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Research Article

Phytochemical Profiling and In vitro Evaluation of Biological Activities of *Costus speciosus* (J. Koenig) SM

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ABSTRACT

Costus speciosus (Koen ex. Retz) Sm. (Costaceae) commonly known as crepe ginger, is a perennial succulent medicinal herb used in Ayurveda and other traditional medicinal systems like Siddha, Unani and Chinese Medicinal System for the treatment of various physical and mental illness. It is an important medicinal plant that draws the attention of the researchers for its diverse pharmacological activity which makes it a source of various important phytochemicals. In the present study, the qualitative screening of methanolic extract of leaf and rhizome of the plant indicated the presence of alkaloids, phenolics, reducing sugar, glycosides, tannins, coumarins, saponins etc. Functional groups including alcohols, phenols, carboxylic acids, carbonyl groups, aromatic rings, ester, amide, and halogens were detected by FTIR spectroscopy analysis. Important chemicals such as diosgenin, phytol, squalene, loliolide, delta-tocopherol, caryophyllene, caryophyllene oxide, 9,12,15-octadecatrienoic acid, methyl ester (α -Linolenic acid methyl ester), beta-sitosterol, androsta-1,4-diene-3,17-dione (ADD), etc. were confirmed to be present by additional GC-MS analysis. Quantitative estimation of phytochemicals revealed that it contains excellent amount of reducing sugar and phenolic compounds. In DPPH assay the IC₅₀ value of leaf and rhizome are found to be 125.9 $\mu\text{g/mL}$ and 167.05 $\mu\text{g/mL}$. The IC₅₀ value of rhizome extract in egg-albumin denaturation assay is calculated to be 57.859 $\mu\text{g/mL}$.


INTRODUCTION

Natural phytochemicals have been found to effectively manage various fatal diseases such as cancer, diabetes, hepatic disorders, nephropathy

and cardiovascular diseases^[1, 2]. In contrast to the extensive use of medicinal plants in Ayurveda and various ethnic medicinal systems, scientists also evaluated the phytochemistry of medicinally valuable plants and isolated various active

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phytochemicals that holds enormous potential to treat modern diseases and insights to the discovery of new therapeutic drugs. Phytochemicals belonging to alkaloids, polyphenols, flavonoids, coumarins, saponins, polysaccharides and essential oils possess significant bioactivities that make them ideal for use not only in pharmaceuticals but also in cosmetic and food industries^[3].

Costus speciosus is an erect, perennial succulent herbaceous medicinal plant that reaches a height of 2.7m^[4]. It contains ginger like rhizomatous roots and the petals of the flowers look like crepe paper, hence often referred to as crepe ginger. It belongs to the family Costaceae, which is characterized by the spiral arrangement of the leaves around the stem^[5]. The plant has thick sessile leaves, which exhibit dark green color, cuspidate and elliptic to oblong in shape. Flowers are showy and white in color that develops at the terminal position from the thick cone like spikes in mature plants. It is globally found in Africa, North America and Oceania and in Asian nations such as India, China, Nepal, Taiwan, Bangladesh, Phillipines, Thailand, Singapore, Hong Kong, Vietnam, Myanmar, Bhutan, Malaysia and Indonesia^[6].

A significant number of phytocompounds have been reported and identified from different parts (seeds, leaves, stem, rhizome, flower) of *C. speciosus*. These phytochemicals belong to different chemical groups such as alkaloids, phenols, saponins, flavonoids, quinines, tannins, carbohydrates, terpenoids, coumarins, steroids, glycosides and cardiac glycosides^[7]. The tribal people of southern and northeastern part of India recognize the therapeutic potential of *C. speciosus* and have many medicinal practices of the plant^[6]. Medicinally the most important and widely used part of *C. speciosus* is its rhizomes. The uses of the plant in the treatment of different diseases are

mentioned in ancient Indian texts like Ayurveda and Samhitas.

In this study, methanolic extract of leaf and rhizome of *C. speciosus* have been evaluated for the presence of various phytochemicals both qualitatively and quantitatively. FTIR analysis was carried out to depict the presence of possible functional groups present in both the extracts. To identify the different bioactive compounds, GC-MS analysis was done. Moreover, antioxidant and anti-inflammatory potential of the extracts were examined using DPPH assay and egg-albumin denaturation assay respectively.

MATERIALS AND METHODS

1. Collection and Authentication of Plant samples

The leaves and the rhizomes of *C. speciosus* were collected in November 2024 from Village Kuthehar, District Kangra of Himachal Pradesh from Lat. 32.186781° Long. 76.225152°. The collected plant material was authenticated from a voucher specimen at CSIR-IHBT Palampur, Himachal Pradesh and an accession number **24692** was provided for further documentation.

2. Preparation of Plant extracts

The healthier collected plant parts were washed to get rid of any dirt particles and shade dried for 8-10 weeks. After complete drying the samples were ground into coarse powder using a laboratory grinder and 20 g each of this powder was Soxhlet extracted with 200 mL of methanol for continuous 12 h at 60 °C. The resulted extracts were concentrated in rotary evaporator to separate the solvent and the concentrated extracts were stored at 4 °C for further analysis.

