



Research Article

PHARMACOGNOSTIC STANDARDIZATION AND HPTLC FINGERPRINTING OF *KARCHUR* (*CURCUMA ZEDOARIA* (CHRISTM.) ROSCOE)

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ABSTRACT

In Ayurvedic medicine, *Karchura* (*Curcuma zedoaria* (Christm.) Roscoe), a perennial rhizomatous herb of the family Zingiberaceae, is traditionally used for its *Deepana*, *Pachana*, *Shothahara*, and *Vedanasthapana* properties. The rhizome is rich in curcuminoids, and volatile oils, which are responsible for responsible for diverse pharmacological effects. **Objective:** To evaluate the pharmacognostic, physicochemical, and phytochemical properties of *Karchur* and develop a High-Performance Thin Layer Chromatographic (HPTLC) fingerprint for authentication and standardization. **Methods:** Collected rhizomes were authenticated and were analysed as per Ayurvedic Pharmacopoeia standards through macroscopic, transverse and powder microscopic examinations. An analysis of physicochemical parameters such as foreign matter moisture loss, ash values, and solvent extractives was carried out. Alcoholic, aqueous and petroleum ether extracts were screened for phytochemicals. High-Performance Thin Layer Chromatographic profiling was performed on silica gel 60 F₂₅₄ plates using toluene:ethyl acetate:Methanol (9:1:0.5), scanned densitometrically at 254 and 366nm. **Results:** The rhizomes were cylindrical, greyish-buff surface and wrinkled, with microscopic features showing epidermis, trichomes, cortex, starch grains, vascular bundles, xylem, tracheids, stone cells and oil cells. Powder microscopy showed the presence of parenchymatous cells, oil cells, stone cells, fibres and hairs. Physicochemical analysis indicated 0.38% foreign matter, 6.12% total ash, 15.53% alcohol-soluble extractives, 18.96% water-soluble extractives, and 16.18% moisture content (LOD). Phytochemical screening confirmed the presence of the presence of steroids, saponins, tannins, reducing sugar, alkaloids flavonoids, and terpenoids, in hydroalcoholic and alcoholic extract, while only steroids, alkaloids and terpenoids were detected only in the petroleum ether extract. The HPTLC fingerprint profile of *Curcuma zedoaria* rhizome extract exhibited 5 resolved bands with R_f values ranging from 0.255 to 0.940 at 254 nm and 6 resolved bands with R_f values ranging from 0.206 to 0.905 at 366nm. **Conclusion:** The study establishes comprehensive pharmacognostic, physicochemical, and HPTLC data for *Karchur* rhizomes, providing a valuable standard for identification and future research applications.

INTRODUCTION

Kachur (*Curcuma zedoaria* Roscoe), also known as zedoary or white turmeric, is a perennial rhizomatous herb belonging to the family

Zingiberaceae. It is indigenous to tropical and subtropical humid regions and grows abundantly in the Eastern Himalayas, the moist deciduous forests of the coastal tract of Kanara, and the hilly regions of Odisha in India.^[1] The plant holds significant ethnomedicinal importance and has been extensively utilized in Ayurveda, Unani, and various folk medicine traditions for centuries. The rhizomes, being the most valued part, have attracted attention for their broad spectrum of pharmacological and therapeutic activities.

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In classical Ayurvedic texts, *Kachur* is categorized under *Shwasahara* (alleviating respiratory disorders), *Vedanasthapana* (analgesic), and *Krimighna* (anthelmintic) groups, highlighting its diverse therapeutic applications. Traditionally, it has been prescribed for the management of respiratory ailments such as asthma, bronchitis, and cough; digestive complaints like loss of appetite, indigestion, colic, and flatulence; and inflammatory disorders including arthritis and wounds.^[2] The rhizome is also valued as a uterine tonic, emmenagogue, and stimulant, thus playing an important role in gynecological disorders. In addition, *Kachur* has been documented to possess antipyretic, hepatoprotective, antiulcer, and antimicrobial properties, which support its wide-ranging use in both preventive and therapeutic contexts.^[3]

