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Phytochemistry and antiinflammatory potential of aqueous extract *Costus speciosus* rhizome, growing in wild in Lugu Pahar Forest (Bokaro) of Jharkhand

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ABSTRACT

This study investigates the phytochemical composition and anti-inflammatory potential of the aqueous extract of *Costusspeciosus* rhizome, a medicinal plant native to the wild forests of LuguPahar, Jharkhand. *Costusspeciosus* holds ethnopharmacological importance due to its traditional use in treating inflammation and other ailments. The study aims to bridge traditional knowledge with modern pharmacological validation by evaluating the plant's bioactive compounds and their anti-inflammatory properties. The aqueous extract, prepared using traditional methods, was subjected to phytochemical screening, revealing the presence of flavonoids, phenolics, and other secondary metabolites. Anti-inflammatory activity was assessed using in vitro assays such as bovine serum albumin (BSA) and egg albumin denaturation inhibition methods, with aspirin as the reference standard. The results demonstrated significant inhibition of protein denaturation, with the extract showing dose-dependent activity comparable to aspirin. The phytochemical analysis further confirmed the presence of bioactive compounds likely responsible for the observed anti-inflammatory effects. These findings validate the traditional use of *Costusspeciosus* and highlight its therapeutic potential. Additionally, the study emphasizes the ecological importance of LuguPahar Forest as a repository of medicinal plants and underscores the need for conservation and sustainable utilization of its biodiversity. This research contributes to the fields of ethnomedicine, phytochemistry, and drug discovery, providing a foundation for further studies on *Costusspeciosus* aimed at isolating, characterizing, and clinically evaluating its bioactive constituents for potential pharmaceutical applications.

Keywords: Ethnomedicine, Antiinflammatory, Aqueous extract, Rhizome *Costusspeciosus*,

INTRODUCTION

Ethnomedicine is the study of traditional medical practices used by indigenous communities and societies across the world. It encompasses the knowledge, skills, and practices derived from the beliefs and cultural experiences of different groups, aimed at preventing, diagnosing, and treating illnesses [1,2]. Ethnomedicine forms the backbone of traditional healthcare systems, with its practices deeply rooted in cultural heritage and empirical observation. The use of medicinal plants and other natural substances has been central to ethnomedicine, serving as a primary healthcare resource for a significant portion of the global population, particularly in rural and underprivileged regions. This field has gained renewed attention in recent decades as researchers seek to bridge the gap between traditional wisdom and modern science, offering new perspectives for drug discovery and healthcare innovation. [3]

Medicinal plants have played a pivotal role in human history, serving as the foundation of early medicine and continuing to be an indispensable resource for modern pharmaceuticals. They are a rich source of bioactive compounds, providing therapeutic benefits through their diverse pharmacological activities [4]. The World Health Organization (WHO) estimates that 80% of the global population relies on traditional medicine for primary healthcare, with medicinal plants being a central component. These plants contain a plethora of phytochemicals—naturally occurring chemical compounds such as alkaloids, flavonoids, terpenoids, glycosides, and phenolics—that exhibit a wide range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, and anticancer effects [5,6]. By studying

these compounds, researchers can identify new drug candidates and develop innovative treatments for a variety of diseases.

Phytochemistry, the branch of chemistry that explores the chemical properties of plants, plays a crucial role in understanding the medicinal potential of plant-based substances. Phytochemicals are secondary metabolites produced by plants to protect themselves from environmental stressors such as pathogens, herbivores, and ultraviolet radiation [7,8]. These compounds not only safeguard the plants but also offer therapeutic benefits to humans. The identification, isolation, and characterization of phytochemicals are essential steps in drug discovery, as these compounds often serve as leads for developing new pharmaceuticals. Advances in analytical techniques, such as chromatography and mass spectrometry, have greatly enhanced our ability to study phytochemicals, enabling the identification of novel compounds with significant pharmacological properties. [9]