3. Qualitative Analysis for Phytochemicals



The methanolic leaf and rhizome extracts of *C. speciosus* were qualitatively analyzed to detect the presence of various phytoconstituents belonging to the different chemical classes. The screening was done through the preliminary detection procedures outlined by^[8].

4. FTIR Spectroscopy Analysis

FTIR spectroscopy analysis of methanolic leaf and rhizome extracts of *C. speciosus* was carried out to identify different functional groups present in the plant extracts. FTIR spectra were captured between range 400 and 4000 cm^{-1} using a Perkin-Elmer Spectrum (version 10.4.40).

5. GC-MS Analysis

The methanolic leaf and rhizome extracts of *C. speciosus* were analysed through GC-MS analysis using Shimadzu GCMS-QP2010 Ultra system. An autosampler injected 1 μL plant sample into the gas chromatograph for analysis. The sample was injected in splitless mode to ensure full transfer of the sample into the gas chromatograph chamber at 280°C. The pressure and flow of gas were automatically regulated by the instrument and the temperature was set to rise steadily from 70°C to 310°C, which allows chemicals to be separated according to their volatility. After the separation of chemicals, they were ionized into the mass spectrometer at 200°C and then detected with high sensitivity. The final result of the analysis revealed the complicated makeup of the samples and provided a thorough chemical profile.

6. Quantitative Estimation of Phytochemicals

Total Reducing Sugar Content

Anthrone method was adopted to estimate the total reducing sugar content (TRS)^[9]. A range of glucose standard solutions from 20 -100 $\mu\text{g}/\text{mL}$ were prepared to draw a standard calibration

curve. 1 mL of methanolic leaf or rhizome extract (1 mg/mL) and 2 mL of anthrone reagent were combined for sample analysis. The combinations were quickly chilled in an ice-cold water after being heated for ten minutes in a boiling water bath. After the incubation period of 30 minutes, absorbance was recorded at 620 nm. TRS was calculated in terms of mg GE/g of dry extract using following equation:

$$TRS = \left(\frac{\text{Absorbance of sample} - \text{Intercept}}{\text{Slope of Standard Curve}} \right)$$

Total Phenolic Content

The total phenolic content (TPC) was determined using Follin-Ciocalteu method^[10]. Standard calibration curve was first prepared using gallic acid solutions at concentrations ranging from 20 - 100 $\mu\text{g}/\text{mL}$. For sample analysis, 1 mL of methanolic leaf or rhizome extracts at concentrations 100 $\mu\text{g}/\text{mL}$ were mixed with 1 mL of 10% FC reagent. After 5-6 minutes, 1 mL of 7.5% sodium carbonate solution was added. The reaction mixtures were left at room temperature in dark for an hour. UV spectrophotometer was used to measure the absorbance of each solution at 765 nm. TPC was calculated as mg GAE/g dry extract using following equation:

$$TPC = \left(\frac{\text{Absorbance of sample} - \text{Intercept}}{\text{Slope of Standard Curve}} \right)$$

Total Flavonoid Content

The total flavonoid content (TFC) was determined using AlCl_3 method^[11]. Quercetin at concentrations of 20, 40, 60, 80 and 100 $\mu\text{g}/\text{mL}$ was used to prepare a standard calibration curve. A test sample was prepared by mixing 1 mL of methanolic leaf or rhizome extract (100 $\mu\text{g}/\text{mL}$) with 150 μL of 5% NaNO_3 . After 10 minutes, 10% AlCl_3 was introduced to each test sample. The



mixture was then neutralized with 1 mL of 1M NaOH after 5 minutes and was allowed to stand at ambient temperature for 40 minutes. A double-beam UV-Vis spectrophotometer was used to measure the absorbance at 415 nm. TFC was calculated as mg QE/g of dry extract using following equation:

$$TFC = \left(\frac{\text{Absorbance of sample} - \text{Intercept}}{\text{Slope of Standard Curve}} \right)$$

7. Evaluation of Biological Activities

Anti-oxidant Activity

The ability of the crude methanolic leaf and rhizome extracts to reduce oxidative stress was assessed through 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay which measures the scavenging activity of free radicals. Ascorbic acid was taken as a standard and for sample analysis, a range of concentrations from 20-100 µg/mL were prepared by diluting the leaf and rhizome extracts. 1 mL of the 0.1mM DPPH solution was introduced in each mixture and incubated in complete darkness for half an hour. An absorbance was measured at 517 nm using a UV-vis spectrophotometer^[12].

The radical scavenging activity was calculated using following equation:

$$\% RSA = \left(\frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \right) \times 100$$

Anti-inflammatory Activity

The inhibition of protein denaturation was taken as a measure for assessing the anti-inflammatory ability of the extracts. Five variable dilutions of both methanolic leaf and rhizome extracts were prepared ranging between 20-100 µg/mL. 1 mL of 1% egg albumin solution and 2 mL of phosphate-buffered saline (pH 7.4) were mixed in each dilution. After incubating for 30 minutes at 37°C, the reaction mixtures were heated at 70°C for 15 minutes in a water bath to cause denaturation of the proteins. The mixtures were cooled to room temperature and absorbance was noted at 280 nm using a UV-vis spectrophotometer. Diclofenac sodium was used as a standard anti-inflammatory agent to compare the activity^[13].