With increasing interest in medicinal plants and the growing need for quality assurance, a detailed pharmacognostical assessment of *Curcuma zedoaria* becomes essential. Such evaluation helps in confirming its identity, identifying diagnostic features, and establishing standard parameters for its safe and effective use in herbal formulations^[4]. This article presents a comprehensive overview of the macroscopic, microscopic, physicochemical, and phytochemical characteristics of *Curcuma zedoaria* to support its authentication and standardisation.^[5]

MATERIALS AND METHODS

Collection and identification of plant material

Fully matured, fresh, and healthy plant materials of *C. zedoaria* were collected from *Zedoaria* was procured from the Vikas Nagar region of Uttarakhand, during January to February, 2025. Herbarium specimen was prepared for identification and authentication of drug. The plant sample were taxonomically identified and authenticated by BGIR-Botanical Garden of Indian Republic, BSI (Botanical Survey of India), Sector 38A, Gautam Budh Nagar, Noida with Authentication no. BSI/BGIR/1/TECH./2025/278 at dated: 19-11-2025.

Plant Sample Processing

The whole and powdered drug samples were stored in airtight, light-resistant containers under ambient conditions in accordance with the standards of the Ayurvedic Pharmacopoeia of India.^[6] Finely sieved (60# mesh) and coarsely powdered samples were used for powder microscopy, physicochemical evaluation, phytochemical screening, and chromatographic studies following standard procedures.

Macroscopy of Plant Material

The plant was evaluated for morphological characteristics such as structure, shape, size, and texture using unaided vision and a simple microscope (Olympus OIC DM). Organoleptic properties including colour, odour, and taste were assessed systematically through sensory evaluation of the whole plant powder.^[7,8]

Sectioning of specimen

The rhizome sample was pre-soaked in water and transversely sectioned using a sharp diamond-edged blade. Temporary mounts of the sections were prepared following standard procedures and examined under a digital trinocular compound microscope (Olympus CX21i fitted with a Magcam DC14 camera). Diagnostic features were observed and photomicrographs were recorded.^[9]

Powder microscopy of Rhizomes

Approximately 2gm of finely dried powder, sieved through 80# mesh, was prepared as wet mounts using 50% glycerine and as dry mounts by the smearing method for uniform distribution. The preparations were examined under a Zeiss AxioCam ICc5 microscope at 10× and 40× magnifications, and photomicrographs were recorded to document the diagnostic microscopic features.

Physicochemical Evaluation

Physicochemical evaluation of the rhizomes of *Curcuma zedoaria*, including loss on drying, ash values, and extractive values, was carried out according to the methods prescribed in the Ayurvedic Pharmacopoeia of India and WHO guidelines. Solvent extractability was assessed using ethanol and water through conventional extraction methods.^[10]

Determination of Moisture Content (Loss on Drying)

This procedure was carried out to determine the amount of volatile matter, mainly moisture content, present in the drug. Approximately 5gm of powdered *C. zedoaria* was taken separately in three petri dishes to obtain the average value. The samples were dried at 105°C for 5 hours, cooled, and weighed. Drying was continued at the same temperature with repeated weighing at one-hour intervals until a constant weight was achieved. The percentage loss on drying was then calculated with reference to the air-dried drug using the standard formula

Weight of the empty petri dish = W1 gm

Weight of the drug sample = X gm

Weight of the petri dish with drug before drying (W3) = (W1 + X)

Weight of petri dish after drying = W2 gm

Loss on drying in % = $[(W3 - W2) / X] \times 100$

Determination of Extractive Values

Determination of Alcohol-Soluble Extractive

Approximately 10gm each of powder *C. zedoaria* were weighed and macerated with 100ml of ethanol. It was then kept in a closed flask for twenty-four hours, shaken frequently during first six hours and then allowed to stand for rest of the eighteen hours. It was then filtered rapidly. Precautions were taken against loss of solvent. Then filtrate was evaporated to dryness in a tarred flat-bottomed shallow petri dish, and dried at 105°C, to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the air-dried drug was calculated using the formula.

