Eksplorium *p*-ISSN 0854-1418
Volume 46 No. 1, May 2025: 325–337 *e*-ISSN 2503-426X

Identification of Bioactive Compounds in *Costus igneus* leaves via Pharmacognostic, Phytochemical Screening, and GC-MS Analysis

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Article Received: 25 Feb 2025, Revised: 20 April 2025, Accepted:06 May 2025

Abstract: The present study aims to investigate the leaves of *Costus igneus* through a comprehensive pharmacognostic study, preliminary phytochemical screening, and Gas Chromatography–Mass Spectrometry (GC-MS) analysis to identify its bioactive constituents. Pharmacognostic analysis was conducted to establish key diagnostic features, including macroscopic and microscopic characteristics, physicochemical parameters, and powder microscopy. Phytochemical screening revealed the presence of important secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides, tannins, and phenolic compounds. GC-MS analysis identified several bioactive compounds with known pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and antidiabetic properties. The findings support the ethnomedicinal use of *Costus igneus* and lay a scientific foundation for further isolation and characterization of therapeutic agents.

Keywords: *Costus igneus*, Pharmacognostical analysis & phytochemical screening, GC-MS analysis

INTRODUCTION

According to the World Health Organization (WHO), approximately 80% of the global population relies on plant-based traditional medicine for the prevention and treatment of various diseases.[1] In addition, although estimates may vary based on the criteria used to define natural product-derived drugs, it is widely accepted that up to 50% of currently marketed pharmaceuticals are derived, directly or indirectly, from natural products.[2] *Costus igneus*, commonly known as the "Insulin Plant," is a medicinal herb belonging to the family Costaceae. It is renowned for its antidiabetic properties, particularly its ability to stimulate insulin production in the human body. In recent years, this plant has gained significant global attention and is increasingly used in Ayurvedic medicine for its therapeutic benefits. Native to Southeast Asia, particularly the Greater Sunda Islands of Indonesia, *Costus igneus* was introduced to India relatively recently from South Central America. In the Indian state of Kerala, it has been cultivated both as an ornamental and a medicinal plant. Traditionally, in herbal medicine, diabetes is managed by chewing the leaves of the insulin plant daily for a period of at least one month, which is believed to help regulate blood glucose levels effectively.[3] An important advantage of using crude plant extracts over isolated compounds is the presence of multiple constituents that may act synergistically with the primary bioactive

molecule, thereby enhancing its therapeutic efficacy.[4] Pharmacognostic studies form the foundation for the standardization and quality control of medicinal plants by identifying key diagnostic characteristics and ensuring the authenticity of the plant material. Furthermore, advanced analytical techniques such as Gas Chromatography–Mass Spectrometry (GC-MS) facilitate the detection and characterization of volatile and semi-volatile phytochemicals with significant pharmacological potential.[5] Therefore, this study aims to explore the pharmacognostic features, phytochemical composition, and bioactive constituents of *Costus igneus* leaves using GC-MS, in an effort to support its traditional uses and contribute to the development of herbal therapeutics.

Figure-1: *Costus igneus* plant

MATERIALS AND METHODS

The fresh leaves of *Costus igneus* were collected in month of July from The National Botanical Research Institute (NBRI) Lucknow- 226001, Uttar Pradesh, India and authenticated by the botanist at (Botany department) Meerut.

Following verification,large quantities of fresh leaves were gathered, washed to get rid of dirt, shadedried, and ground into powder using a machine.to create coarse powder, the dried leaf po wder was sieved through 40 mesh and kept in a tight container until it was needed again. The Qualitative, quantitative & powder microscopy of Leaf were studied.[6] The physicochemical constants were determined by pharmacopeial methods.[7] The successive extraction of the leaves powder was done by continuous hot extraction method using soxhlet apparatus. The various solvents used such as Petroleum ether, chloroform, ethanol and water.[8] The preliminary phytochemical studies of different extracts were performed.[9,10]

Gas chromatography-mass spectroscopic analysis of *Costus igneus*

GC-MS analysis of the Ethanolic extract of *Costus igneus* leaves was performed using the equipment GCMS-QP2010 is a single quadrupole Gas Chromatograph-Mass Spectrometer. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 μm films. The carrier gas used is Helium with at low of

1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60 °C for 15 min, then gradually increased to 280 °C at 3 min. The identification of components was based on NIST14.L libraries as well as comparison of their retention indices. The constituents were identified after comparison with those available in the computer library (NIST14.L) attached to the GC-MS instrument and the results obtained have been tabulated.[11,12]