Following equation was used to determine the % inhibition:

$$\% \text{ Inhibition} = \left(\frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \right) \times 100$$

RESULTS AND DISCUSSION

1. Qualitative Analysis of Phytochemicals

The qualitative estimation of bioactive compounds in the methanolic leaf and rhizome extracts showed the existence of several valuable phytochemical classes including alkaloids, glycosides, terpenoids, triterpenoids, saponins, tannins, coumarins etc. The results of the preliminary tests for phytochemical analysis are provided in Table 1.

Table 1. Phytochemicals present in methanolic leaf and rhizome extracts

Sr. No.	Phytoconstituents	Test(s) performed	Leaf	Rhizome
1.	Alkaloids	Mayer's test	+	+
		Wagner's test	-	-
		Picric acid test	+	-
		Iodine test	-	-
2.	Reducing sugars	Benedict's test	+	-
		Fehling's test	-	-



3.	Glycosides	Modified Borntrager's test	+	+
		10% NaOH test	-	-
		Aqueous NaOH test	+	-
		Concentrated H ₂ SO ₄ test	+	+
4.	Flavonoids	Alkaline reagent test	+	-
		Ammonia test	+	-
		Conc. H ₂ SO ₄ test	+	-
5.	Proteins and Amino acids	Ninhydrin test	-	-
		Xanthoproteic test	+	-
6.	Phenolic compounds	Iodine test	+	+
		Gelatin test	-	-
		Lead acetate test	+	+
7.	Tannins	Gelatin test	-	-
		Braymer's test	-	-
		10% NaOH test	+	+
8.	Phytosterol	Salkowski's test	-	+
9.	Terpenoids		-	-
10.	Triterpenoids	Salkowski's test	-	+
11.	Quinones	Alcoholic KOH test	-	-
		Conc. HCl test	-	-
12.	Anthraquinones	Borntrager's test	-	-
13.	Anthocyanins	HCl test	-	+
14.	Leucoanthocyanins	Isoamyl alcohol test	+	-
15.	Coumarins	NaOH paper test	+	+
16.	Saponins	Foam test	+	+

- indicates absence; + indicates presence of phytoconstituents

2. FTIR Spectroscopy

The FTIR spectra of methanolic leaf and rhizome extracts have been shown in Figure 1 and Figure 2, respectively. The interpretations of spectral data are tabulated in Table 2 and Table 3. The FTIR spectra of samples recorded a range of functional groups reflecting their chemically rich and diverse compositions. The presence of broad absorption bands between 3325–3338 cm⁻¹ suggest O–H stretching, which is typical for alcohols or phenolic substances. The peaks at 2944–2945 cm⁻¹ and 2832–2833 cm⁻¹ indicate the presence of C–H stretching vibrations typical of alkanes. Absorptions in the vicinity of 1706–1708 cm⁻¹

indicate the existence of carbonyl group like those present in ketones, aldehydes, or carboxylic acids. The bands in the region 1652–1450 cm⁻¹ may be due to C=C stretching by the aromatic rings and other structures associated with carbonyls. Strong bands at 1230–1110 cm⁻¹ are typical of C–O or C–N stretching vibrations indicating presence of ethers, esters, or amines. Further, sharp peaks at 1020–1021 cm⁻¹, as well as bending vibrations at 880 cm⁻¹ and 620–632 cm⁻¹, can suggest aromatic compounds and potentially halogenated groups^[14]. The results overall verify the presence of alcohols, alkanes, carbonyls, aromatic rings, ethers, and halogens in the samples.

