cell Viability)

S. No.	Concentration (mg/mL)	Cell Viability (%)	Reference (Doxorubicin) % Cell Viability
1	30	76.3	50.3
2	60	58.0	40.1

3	120	38.7	30.0
---	-----	------	------

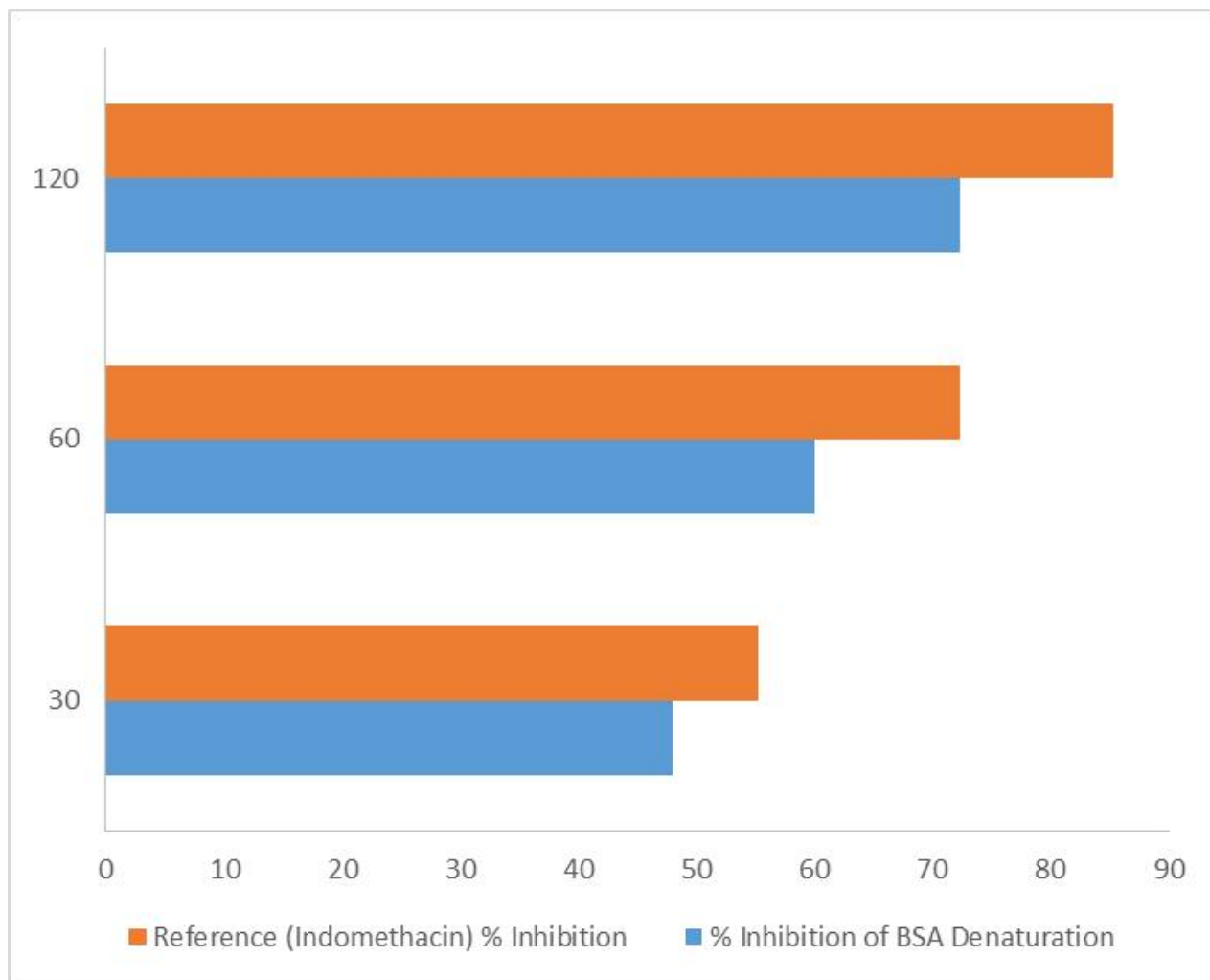


BSA Denaturation Assay

The anti-inflammatory assay demonstrated significant inhibition of BSA denaturation by the rhizome extract (Table 4.11). At 120 mg/mL, the extract achieved 72.4% inhibition, which was close to the activity of indomethacin (90%).

Table 4.11: BSA Denaturation Assay

S.No.	Concentration (mg/mL)	% Inhibition of BSA Denaturation	Reference (Indomethacin) % Inhibition
1	30	48.0	55.2
2	60	60.0	72.3
3	120	72.4	85.4



Discussion

The phytochemical analysis revealed a diverse array of bioactive compounds in the rhizome aqueous extract of *C. speciosus*. The predominance of 2-Pentanone derivatives aligns with their reported cytotoxic and anti-inflammatory properties. These findings corroborate the traditional use of *C. speciosus* rhizomes in treating inflammation and cancer.