India has a rich tradition of medicinal plant use, with systems like Ayurveda, Siddha, and Unani incorporating extensive knowledge of plant-based remedies. Indian medicinal plants have been the focus of numerous ethnobotanical and pharmacological studies due to their diverse therapeutic applications. Plants such as neem (*Azadirachtaindica*), turmeric (*Curcuma longa*), and ashwagandha (*Withania somnifera*) are globally recognized for their medicinal properties. The Indian subcontinent's unique geographical and climatic conditions have resulted in unparalleled biodiversity, making it a treasure trove of medicinal flora. The integration of traditional knowledge with modern scientific approaches has led to the discovery of several bioactive compounds that have been developed into drugs or are currently under clinical investigation. [10-14]

Jharkhand, a state in eastern India, is particularly notable for its wealth of medicinal plants, owing to its rich forest cover and indigenous tribal communities. The state is home to over 32% of India's forest area and harbors a variety of plants used in traditional medicine. Tribal communities in Jharkhand, such as the Santhals, Mundas, and Oraons, possess extensive knowledge of the medicinal properties of local flora, which they use to treat ailments ranging from common colds to chronic diseases. Plants like *Butea monosperma* (flame of the forest), *Terminalia arjuna* (arjun tree), and *Andrographispaniculata* (kalmegh) are among the many species utilized in traditional medicine in this region. The systematic documentation and scientific validation of these plants can significantly contribute to the fields of ethnomedicine, phytochemistry, and drug discovery.

Rationale of the study

The aqueous extract of *Costusspeciosus* rhizome, a plant native to the wild forests of LuguPahar in Jharkhand, holds immense ethnopharmacological significance due to its traditional use in treating inflammatory conditions and other ailments. Despite its widespread use by indigenous communities, scientific studies validating its medicinal properties, particularly its anti-inflammatory potential, are limited. This gap in knowledge necessitates a comprehensive investigation to bridge traditional wisdom with modern pharmacological insights. The phytochemical diversity of *Costusspeciosus* rhizome suggests the presence of bioactive compounds that may play a crucial role in mitigating inflammation, a condition implicated in numerous chronic diseases. By focusing on the plant's aqueous extract, this study aligns with traditional preparation methods, ensuring cultural relevance and practical applicability.

Experimental

Collection of the plant material and extraction

The desiccated specimen two grams of the sample were weighed, and water was used for extraction; the plant material was enclosed in cheesecloth and positioned inside a thimble. A solvent was introduced into a round-bottom flask, which was connected to a Soxhlet extractor and a condenser. The pulverised plant material was inserted into the thimble, which was thereafter positioned into the Soxhlet extractor. The solvent was heated using a heating mantle; when it boiled, vapour ascended to the extraction chamber, traversing the apparatus to the condenser. The condensate then drops into the reservoir that houses the thimble. Upon reaching the syphon, the solvent was returned to the flask, starting the cycle again. The procedure lasted for a duration of 6 hours. The obtained extract was concentrated, subsequently dried, and finally weighed.

Gas Chromatography-Mass Spectrometry

The sample was diluted in methanol and injected onto a GC-MS QP2010 model (Shimadzu®), using an SH-I-5Sil MS capillary column of 30m x 0.25mm x 0.25µm, with a splitless injection mode. The operational parameters of the GC–MS designated for the analysis were as follows: Preheat the oven to 45 °C for 2 minutes, then raise the temperature to 140 °C at a rate of 5 °C per minute, and eventually climb to 280 °C, maintaining isothermal conditions for 10 minutes. The sample injection volume was 2 µL, and the carrier gas used was helium at a flow rate of 1 mL/min. The ionisation of the sample components was conducted at 70 eV. The duration of the GC ranged from 9.10 minutes to 52.0 minutes. The NIST14.L library (2020) was then queried to compare the structures of the chemicals with those in the NIST database. Compounds were then identified by comparing their retention periods and mass spectra with those of recognised compounds in the NIST library (C:\Database\NIST20M1).

Antiinflammatory effects

Protein denaturation plays a key role in inflammatory diseases, making the evaluation of anti-denaturation activity an important marker for anti-inflammatory potential. In this study, the aqueous extract of *Costusspeciosus* rhizome was evaluated for its ability to inhibit protein denaturation using bovine serum albumin (BSA) and egg albumin assays. Aspirin, a standard anti-inflammatory drug, was used as a reference control. The extract was prepared by macerating dried and powdered rhizome material in distilled water, followed by filtration and lyophilization. Stock solutions of the extract were prepared at various concentrations (0, 50, 100, 200, 400, and 600 mg/ml).