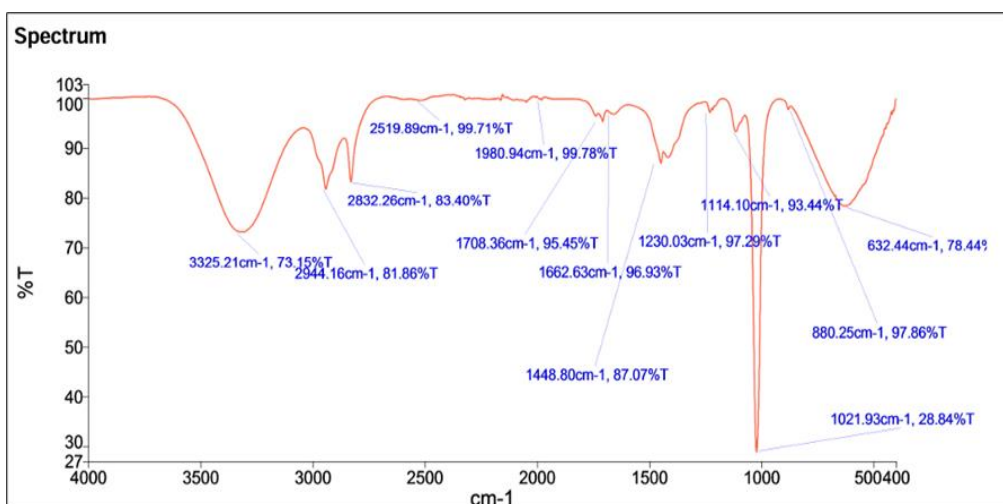


Figure 1. FTIR spectrum of methanolic extract of leaf

Table 1. Analysis of FTIR spectrum of leaf extract

Peak (cm ⁻¹)	%T	Interpreted functional group(s)
3325.21	73.15	O–H stretching (alcohols/ phenols)
2994.16	81.86	C–H stretching (alkane, CH ₂ /CH ₃)
2832.26	83.40	C–H stretching (alkane, CH ₂ /CH ₃)
2519.89	99.71	C≡C–H terminal alkyne, possibly COOH group (carboxylic acid)
1980.94	99.78	-
1708.36	95.45	C=O stretch, possibly from carboxylic acid, aldehyde, ketone, ester or amide.
1662.63	96.93	C=C stretching, possibly from alkene or aromatic ring
1448.80	87.07	CH ₂ /CH ₃ Bending (Methyl/methylene groups)
1230.03	97.29	C–O Stretch (Ester, ether, alcohol)
1114.10	93.44	C–N Stretch or Aromatic C–H Bending
1021.93	28.84	Possibly C–O–C (ether) or sulfoxide S=O stretch
880.25	97.86	Could be =C–H out-of-plane bending (alkenes or aromatics)
632.44	78.49	C–Cl or C–I bonds

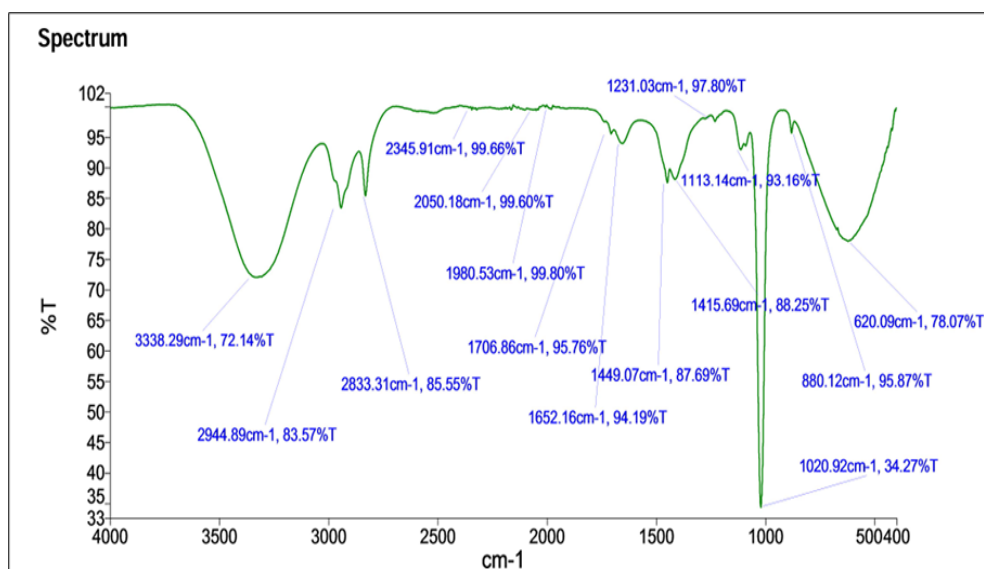


Figure 2. FTIR spectrum of methanolic extract of rhizome

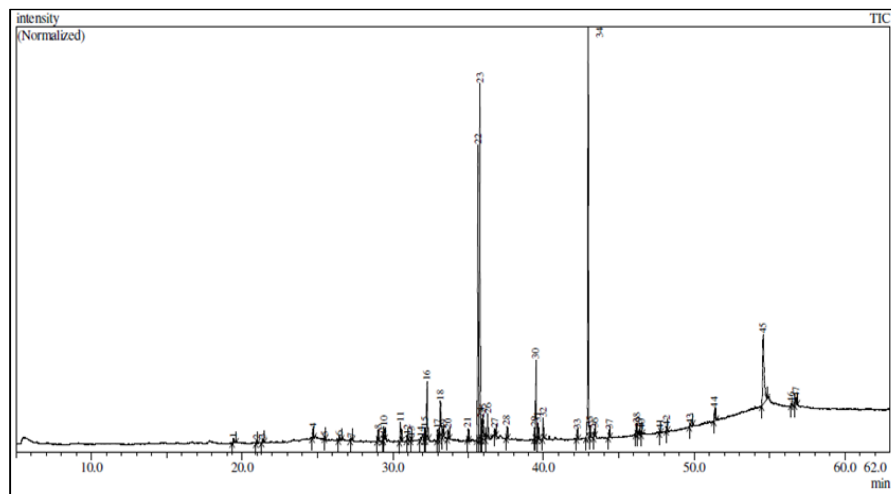
Table 2. Analysis of FTIR spectrum of rhizome extract

Peak (cm ⁻¹)	%T	Interpreted functional group(s)
3338.29	72.14	O-H stretching (alcohols/ phenols, hydrogen bonded)
2944.89	83.57	C-H stretching (alkane, CH ₂ /CH ₃)
2833.31	85.55	C-H stretching (alkane, CH ₂ /CH ₃)
2345.91	99.66	-
2050.18	99.60	-
1980.53	99.80	-
1706.86	95.76	C=O stretch, possibly from carboxylic acid, aldehyde, ketone, ester or amide.
1652.16	94.19	C=C stretching, possibly from alkene or aromatic ring or amide
1449.07	87.69	CH ₂ /CH ₃ bending or aromatic C-C (Alkane or Aromatic)
1415.69	88.25	CH ₂ bending or aromatic ring mode (Alkane or Aromatic)
1231.03	97.80	C-O stretch (Ester, Ether, or Alcohol)
1113.14	93.16	C-O or C-N stretch (Ether, Alcohol, or Amine)
1020.92	34.27	Strong C-O-C or S=O (Ether, Sulfoxide)
880.12	95.87	=C-H out-of-plane bending (Aromatic or Alkenes)
620.09	78.07	C-Cl or aromatic ring deformation (halogen or aromatic)