Weight of the drug material = X gm

Weight of the empty petri dish = W1 gm

Weight of the petri dish with dried extract = W2 gm

Percentage of extractive value = $[W2 - W1/X] \times 100$

Determination of Water-soluble Extractive

The procedure was carried out in the same manner as for the alcohol-soluble extractive determination, using distilled water in place of ethanol.

Determination of Ash Values

Approximately 2gm of *C. zedoaria* powder was accurately weighed into a pre-weighed silica crucible and incinerated at the required temperature. 500-600°C until free from carbon. It was then cooled under desiccator and weighed. The ash content was determined as a percentage of the air-dried drug using the standard formula.

Wt. of Empty Silica Crucible = A1 gm

Wt. of Sample (X) = X gm

Wt. of the Crucible with Ash = A2 gm

Percentage of Total Ash = $[A2 - A1/X] \times 100$

Determination of Acid-Insoluble Ash (AIA)

Each crucible containing the total ash of *C. zedoaria* was treated with 25ml of dilute HCl. Then these crucibles were kept in heating mantle until they start to boil after which the solution was filtered using the ashless filter paper (Whatman 41). The insoluble matter was collected on the filter paper and washed with hot water until the filtrate was neutral. After collecting the insoluble matter on filter paper, it was transferred to the same crucible, dried in the muffle furnace, and subjected to ignition until a stable weight was obtained. The residue was cooled in a desiccator for 30 minutes, weighed immediately, and the percentage of acid-insoluble ash relative to the air-dried sample was calculated using the standard formula.

Wt. of drug sample = X gm

Wt. of Crucible = G1 gm

Wt. of Crucible with insoluble Ash = G2 gm

Wt. of insoluble ash (G3) = G2-G1

Percentage of acid insoluble ash = $G3/X \times 100$

Preliminary Phytochemical Analysis

Approximately 5gm of powdered *C. zedoaria* was taken in conical flask, and solvents (ethanol, distilled water and Petroleum Ether) were added in a 1:10 ratio (50mL per sample). The samples were subjected to rotary shaking for six hours, allowed to settle, and subsequently passed through Whatman filter paper to yield the extracts. The filtrate was taken for screening of phytochemicals by using standard chemical tests.^[11,12]

Test for Alkaloids

Dragendroff's Test – 2ml alcoholic/aqueous extract of powder of *C. zedoaria* were taken separately in the test tubes and few drops of Diluted Hydrochloric Acid (dil. HCl) were added to them respectively. The solution formed was then filtered and 1ml of Dragendroff's reagent (potassium bismuth iodide solution) was added to each. Formation of orange precipitate indicates the presence of alkaloids.

Test for Saponins

Foam Test– 2.5ml alcoholic/aqueous extract of powder of *C. zedoaria* were taken separately in the test tubes and 10ml distilled water was added to them. The mixture was shaken thoroughly and allowed to stand for two minutes. Formation of honeycomb-like froth indicated the presence of saponins and visa-versa.

Test for Tannins

Ferric Chloride Test– 1ml alcoholic/aqueous extract of powder of *C. zedoaria* were taken separately in the test tubes and 5ml distilled water was added to them. Then, few drops of 10% FeCl₃ solution were added to the respective test tubes. A greenish-black or bluish-black precipitate appears, indicating tannins are present.

Test for Glycosides

Keller-Killiani test– 1ml alcoholic/aqueous extract of powder of *C. zedoaria* were taken separately in the test tubes after which the extracts were dissolved in approximately 1ml of glacial acetic acid, 10% ferric chloride solution, and concentrated sulfuric acid. A reddish-brown colour ring at the junction of two layers indicated the presence of glycosides.

Test for Flavonoids

Alkaline Reagent Test– In separate test tubes containing 1ml of alcoholic/aqueous extract of *C. zedoaria*, a few drops of 20% sodium hydroxide solution were added, resulting in the formation of a yellow precipitate. A few drops of dilute hydrochloric acid make the solution colourless, thereby confirming flavonoid content.