The MTT assay results highlight the extract's potent cytotoxicity, especially at higher concentrations. The observed activity suggests potential applications in developing anticancer therapeutics. Similarly, the BSA denaturation assay confirmed the anti-inflammatory efficacy of the rhizome extract, further validating its traditional use in inflammation management. By providing empirical evidence for these activities, this study bridges traditional knowledge with modern pharmacological research. [9,10]

The diversity of bioactive compounds identified in the extract reflects the complex interactions between these constituents, contributing to its overall bioactivity. It also underscores the importance of considering the synergistic effects of phytochemicals in herbal medicine. The high abundance of 2-Pentanone derivatives, coupled with other minor compounds, suggests a multidimensional approach to targeting cellular pathways involved in inflammation and tumor growth. [11,12]

Comparisons with standard drugs like doxorubicin and indomethacin highlight the extract's competitive pharmacological activities, making it a promising candidate for further studies. Future research should focus on isolating and characterizing these bioactive compounds to elucidate their mechanisms of action. Additionally, advanced pharmacokinetic and toxicological studies are necessary to translate these findings into practical applications. [13]

The ecological significance of Bermo's unique flora also highlights the need for conservation efforts. The findings of this study could contribute to sustainable utilization practices, ensuring that *C. speciosus* and other medicinal plants remain available for future generations.

Conclusion

Costus speciosus rhizome exhibits significant phytochemical diversity and pharmacological activities, particularly in cytotoxicity and anti-inflammation. These results underline its potential as a source of bioactive compounds for therapeutic applications. Further studies are warranted to isolate and characterize the active constituents and elucidate their mechanisms of action.

References

1. Sohrab S, Mishra P, Mishra SK. Phytochemical competence and pharmacological perspectives of an endangered boon—*Costus speciosus* (Koen.) Sm.: a comprehensive review. *Bulletin of the National Research Centre*. 2021 Dec;45:1-27.
2. Delarosa A, Hendrawan RP, Halimah E. Screening of *Costus speciosus* and Determination of Antioxidant Potential Using DPPH Method: A Review. *European Journal of Medicinal Plants*. 2023 Jul 25;34(7):17-28.
3. Shaikh SS, Bawazir AS, Yahya BA. Phytochemical, histochemical and in vitro antimicrobial study of various solvent extracts of *costus speciosus* (j. koenig) sm. and *costus pictus* d. don. *Turkish Journal of Pharmaceutical Sciences*. 2022 Apr;19(2):145.
4. Zishan SA, Uddin MM, Mohammad M, Asadul Karim Azad SM, Naima J, Ibban SS, Saiful Islam Arman M. *Costus speciosus* leaf and seed extracts for wound healing: a comparative evaluation using mice excision wound models. *Clinical Phytoscience*. 2024 Feb 21;10(1):5.
5. Kodagoda YK, Jayasinghe CV, Dharmadasa RM. In vitro antioxidant activity and antidiabetic potential of five spiral ginger (*Costus speciosus* (J. Koenig) Sm.) populations available in Sri Lanka. *Journal of Agriculture and Food Research*. 2023 Jun 1;12:100553.
6. Halimah E, Wilar G, Sofian FF, Megantara S, Levita J. The cytotoxic and apoptotic activity of *Costus speciosus* (Koenig) Smith (Costaceae) leaves against MCF-7 and HeLa cells. *Journal of Herbmed Pharmacology*. 2024 Jun 27;13(3):491-500.
7. El-Far A, Shaheen H, Alsenosy A, El-Sayed Y, Al Jaouni S, Mousa S. *Costus speciosus*: Traditional uses, phytochemistry, and therapeutic potentials. *Pharmacognosy Reviews*. 2018;12(23).
8. Elkady AI. Targeting prostate cancer cell proliferation, stemness and metastatic potential using *Costus speciosus* derived phytochemicals. *American journal of translational research*. 2019;11(4):2550.
9. Al-Attas AA, El-Shaer NS, Mohamed GA, Ibrahim SR, Esmat A. Anti-inflammatory sesquiterpenes from *Costus speciosus* rhizomes. *Journal of ethnopharmacology*. 2015 Dec 24;176:365-74.
10. Selim S, Al Jaouni S. Anti-inflammatory, antioxidant and antiangiogenic activities of diosgenin isolated from traditional medicinal plant, *Costus speciosus* (Koen ex. Retz.) Sm. *Natural product research*. 2016 Aug 17;30(16):1830-3.
11. Azam S, Ansari P, Jalil S, Ibrahim AH, Sultana N, Hossain MM, Naveed JM, Hossain MF. Antinociceptive activity investigation of the methanolic crude extract of *Costus speciosus* in Mice. *Progress in Nutrition*. 2016 Dec 1;18(4):436-42.
12. Naznin NE, Mazumder T, Reza MS, Jafrin S, Alshahrani SM, Alqahtani AM, Alqahtani T, Daula AS. Molecular docking supported investigation of antioxidant, analgesic and diuretic effects of *Costus speciosus* rhizome. *Bulletin of the Chemical Society of Ethiopia*. 2022 Jul 15;36(3):627-40.
13. Hussain MD, Mazumder T. A comprehensive review of pharmacological and toxicological properties of *Cheilocostus speciosus* (J. Koenig) CD Specht. *Trends in Phytochemical Research*. 2021 Mar 1;5(1):1-2.