3. GC-MS Analysis

The GC-MS analysis of the methanolic leaf extract detected the presence of 45 phytochemicals

(Figure 3) and are summarized in Table 3 and that of rhizome extract reported the presence of 42 bioactive compounds (Figure 4) that are tabulated in Table 4.

**Figure 3. GC-MS chromatogram of methanolic leaf extract****Table 3. Phytochemicals identified in leaf extract**

Sr. No.	Name of compound	RT	Peak %	Molecular formula	Molecular weight
1	1-Tridecene	19.433	0.3	C ₁₃ H ₂₆	182
2	Cyclohexane, octyl-	20.985	0.14	C ₁₄ H ₂₈	196
3	Cycloheptasiloxane, tetradecamethyl-	21.368	0.18	C ₁₄ H ₄₂ O ₇ Si ₇	518
4	1-Heptadecene	24.704	0.52	C ₁₇ H ₃₄	238
5	Cyclooctasiloxane, hexadecamethyl-	25.478	0.09	C ₁₆ H ₄₈ O ₈ Si ₈	592

6	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	26.436	0.32	C ₁₃ H ₁₆ O ₃	220
7	1-Methylheptyl 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylate	27.213	0.17	C ₁₈ H ₃₂ O ₂	280
8	Cyclononasiloxane, octadecamethyl-	29.001	0.47	C ₁₈ H ₅₄ O ₉ Si ₉	666
9	Loliolide	29.342	0.49	C ₁₁ H ₁₆ O ₃	196
10	9-Eicosene, (E)-	29.406	0.73	C ₂₀ H ₄₀	280
11	2-Pentadecanone, 6,10,14-trimethyl-	30.499	1.06	C ₁₈ H ₃₆ O	268
12	Phthalic acid, diisobutyl ester	30.953	0.29	C ₁₆ H ₂₂ O ₄	278
13	8-Octadecanone	31.206	0.12	C ₁₈ H ₃₆ O	268
14	7-Hexadecenoic acid, methyl ester, (Z)-	31.833	0.7	C ₁₇ H ₃₂ O ₂	268
15	Cyclodecasiloxane, eicosamethyl-	32.128	0.58	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	740
16	Hexadecanoic acid, methyl ester	32.278	3.2	C ₁₇ H ₃₄ O ₂	270
17	Dibutyl phthalate	32.974	0.62	C ₁₆ H ₂₂ O ₄	278
18	n-Hexadecanoic acid	33.158	3.46	C ₁₆ H ₃₂ O ₂	256
19	Phthalic acid, butyl isohexyl ester	33.325	0.5	C ₁₈ H ₂₆ O ₄	306
20	3-Eicosene, (E)-	33.669	0.49	C ₂₀ H ₄₀	280
21	Cyclooctasiloxane, hexadecamethyl-	34.985	0.49	C ₁₆ H ₄₈ O ₈ Si ₈	592
22	9,12-Octadecadienoic acid, methyl ester	35.649	15.49	C ₁₉ H ₃₄ O ₂	294
23	8,11,14-Docosatrienoic acid, methyl ester	35.776	19.62	C ₂₃ H ₄₀ O ₂	348
24	Methyl cis-octadec-11-enoate	35.888	1.19	C ₁₉ H ₃₆ O ₂	296
25	Phytol	35.984	2.11	C ₂₀ H ₄₀ O	296
26	Methyl stearate	36.291	1.54	C ₁₉ H ₃₈ O ₂	298
27	Ethyl 2-(2-(2-butoxyethoxy)ethoxy)acetate	36.764	0.45	C ₁₂ H ₂₄ O ₅	248
28	Cyclononasiloxane, octadecamethyl-	37.572	0.62	C ₁₈ H ₅₄ O ₉ Si ₉	666
29	11,13-Eicosadienoic acid, methyl ester	39.41	0.61	C ₂₁ H ₃₈ O ₂	322
30	cis-Methyl 11-eicosenoate	39.508	4.26	C ₂₁ H ₄₀ O ₂	324
31	cis-Methyl 11-eicosenoate	39.629	0.88	C ₂₁ H ₄₀ O ₂	324
32	Eicosanoic acid, methyl ester	39.973	1.06	C ₂₁ H ₄₂ O ₂	326
33	Cyclononasiloxane, octadecamethyl-	42.227	0.47	C ₁₈ H ₅₄ O ₉ Si ₉	666
34	Methyl (Z)-13-docosenoate	42.962	23.61	C ₂₃ H ₄₄ O ₂	352
35	Methyl (Z)-13-docosenoate	43.071	0.58	C ₂₃ H ₄₄ O ₂	352
36	13-Docosanoic acid, methyl ester	43.376	0.41	C ₂₃ H ₄₆ O ₂	354
37	Cyclononasiloxane, octadecamethyl-	44.346	0.35	C ₁₈ H ₅₄ O ₉ Si ₉	666
38	15-Tetracosenoic acid, methyl ester, (Z)-	46.156	0.61	C ₂₅ H ₄₈ O ₂	380
39	Cyclononasiloxane, octadecamethyl-	46.331	0.31	C ₁₈ H ₅₄ O ₉ Si ₉	666
40	Tetracosanoic acid, methyl ester	46.524	0.15	C ₂₅ H ₅₀ O ₂	382
41	Squalene	47.729	0.21	C ₃₀ H ₅₀	410
42	Cyclononasiloxane, octadecamethyl-	48.186	0.15	C ₁₈ H ₅₄ O ₉ Si ₉	666
43	.delta.-Tocopherol	49.7	0.03	C ₂₇ H ₄₆ O ₂	402
44	Diosgenin acetate	51.36	0.87	C ₂₉ H ₄₄ O ₄	456
45	Diosgenin	54.582	8.68	C ₂₇ H ₄₂ O ₃	414
46	Diosgenin acetate	56.433	0.09	C ₂₉ H ₄₄ O ₄	456
47	7.beta.-Hydroxydiosgenin	56.749	0.71	C ₂₇ H ₄₂ O ₄	430