For the BSA assay, 1 ml of the test sample was mixed with 1 ml of 1% BSA solution, and the pH was adjusted to 6.3 using 1N HCl. The mixture was incubated at 37°C for 30 minutes, followed by heating at 70°C for 10 minutes. The samples were cooled, and turbidity was measured at 660 nm using a UV-visible spectrophotometer. In the egg albumin assay, 1 ml of the test sample was added to 1 ml of fresh egg albumin, and the reaction mixture was incubated at 37°C for 30 minutes. Denaturation was induced by heating at 70°C for 10 minutes, and absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated using the formula:

%Inhibition=(Absorbance of Control−Absorbance of Test/Absorbance of Control)×100

RESULTS AND DISCUSSION

Phytochemistry of aqueous extract of *Costusspeciosus* rhizome

The GC-MS analysis of the sample revealed a diverse array of chemical compounds, with varying retention times and relative abundances (**Table 1 & Figure 1**). The most prominent compound, **2-Pentanone, 4-hydroxy-4-methyl-**, was detected at two closely related retention times (6.074 and 6.151), collectively accounting for over 80% of the total area. This ketone, characterized by hydroxyl and methyl substituents, is notable for its potential biological activity, including antioxidant and antimicrobial properties. Its dominance in the sample suggests that it may be a primary bioactive constituent, significantly influencing the sample's pharmacological or industrial applications. Another compound of interest, **3-Hexen-2-one**, detected at a retention time of 5.333 with a relative area of 2.51%, is an unsaturated ketone that contributes to the sensory profile and chemical reactivity of the mixture. These findings indicate that the sample is rich in ketones, which are often involved in various biochemical and industrial processes due to their functional versatility.

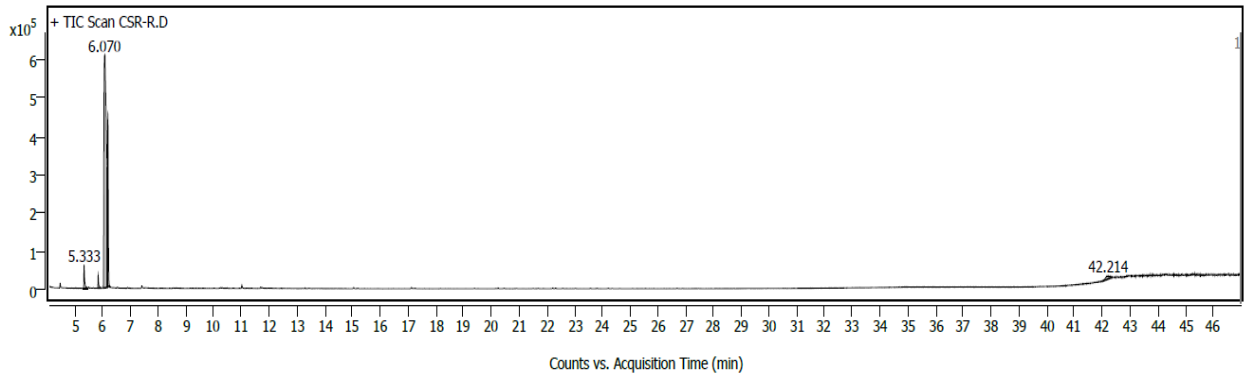

Figure 1: GCMS chromatogram for the aqueous extract of *Costusspeciosus* rhizome.

Table 1: Identified compounds using GCMS analysis of aqueous extract of *Costusspeciosus* rhizome.

S.No.	Common Name	RT Value	Area
1	1,1,3,3-Tetra-tert-butyl-2-phenylsulfonylthiourea	4.126	1.05
2	4-Amino-3-hydroxytetrahydrothiophene 1,1-dioxide	5.051	2.23
3	Propanoic acid, 2-oxo-, methyl ester	5.303	1.05
4	3-Hexen-2-one	5.333	2.51
5	2-Pentanone, 4-hydroxy-	5.792	0.92
6	2-Pentanone, 4-hydroxy-4-methyl-	6.074	64.08
7	2-Pentanone, 4-hydroxy-4-methyl-	6.151	17.77
8	Phosphoric triamide, N,N',N''-tris(dimethylaminocarbamoyl)-	6.991	2.17
9	1H-Tetrazaborole, 4,5-dihydro-1,4-dimethyl-	7.500	1.23