RESULT & DISCUSSION

1. Macroscopic Characters

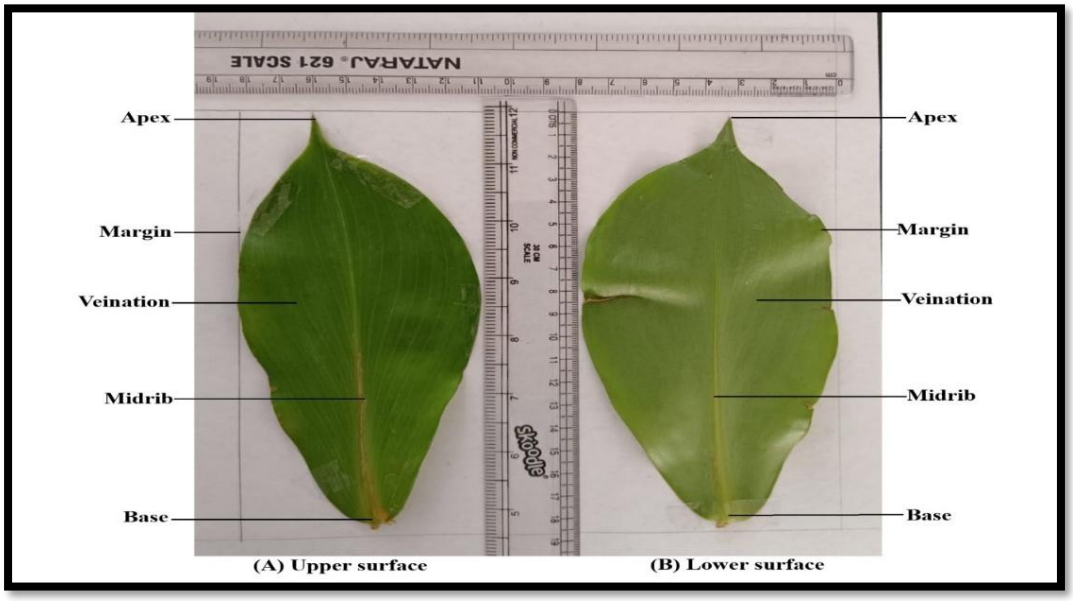


Figure- 2: Macroscopic View of Upper and Lower Surface of Leaf

Table 1: Macroscopic characters of *Costus igneus* (leaves)

S. No	Macroscopic characters	Observations
1.	Size	7-18cm length, 5 to 8 cm breadth and 1 mm thickness.
2.	Shape	lance
3.	Taste	Bitter
4.	Colour	Dark green above and lighter green below
5.	Odour	Characteristic
6.	Texture	Simple, sessile, alternate, thin with smooth glaborous leathery surface
7.	Fracture	Soft

Macroscopically characters of leaves of *Costus igneus* was examined by size, shape, taste, colour, odour, texture and fracture. After examination it was found that *Costus igneus* has a Leaves are Simple, sessile, alternate, thin with smooth glaborous leathery surface. The leaves colour were dark green above and lighter green below and characteristic odour. The size of leaves was about 4-11cm length, 5 to 8 cm breadth and 1 mm thickness. All above examination helps to identify the plant.

2. Quantitative Microscopy of Leaf Constants of *Costus igneus*

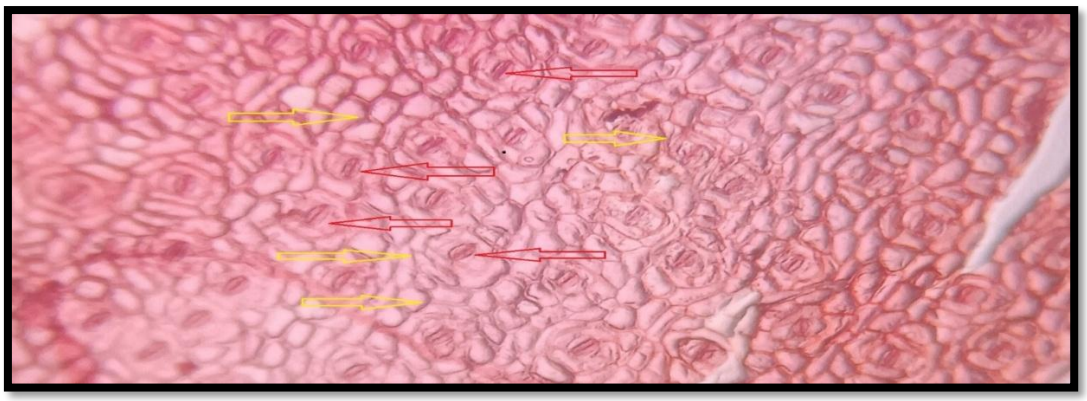


Figure- 3: Stomata and epidermal cells of CI leaf (Upper side) Yellow color arrow represents the epidermal cells & Red color arrow represents the Stomata