Test for Carbohydrates

Molisch's Test- About 1ml of the aqueous/alcoholic extract of *C. zedoaria* was taken in a test tube, to which 2–3 drops of Molisch's reagent were added. Subsequently, concentrated sulphuric acid was carefully poured along the side of the tube to form a separate layer. The formation of a violet ring at the interface indicated the presence of carbohydrates.

Test for Steroids

Salkowski test- 2ml alcoholic/aqueous extract of powder of *C. zedoaria*, were taken separately in the test tubes to which 2ml of chloroform along with equivalent quantity (2ml) of conc. H_2SO_4 was added, respectively. The formation of the pink/red ring indicated the presence of steroids.

Test for Tannins

Ferric Chloride Test- 2ml of the alcoholic/aqueous extract of *Curcuma zedoaria* powder were taken separately in test tubes. To each, 2–3 drops of 5% ferric chloride ($FeCl_3$) solution were added. The appearance of a bluish-black colour (hydrolysable tannins) or greenish-black colour (condensed tannins) indicated the presence of tannins.

Fingerprint Analysis by High-Performance Thin Layer Chromatography (HPTLC)

The filtrate was concentrated to 1gm per 10ml and then subjected to HPTLC profiling. The extract (4 μ L) was spotted on a pre-coated silica gel GF 60 254 aluminium plate using a Camag Linomat V sample applicator equipped with a 100 L Hamilton syringe. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as Toluene, Ethyl Acetate, Formic Acid (5:4:1) (v/v/v) to a distance of 90mm, dried for 5 min in ambient air. After development, a densitometric scan was done with a Camag TLC scanner III in reflectance absorbance mode at UV detection wavelengths of 254 nm and 366 nm using Win CATS Software (V 1.2.1. Camag).

RESULTS**Macroscopic characters**

The rhizomes of *Curcuma zedoaria* are cylindrical, reaching up to 6 cm in length, with transversely cut pieces measuring 2 to 3.5cm in diameter. The external surface is characteristically rough, marked by longitudinal wrinkles, occasional protuberances, and distinct nodes and internodes. Additionally, some specimens feature thin roots and root scars. In terms of appearance, the rhizome exhibits a greyish-buff external color, while the internal surface is cream-colored. Organoleptically, it is defined by a camphoraceous odor and a slightly bitter taste. [Table 1, Fig 1and Fig 2]

Table 1: Organoleptic features of *Curcuma zedoaria* rhizome and rhizome powder

S.No.	Character	<i>Curcuma zedoaria</i> Rhizome	Rhizome powder
1.	<i>Shabda</i> (sound)	-	-
2.	<i>Sparsha</i> (touch)	Smooth	Fine
3.	<i>Roopa</i> (colour)	Pale yellow	Yellowish-brown
4.	<i>Rasa</i> (taste)	<i>Katu</i> (pungent)- <i>Tikta</i> (bitter)	<i>Katu</i> (pungent) – <i>Tikta</i> (bitter)
5.	<i>Gandha</i> (odor)	Aromatic / odoriferous	Aromatic / odoriferous

**Fig. 1 Karchur (*Curcuma zedoaria* (Christm.)Roscoe)**



Fig. 2 *Karchur (Curcuma zedoaria (Christm.) Roscoe)* (A) Dry Rhizomes and (B) Powder