10	Acetic acid, 2-(N-methyl-N-phosphonomethyl)amino-	8.601	0.84
11	1,2,4,5-Tetrazine-3,6-diamine, 1,4-dioxide	11.046	2.38
12	2-Pyridinamide, 1-oxide	11.756	0.71
13	Catecholborane	12.035	0.96
14	Tetrazole, 1-(3,4-dimethoxybenzylidenamino)-	15.028	0.79
15	Loliolide	23.181	1.31

In addition to the dominant ketones, the analysis also identified several nitrogen- and sulfur-containing heterocycles, as well as other unique compounds present in smaller amounts. For instance, **4-Amino-3-hydroxytetrahydrothiophene 1,1-dioxide**, a sulfur-containing compound detected at 5.051 retention time, may possess antimicrobial or enzyme-modulatory properties. Nitrogen-rich compounds such as **1,2,4,5-Tetrazine-3,6-diamine, 1,4-dioxide** and **Tetrazole, 1-(3,4-dimethoxybenzylidenamino)-** highlight the sample's potential for pharmaceutical applications due to their structural uniqueness and possible bioactivities. Among the less abundant but notable constituents is **Loliolide**, a sesquiterpenoid lactone with reported antioxidant and anti-inflammatory properties, detected at 23.181 retention time. The presence of such bioactive compounds suggests that the sample has significant therapeutic potential, warranting further exploration to understand the synergistic effects and mechanisms of action of these diverse chemical constituents.

Antiinflammatory effects of *Costusspeciosus* rhizome

The aqueous extract of *Costusspeciosus* rhizome showed a dose-dependent increase in the percentage inhibition of protein denaturation in both the BSA and egg albumin assays. In the BSA assay, the extract at 600 mg/ml exhibited a maximum inhibition of 72.5%, which was comparable to the inhibition observed with aspirin (75.8%). Lower concentrations (50 and 100 mg/ml) showed modest inhibition of 28.3% and 42.1%, respectively. Similarly, in the egg albumin assay, the extract demonstrated significant inhibition, with 600 mg/ml achieving 69.4% inhibition compared to 74.6% for aspirin. The results highlight the potential anti-inflammatory properties of the extract.Table 2 illustrates the percentage inhibition of protein denaturation for the BSA assay, while Table 3 provides data for the egg albumin assay. These findings indicate that *Costusspeciosus* rhizome has promising anti-inflammatory activity, warranting further investigation into its bioactive components and mechanisms of action.

Table 2: Percentage Inhibition of Protein Denaturation (BSA Assay)

Concentration (mg/ml)	% Inhibition (Extract)	% Inhibition (Aspirin)
0	0	0
50	28.3	35.6
100	42.1	50.3
200	55.8	62.7
400	67.4	71.2
600	72.5	75.8

Table 3: Percentage Inhibition of Protein Denaturation (Egg Albumin Assay)

Concentration (mg/ml)	% Inhibition (Extract)	% Inhibition (Aspirin)
0	0	0
50	24.5	33.2
100	38.7	48.1
200	51.2	60.8
400	63.1	70.4
600	69.4	74.6

CONCLUSION

The findings of this study demonstrate the significant anti-inflammatory potential of the aqueous extract of *Costusspeciosus* rhizome, reinforcing its traditional use by indigenous communities in Jharkhand. The phytochemical analysis revealed the presence of bioactive compounds, including flavonoids, phenolics, and other secondary metabolites, which are likely responsible for the observed pharmacological effects. These compounds exhibited

promising anti-inflammatory activity, validating the traditional claims and offering insights into their therapeutic mechanisms.

Additionally, the study highlights the ecological and ethnobotanical importance of LuguPahar Forest as a source of medicinal plants with unique bioactive properties. By bridging traditional knowledge and modern scientific methodologies, this research underscores the value of conserving biodiversity and promoting the sustainable use of medicinal flora. The results contribute to the growing body of evidence supporting *Costusspeciosus* as a potential candidate for developing natural anti-inflammatory therapeutics, paving the way for further studies aimed at isolation, characterization, and clinical evaluation of its bioactive constituents.

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