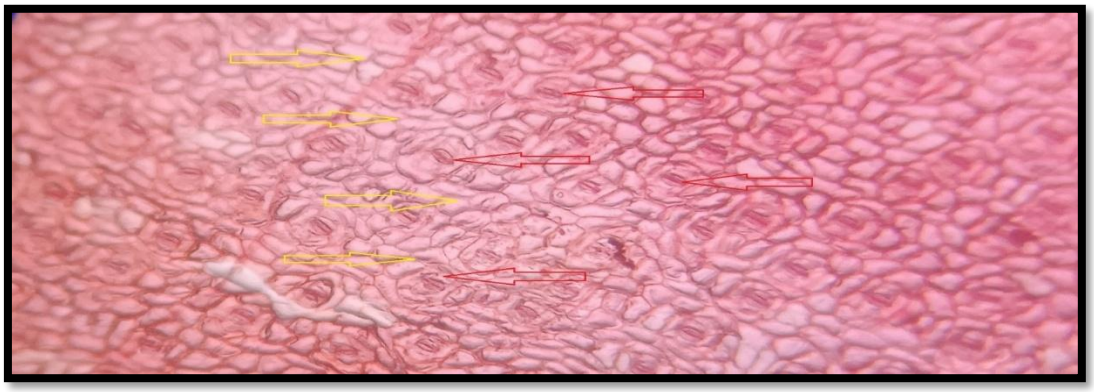


Figure- 4: Stomata and epidermal cells of CI leaf (Lower side) Yellow color arrow represent the epidermal cells & Red color arrow represents the Stomata

Table 2: Stomatal Number and Stomatal Index

Value	Stomatal number	Stomatal index	Vein islet Number	Vein termination number
Per sq. mm	42.25	17.12	22.85	18.40