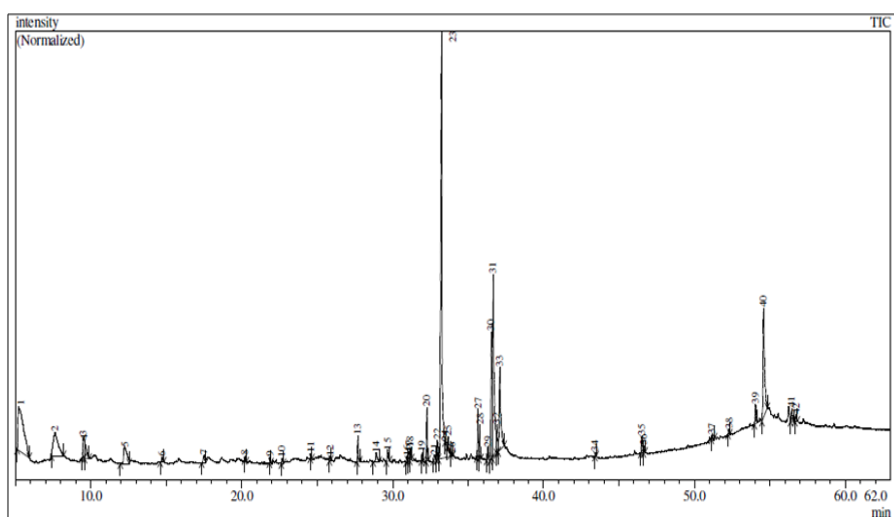


Figure 4. GC-MS chromatogram of methanolic rhizome extract

Table 4. Phytochemicals identified in rhizome extract

Sr. No.	Name of compound	RT	Peak %	Molecular formula	Molecular weight
1	2-Cyclopenten-1-one, 2-hydroxy-	5.224	11.65	C ₅ H ₆ O ₂	98
2	Glycerin	7.634	5.31	C ₃ H ₈ O ₃	92
3	Furaneol	9.517	1.37	C ₆ H ₈ O ₃	128
4	4-Heptanone, 2-methyl-	9.592	1.42	C ₈ H ₁₆ O	128
5	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	12.243	2.62	C ₆ H ₈ O ₄	144
6	6-Tridecen-4-yne, (Z)-	14.721	0.31	C ₁₃ H ₂₂	178
7	2-Methoxy-4-vinylphenol	17.524	0.67	C ₉ H ₁₀ O ₂	150
8	Caryophyllene	20.223	0.12	C ₁₅ H ₂₄	204
9	Curcumene	21.885	0.14	C ₁₅ H ₂₂	202
10	2,4-Di-tert-butylphenol	22.677	0.24	C ₁₄ H ₂₂ O	206
11	Caryophyllene oxide	24.583	0.13	C ₁₅ H ₂₄ O	220
12	beta-Acorenol	25.845	0.12	C ₁₅ H ₂₆ O	222
13	Pentadecanal-	27.694	0.9	C ₁₅ H ₃₀ O	226
14	Tetradecanoic acid	28.903	0.65	C ₁₄ H ₂₈ O ₂	228
15	4-Hydroxy-3,5,5-trimethyl-4-[3-oxo-1-butenyl]-2-cyclohexen-1-one	29.662	0.64	C ₁₃ H ₁₈ O ₃	222
16	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	30.942	0.15	C ₁₆ H ₂₂ O ₄	278
17	Pentadecanoic acid	31.075	0.45	C ₁₅ H ₃₀ O ₂	242
18	Methyl 3-hydroxytetradecanoate	31.193	0.47	C ₁₅ H ₃₀ O ₃	258