Microscopic characters

Transverse section of Rhizomes

The safranin-stained transverse section of the rhizome of *Curcuma zedoaria* (Christm.) Roscoe examined under 10× magnification revealed well-defined anatomical features characteristic of the species. The outermost region consists of a single-layered epidermis bearing long, uniseriate unicellular covering trichomes, which arise from the epidermal cells and serve as a prominent diagnostic character (Fig. 3A & Fig. 3B). Beneath the epidermis lies the cortical region, composed of several layers of thin-walled, polygonal to rounded parenchymatous cells. These cells constitute the major ground tissue and are loosely arranged with distinct intercellular spaces. Numerous closed collateral vascular bundles are embedded within the cortex and extend throughout the ground tissue (Fig. 3A). The vascular bundles consist of xylem and phloem elements, with xylem tracheids forming the principal water-conducting tissue. The vascular bundles are scattered within the parenchymatous ground tissue, a characteristic feature of monocotyledonous rhizomes (Fig. 3C). The powdered tissue and medullary parenchyma contain abundant simple starch grains, which are predominantly rounded to oval in shape and occupy the majority of the parenchymatous cells. In addition, numerous yellowish oil globules (oleoresin cells) are distributed throughout the ground tissue, representing the storage sites of volatile oil and resinous constituents responsible for the medicinal properties of the drug (Fig. 3D). Occasional stone cells (sclereids) with thick lignified walls are observed scattered among the parenchymatous tissue, providing mechanical support to the rhizome (Fig. 3D). The combined occurrence of unicellular covering trichomes, parenchymatous cortex rich in starch grains, scattered collateral vascular bundles with xylem tracheids, oil globules, and isolated stone cells constitutes the principal diagnostic microscopic characteristics of *Curcuma zedoaria*.

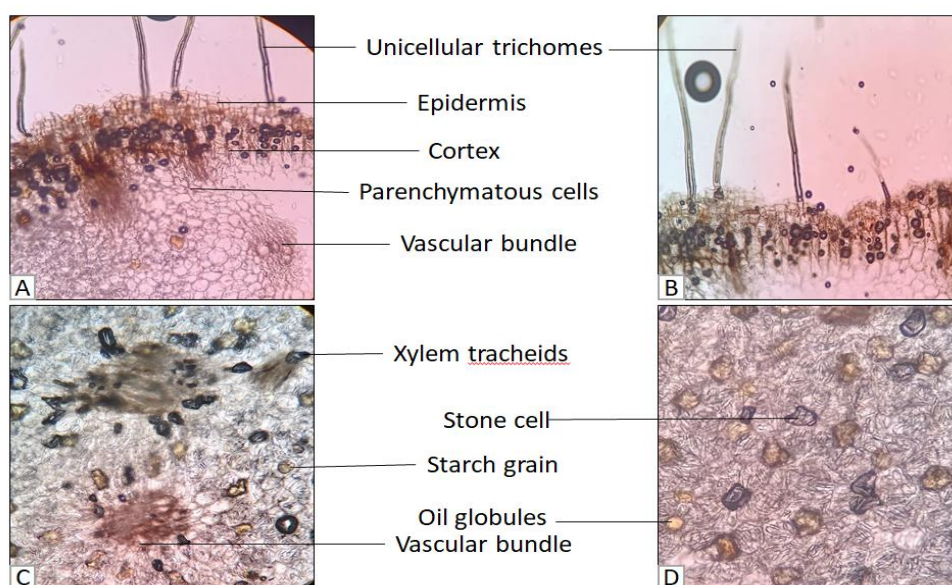


Figure 3. *Curcuma zedoaria* (Christm.) Roscoe; Transverse section of rhizome showing at 10× A & B: Unicellular trichomes, epidermis, cortex, parenchymatous cells, vascular bundles

Powder Microscopy of Rhizomes

The safranin-stained powder of *Curcuma zedoaria* (Christm.) Roscoe examined under 40× magnification revealed abundant simple starch grains, predominantly circular, oval to sac-shaped, occurring singly or within parenchymatous cells (Fig. 4A, 4C & 4D). Numerous oil cells (oleoresin cells) containing yellowish contents were observed (Fig. 4A). Stone cells (sclereids) with thick lignified walls were scattered throughout the powder (Fig. 4B). Fragments of fibres and long unicellular covering hairs were also present, serving as important diagnostic feature (Fig. 4E & 4F).