3. Qualitative Microscopy of *Costus igneus* leaves & stem

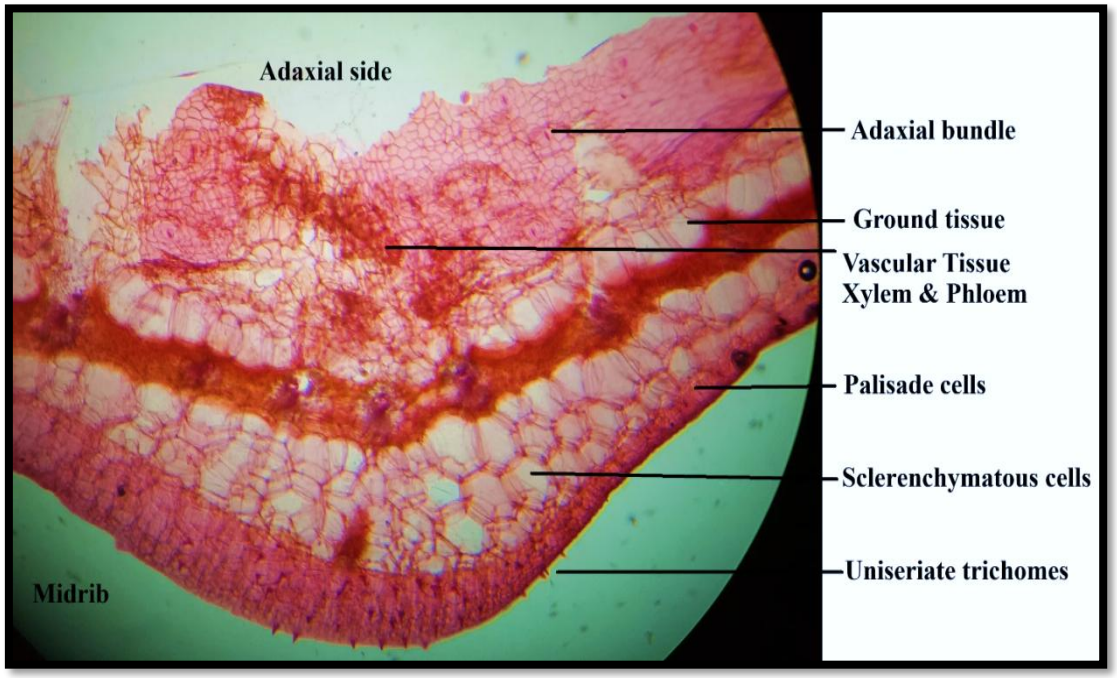


Figure- 5: T.S. of midrib leaf under 100X

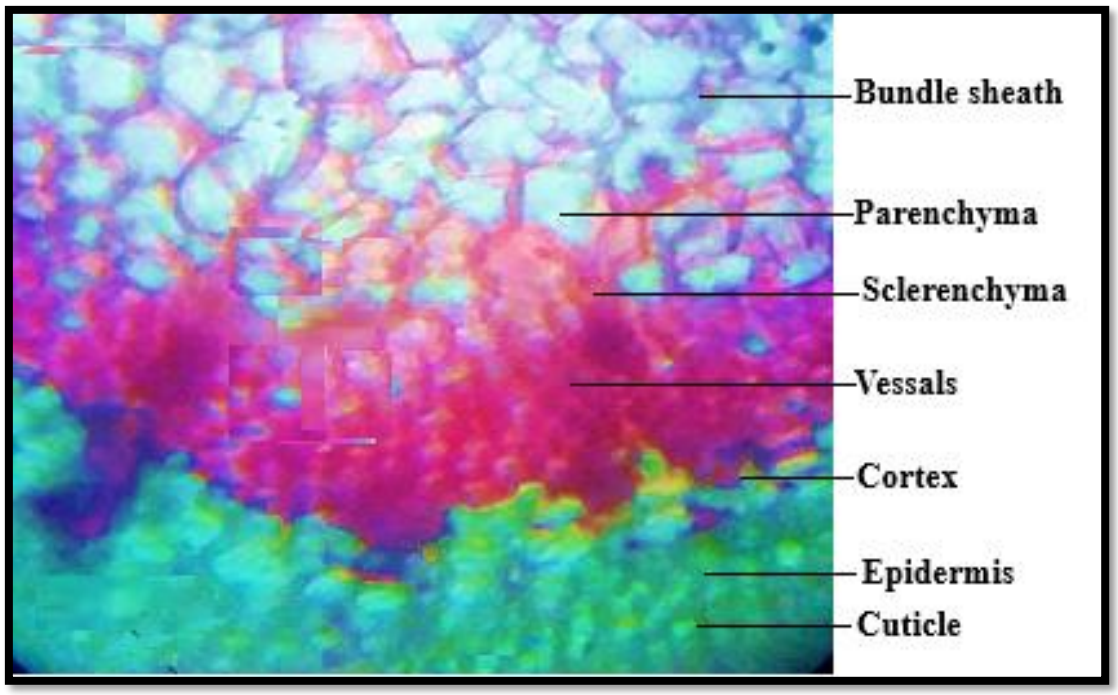
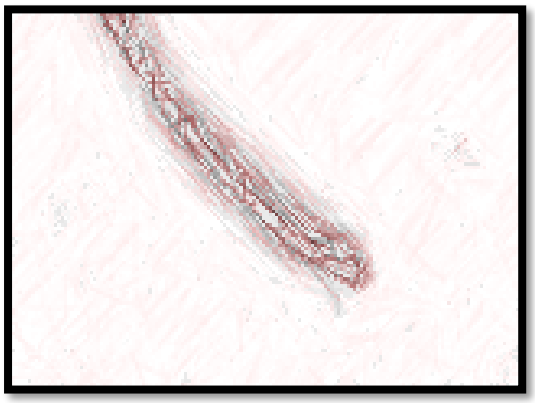
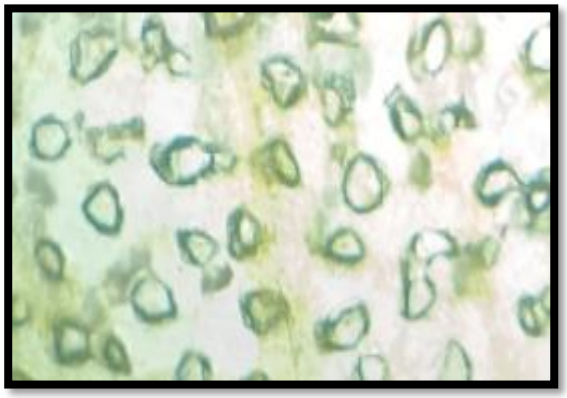