19	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	31.954	0.28	C ₁₆ H ₂₂ O ₄	278
20	Hexadecanoic acid, methyl ester	32.273	1.63	C ₁₇ H ₃₄ O ₂	270
21	cis-7-Hexadecenoic acid	32.75	0.3	C ₁₆ H ₃₀ O ₂	254
22	Dibutyl phthalate	32.961	0.68	C ₁₆ H ₂₂ O ₄	278
23	n-Hexadecanoic acid	33.248	28.93	C ₁₆ H ₃₂ O ₂	256
24	4-Fluoro-1-methyl-5-carboxylic acid, ethyl (ester)	33.508	0.01	C ₇ H ₉ FN ₂ O ₂	172
25	Docosanoic acid, ethyl ester	33.676	0.58	C ₂₄ H ₄₈ O ₂	368
26	Phthalic acid, butyl octyl ester	33.942	0.09	C ₂₀ H ₃₀ O ₄	334
27	9,12-Octadecadienoic acid, methyl ester	35.646	1.48	C ₁₉ H ₃₄ O ₂	294
28	Linolenic acid, methyl ester	35.77	1.09	C ₁₉ H ₃₂ O ₂	292
29	Methyl stearate	36.29	0.37	C ₁₉ H ₃₈ O ₂	298
30	10E,12Z-Octadecadienoic acid	36.543	6.1	C ₁₈ H ₃₂ O ₂	280
31	7-Tetradecenal, (Z)-	36.666	12.51	C ₁₄ H ₂₆ O	210
32	Linoleic acid ethyl ester	36.921	1.39	C ₂₀ H ₃₆ O ₂	308
33	Octadecanoic acid	37.099	5.86	C ₁₈ H ₃₆ O ₂	284
34	Bis(2-ethylhexyl) phthalate	43.44	0.06	C ₂₄ H ₃₈ O ₄	390
35	Androsta-1,4-diene-3,17-dione	46.509	0.95	C ₁₉ H ₂₄ O ₂	284
36	cis-11-Eicosenoic acid	46.661	0.22	C ₂₀ H ₃₈ O ₂	310
37	2H-1-Benzopyran, 3,4-dihydro-6-methoxy-2,8-dimethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-, (2R)	51.15	0.03	C ₂₈ H ₄₈ O ₂	416
38	1-Eicosanol	52.29	0.16	C ₂₀ H ₄₂ O	298
39	4,4-Dimethylcholest-5-enol	54.052	0.87	C ₂₉ H ₅₀ O	414
40	Diosgenin	54.585	7.88	C ₂₇ H ₄₂ O ₃	414
41	Diosgenin acetate	56.448	0.77	C ₂₉ H ₄₄ O ₄	456
42	7-beta-Hydroxydiosgenin	56.742	0.37	C ₂₇ H ₄₂ O ₄	430

The major compounds in leaf extract were Methyl (Z)-13-docosenoate (23.61%) followed by 8,11,14-Docosatrienoic acid, methyl ester (19.62%), 9,12-Octadecadienoic acid, methyl ester (15.49), Diosgenin (8.68%), cis-Methyl 11-eicosenoate (4.26), n-Hexadecanoic acid (3.46%), Hexadecanoic acid, methyl ester (3.2%), Phytol (2.11%) etc. The important phytochemicals that possess high medicinal values are Loliolide (0.49%), Phytol (2.11%), Squalene (0.21%), Diosgenin (8.68%), delta-Tocopherol (0.03%), 2-Pentadecanone, 6,10,14-trimethyl- (1.06%), 9,12-

Octadecadienoic acid, methyl ester (15.49%), 8,11,14-Docosatrienoic acid, methyl ester (19.62%), 13-Docosenoic acid, methyl ester (0.41%), Diosgenin acetate (0.09%) etc. Tridecene shows antimicrobial and cytotoxic activity^[15]. Loliolide is a phytochemical that possesses cell protective and neuroprotective property^[3,16]. Phytol exhibits cytotoxic and antimicrobial potential^[17,18].

The major compounds contributing to chemical composition of the rhizome extract were n-



Hexadecanoic acid (28.93%), 7-Tetradecenal, (Z)- (12.51%), Diosgenin (7.88%), 10E,12Z-Octadecadienoic acid (6.1%), Octadecanoic acid (5.86%) etc. The compounds belonging to sesquiterpene category such as Diosgenin (7.88%), Diosgenin acetate (0.77%), 7 β Hydroxydiosgenin (0.37%), Caryophyllene (0.12%), Caryophyllene oxide (0.13%), Androsta-1,4-diene-3,17-dione (0.95%), 2-Methoxy-4-vinylphenol (0.67%), Linolenic acid, methyl ester (1.09%) etc. are of more pharmacological value. Most of these compounds are potential antioxidants. Diosgenin is a steroidal sapogenin and reported to have anti-inflammatory, anticancer and antidiabetic activities^[19]. Caryophyllene is a flavoring agent and can regulate cell signaling pathways to mitigate conditions like inflammation, oxidative stress, neuronal death and reduced plasticity caused due to sleep loss^[20]. Caryophyllene oxide is a food preservative that exhibit antifungal activity against dermatophytes^[21]. 2,4-Di-tert-butylphenol is auto-toxic and inhibits the growth of various bacteria and fungi that produce it^[22]. n-Hexadecanoic also known as palmitic acid can inhibit phospholipase A2 and reduce inflammation^[23]. Linolenic and linoleic acids possess anti-plasmodial and antifungal activity^[24, 25]. Androsta-1,4-diene-3,17-dione (ADD) is an important steroidal drug and used in the production of steroids in pharmaceuticals^[26]. Diosgenin and some of its derivatives were found in both the leaf and rhizome extracts and are significantly used in type 2 diabetes and cancer therapy^[27].