Figure- 6: T.S. of stem under 100X

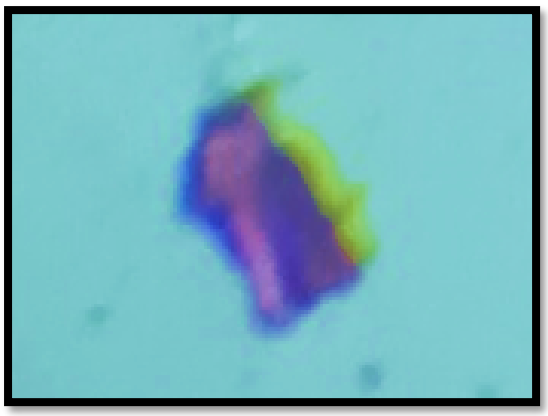
4. Powder Microscopy of *Costus igneus* leaves



(A) Unicellular Covering Trichome

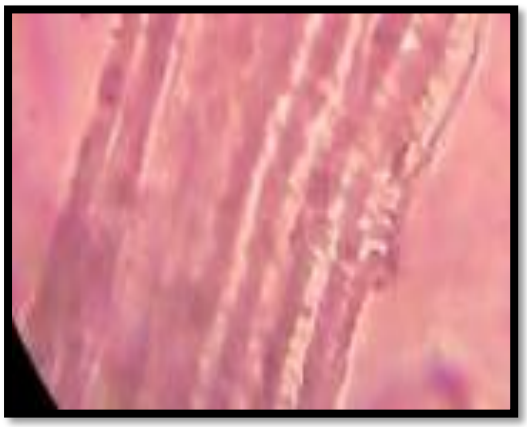


(B) Starch grain

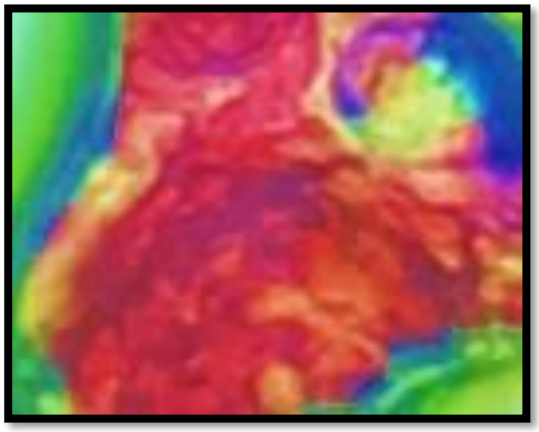


(C) Lignified Fiber

(D) Calcium oxalate



(E) Xylem Vessel (Bordered Pitted)



(F) Sclereids (Stone Cell)

Figure- 7: Microscopy of powder under 100X

5. Physico-chemical studies

Table 3: Physico-chemical Characteristics of of *Costus igneus* (Leaves)

S. No.	Parameters	Results
1.	Foreign organic content	0.07%
2.	Moisture content	0.87%
3.	Swelling Factor	1.2 ml.
4.	Foaming Index	Less than 100
5.	**Ash Values** Total Ash Acid Insoluble Ash Water Soluble Ash Sulphated ash	 14.72% w/w 3.10% w/w 1.85% w/w 2.73% w/w
6.	Alcohol soluble extractive value	11.65% w/w
7.	Water soluble extractive value	14.75% w/w

6. Successive Extraction with Different Solvents of *Costus igneus* leaves

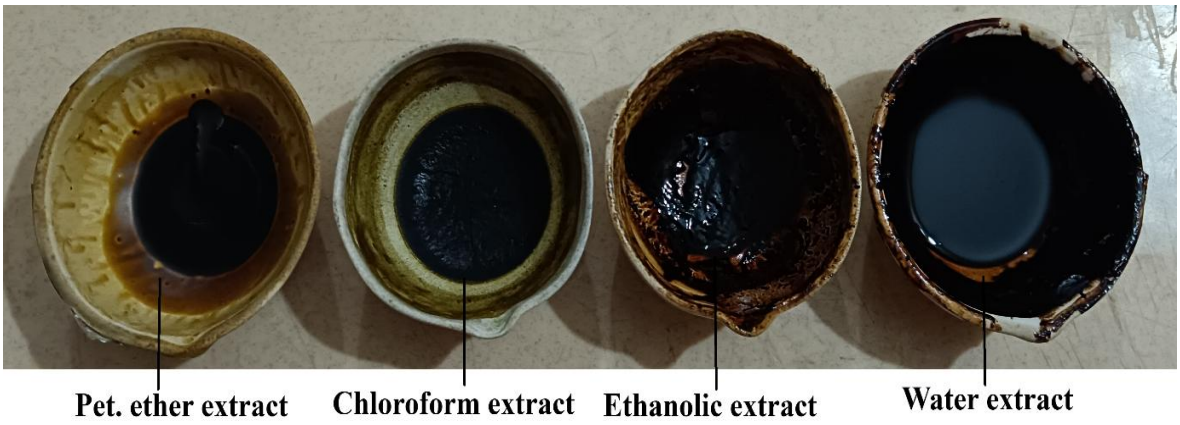


Figure- 8: Extract with Different Solvents

Table 4: Yield of Successive Extraction with Different Solvents

S. No.	Solvents	Appearance	Yield (%w/w)
1.	**Pet. ether**	Light brown	5.25
2.	**Chloroform**	Light Green	4.86
3.	**Ethanol**	Dark green	8.08
4.	**Aqueous**	Light black	6.16

7. Preliminary Phytochemical Screening

Table 5: Qualitative chemical examination of *Costus igneus* extract

S. No.	Phytoconstituents	Pet. ether extract	Chloroform extract	Ethanolic extract	Aqueous extract
1.	Alkaloids	—	—	+ +	+
	Saponin	+	+	++	+
2.	Carbohydrates	—	—	+	+
3.	Glycosides	—	—	++	+
4.	Steroids	+	—	++	—
6.	Resins	—	—	—	—
7.	Fats and Oils	+	—	+	—
8.	Flavonoids	—	+	++	+
9.	Tannin	—	—	++	+
10.	Phenols	—	—	+	+
11.	Mucilage	—	—	+	+
12.	Terpenoids	+	+	+	—

Where: (+ +) Strongly Present; (+) Mild Present; (—) Absent

8. Thin Layer Chromatography of ethanolic leaves extract *Costus igneus*

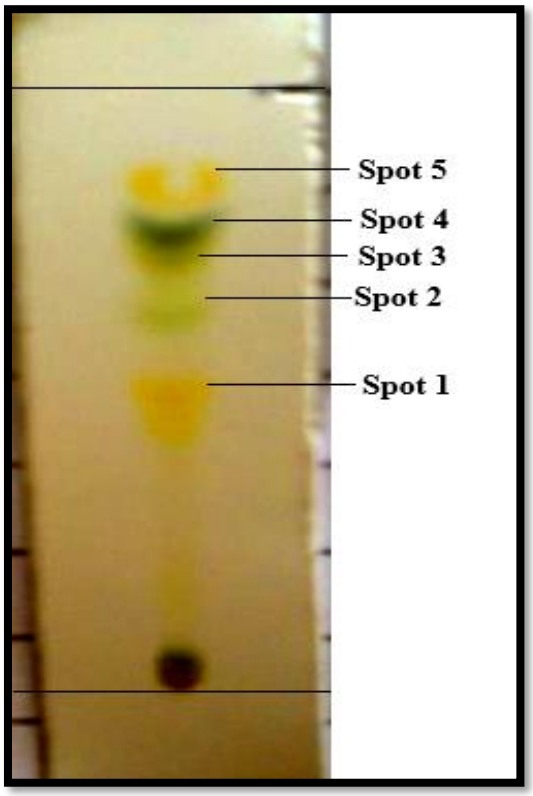


Figure- 9: TLC of ethanolic extract

Table 6: TLC of ethanolic extract

S.N.	Fractions	Solvent systems	Detecting reagents	Color	No. of spots	Rf value of spots
1	Ethanol	n-hexane: dichloromethane: ethanol (5:2:1)	Anisaldehyde sulphuric acid, heated at 1000C for 5 Min.	Green & yellow	Green & yellow	0.35, 0.40, 0.53, 0.63, 0.68

9. Identification of Phytocomponent by GC-MS of ethanolic extract of *Costus igneus* leaf

This study aimed to identify the phytochemical constituents present in the ethanolic leaf extracts of *Costus igneus*, a species belonging to the genus *Costus* and the family *Costaceae*. Phytochemical screening was carried out to determine the nature of secondary metabolites, while Gas Chromatography–Mass Spectrometry (GC-MS) was employed to identify specific phytocompounds within the ethanolic extract. The phytochemicals were identified based on their molecular weights (m/z) obtained from the GC-MS chromatograms. Compound identification was achieved by interpreting the spectral peaks and comparing them with reference data from the National Institute of Standards and Technology (NIST) library.

Preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides, steroids, tannins, saponins, and phenolic compounds. This study reports the presence of several phytochemical compounds in the ethanolic leaf extract of *Costus igneus*, some of which are identified for the first time within this genus and species. The pharmacological relevance of these compounds supports the ethnomedicinal use of *Costus igneus*, particularly in the treatment of various ailments, including fungal and bacterial infections, inflammatory conditions, cancer, and diabetes.

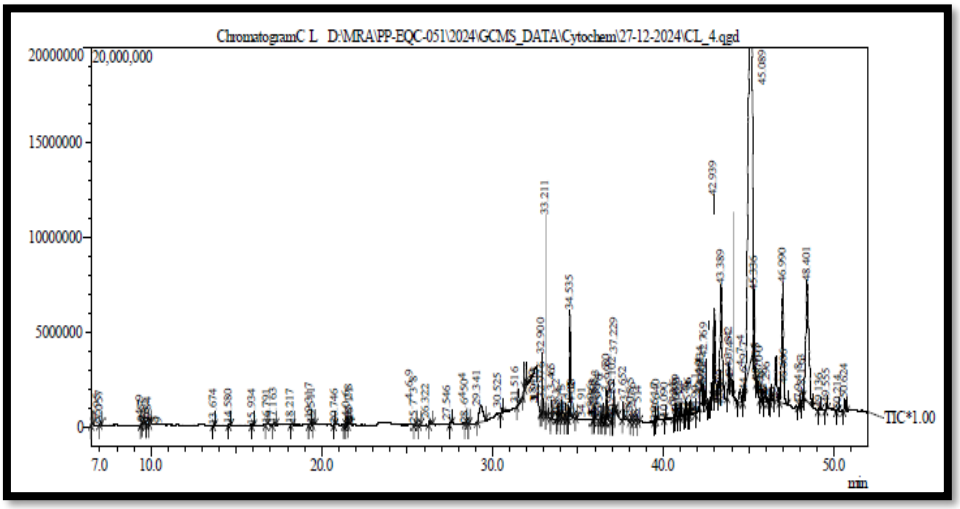
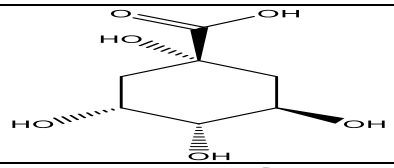
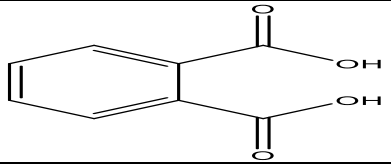
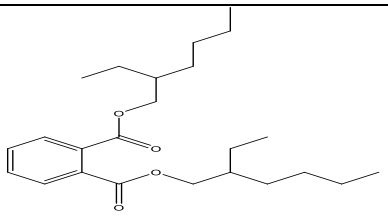
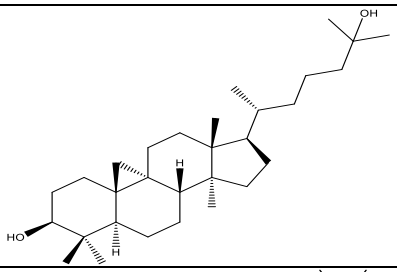
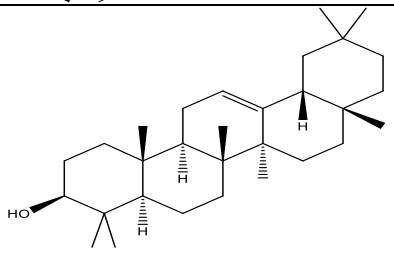
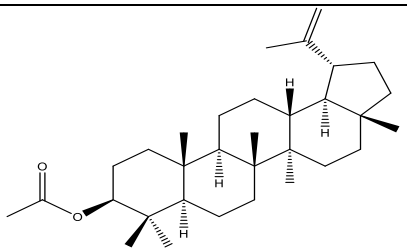
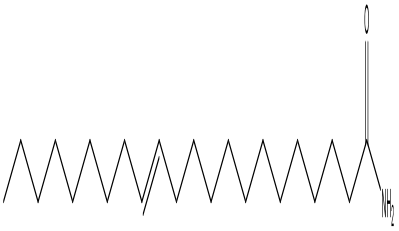
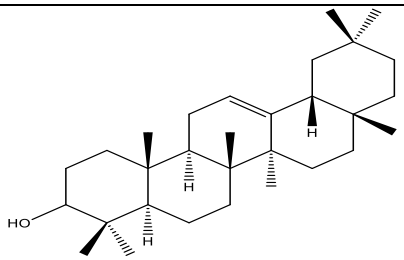
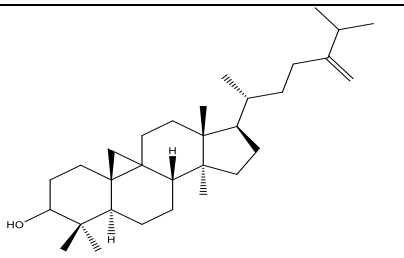
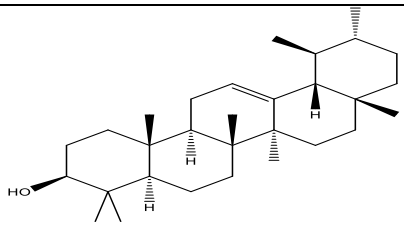