4. Quantitative Estimation of Phytochemicals

Total Reducing Sugar Content

The estimation of TRS present in methanolic leaf and rhizome extracts of *C. speciosus* was done by using anthrone method and found to be 323.2 mg GE/g and 618.2 mg GE/g dry extract of leaf and

rhizome, respectively. The study revealed that the rhizome contains much higher amount of reducing sugar in comparison to leaf (Table 5).

Total Phenolic Content

The Folin-Ciocalteu technique was used to calculate the quantity of TPC in the methanolic leaf and rhizome extract of *C. speciosus*. The study revealed that the leaf and rhizome extracts contain total phenolic amount of 64.508 mg GAE/g and 65.643 mg GAE/g dry extracts of leaf and rhizome, respectively (Table 5).

Total Flavonoid Content

With the help of the calorimetric assay, the TFC in the methanolic leaf and rhizome extracts of *C. speciosus* was assessed and was estimated to be 9.571 mg QE/g and 7.786 mg QE/g dry extracts of leaf and rhizome, respectively (Table 5).

Table 5. Quantification of important phytoconstituents

Quantitative test	Amount	
	Leaf	Rhizome
TRS	323.2 mg GE/g	618.2 mg GE/g
TPC	64.508 mg GAE/g	65.643 mg GAE/g
TFC	9.571 mg QE/g	7.786 QE/g

5. Evaluation of Biological Activities

Antioxidant Activity

The IC₅₀ value for ascorbic acid standard was found to be 58.22 μ g/mL which was required for the 50% inhibition of DPPH free radicals. The leaf extract showed more suppression of the free radical as compared to the rhizome extract. The IC₅₀ values of methanolic leaf and rhizome extracts were observed to be 125.9 μ g/mL and 167.05 μ g/mL, respectively. In the present study, the rhizome extract (IC₅₀ = 167.05 μ g/mL) was considerably more potent than the value noted by



Naznin et. al. ($IC_{50} = 1699 \pm 62 \mu\text{g/mL}$)^[28] but less potent than that reported by Jha et. al. ($IC_{50} = 50.38 \mu\text{g/mL}$)^[29].

Anti-inflammatory Activity

The denaturation of egg-albumin decreased with an increase in the concentration of diclofenac sodium and the IC_{50} value was calculated to be $49.434 \mu\text{g/mL}$ which was required for the 50% renaturation of egg-albumin. The IC_{50} value obtained for methanolic rhizome extract was $57.859 \mu\text{g/mL}$ which was comparable to the standard drug diclofenac sodium ($49.434 \mu\text{g/mL}$). The value obtained in the present study was quite lower than the values reported earlier (IC_{50} value 2.23 mg/mL)^[30] which revealed that the rhizome of *C. speciosus* exhibit good anti-inflammatory property.

CONCLUSION

The phytochemical profiling of *C. speciosus* disclosed a range of secondary metabolites supporting its use in ethnic medicine. The spectrometric analysis confirmed the presence of important chemicals like diosgenin, phytol, squalene, loliolide, delta-tocopherol, caryophyllene, caryophyllene oxide, 9,12,15-octadecatrienoic acid, methyl ester (α -Linolenic acid methyl ester), beta-sitosterol, androsta-1,4-diene-3,17-dione (ADD) etc. which have important medicinal and economic values. Quantitative estimation of TRS, TPC and TFC showed the presence of significant amount of these phytoconstituents. The TRS in leaf and rhizome were found to be 323.2 mg GE/g and 618.2 mg GE/g of dry extract of leaf and rhizome, respectively. The TPC in the leaf and rhizome extracts were calculated to be 64.508 mg GAE/g and 65.643 mg GAE/g , respectively, while the TFCs were assessed to be 9.571 mg QE/g and 7.786 mg QE/g of dry extract, respectively. The

DPPH assay concluded that the antioxidant activity of methanolic leaf and rhizome extracts (IC_{50} value $125.9 \mu\text{g/mL}$ and $167.05 \mu\text{g/mL}$) were comparatively lower than the standard ascorbic acid (IC_{50} value $58.22 \mu\text{g/mL}$). Analysis of anti-inflammatory property by egg-albumin denaturation method proved that the soluble extract of rhizome in methanol exhibit notable anti-inflammatory effects. Perhaps the anti-inflammatory activities of the extract were enhanced because of the existence of steroidal sapogenins and monounsaturated fatty acids which were validated by GC-MS analysis. Ultimately, this work opens the door for additional exploration of *C. speciosus* and its conservation for the future use in the field of research to isolate the important phytochemicals and new discoveries for the treatment of fatal illnesses.

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Declarations

Author Contributions

Conceptualization, Methodology, Data curation, Writing original draft, Software: **Biplab Singha Deka, Raj Kumar and Sachin Thakur**; Conceptualization, Reviewing and editing, Supervision: **Munish Sharma**.

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Conflict of Interest



The authors declare that the study was conducted without any kind of financial or other relationships that could be regarded as a potential conflict of interest.

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