Figure- 10: Chromatogram of *Costus igneus*

Table 7: GC-MS spectral analysis of ethanolic extract of *Costus igneus leaves*

No.	R. Time	Name	Molecular formula	Molecular weight	Area %	Structure	Category
1	29.341	Quinic acid	$C_7H_{12}O_6$	192	1.84		Glycosides
2	33.211	1,2-Benzenedicarboxylic acid	$C_{16}H_{22}O_4$	278	2.64		Tannins
3	34.535	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.24		Fatty acid
4	37.229	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278	1.38		Fatty acid
5	42.939	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	3.69		Flavonoid
6	42.999	9,19-Cyclo-9. beta-lanostane-3. beta, 25-diol	$C_{30}H_{52}O_2$	444	2.30		Triterpenoid
7	43.389	beta-Amyrin	$C_{30}H_{50}O$	426	6.21		Glycosides

8	45.089	Lup-20(29)-en-3-ol, acetate, (3. beta.)	$C_{32}H_{52}O_2$	468	47.11		Terpenoid
9	45.336	13-Docosenamide	$C_{22}H_{43}NO$	337	1.21		Fatty acid
10	46.606	Olean-12-en-3-ol acetate	$C_{32}H_{52}O_2$	468	2.26		Terpenoid
11	46.990	9,19-Cyclolanostan-3-ol, 24-methylene	$C_{31}H_{52}O$	440	6.72		Terpenoid
12	48.401	alpha-Amyrin	$C_{30}H_{50}O$	426	10.32		Pentacyclic triterpenoid

DISCUSSION

Existing research on *Costus igneus* leaves describes them as measuring approximately 7–18 cm in length, 5–10 cm in breadth, and about 1 mm in thickness. The leaves exhibit a lanceolate shape, an acute apex, a bitter taste, and a characteristic odour. This plant has been widely recognized for its broad spectrum of pharmacological benefits. Phytochemical investigations have confirmed the presence of essential phytoconstituents such as alkaloids, glycosides, flavonoids, tannins, and others. These bioactive compounds are associated with a variety of therapeutic activities, including antidiabetic, antimicrobial, anti-inflammatory, antioxidant, and hypolipidemic effects. Given its diverse pharmacological potential, further

in-depth research is warranted to explore and isolate the specific constituents responsible for these medicinal properties. Such studies will be instrumental in the development of more effective, reliable, and safe plant-based treatments for various health conditions, ultimately contributing to the betterment of human health and well-being.

CONCLUSION

In conclusion, pharmacognostic evaluation, physicochemical analysis, phytochemical screening, thin-layer chromatography (TLC), and GC-MS analysis serve as essential tools for the authentication and standardization of *Costus igneus*.

Declaration

Ethics approval and consent to participate- Not applicable

Consent for publication - Not applicable

Availability of data and materials – All data and material are available upon request.

Competing Interests- The author has declared that no conflicts of interest exist.

Funding- No financial support

Authors' Contributions - Ramesh pratap chaudhary contributed to the study conception, design & performed data collection and analysis, Dr Munesh Mani contributed to manuscript preparation and editing.

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