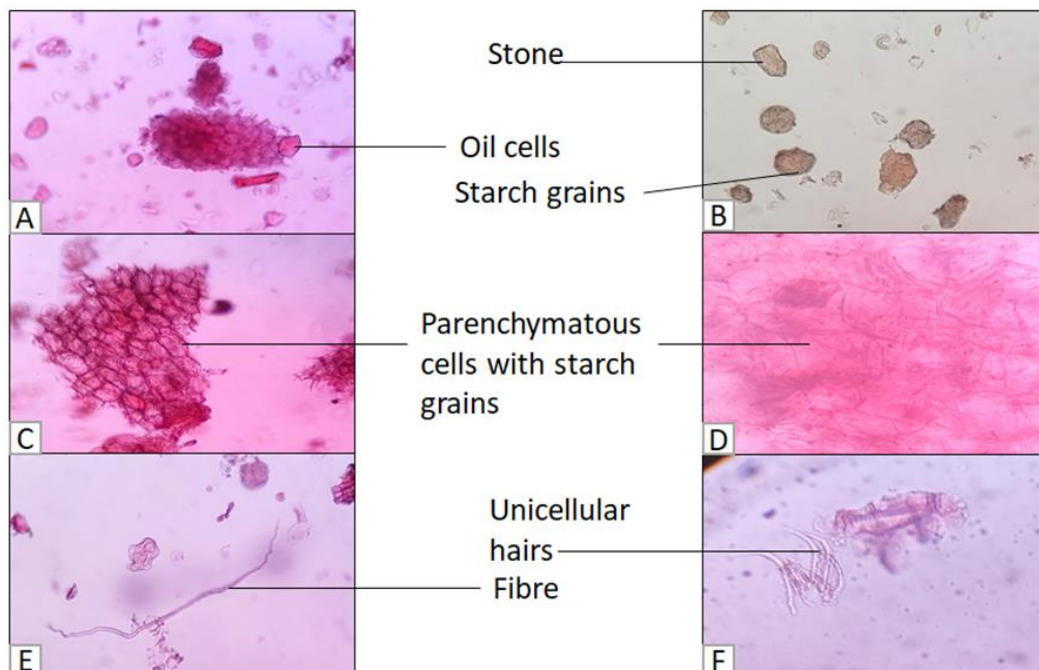


Figure 4. *Curcuma zedoaria* (Christm.) Roscoe; rhizome powder stained with safranin showing characteristic powder microscopic features at 40×: A. Oil cells B. Stone cells and starch grains C. Parenchymatous cells with starch grains D. Starch-filled parenchymatous cells E. Fibre and unicellular hair F. Unicellular covering hairs.

Physicochemical Parameters

The rhizome powder was analysed for foreign matter, loss on drying, total ash, acid-insoluble ash, and water and alcohol extractive values. [Table 2]. All tests were performed using the standard protocols recommended by the Ayurvedic Pharmacopoeia of India and the WHO.^[13,14]

Table 2: Physicochemical observations of *Curcuma zedoaria* rhizome

S.No.	Test	Observations
1.	Foreign matter	0.38%
2.	Extractive value (alcohol soluble)	15.53%
3.	Extractive value (water-soluble)	18.96%
4.	Total ash	6.12%
5.	Acid insoluble ash	0.64%
6.	Moisture content (LOD)	16.18%

Preliminary Phytochemical Analysis

Aqueous, ethanolic and petroleum Ether extracts, prepared as outlined in the preceding section, were subjected to preliminary phytochemical screening using approximately 2ml of each extract for individual tests. The findings are presented in Table 3.

Table 3: Phytochemical Observations of *Curcuma zedoaria* rhizome

S.No.	Phytochemical	Alcohol extract	Aqueous extract	Petroleum ether extract
1.	Alkaloids- Dragendroff's Test	+	+	+
2.	Saponins- Foam test	+	+	-
3.	Tannins- FeCl ₃ test	+	+	-
4.	Glycosides- Keller–Killiani test	+	+	-
5.	Flavonoids- Alkaline Reagent Test	+	+	-
6.	Carbohydrates- Molisch's test	-	+	-
7.	Steroids- Salkowski Test	+	+	+
8.	Terpenoids	+	+	+

Note: (-): Absent, (+): Present

HPTLC Analysis of *Curcuma zedoaria*

HPTLC analysis of *Curcuma zedoaria* rhizome extract was performed using pre-coated silica gel 60 F₂₅₄ plates. The sample was applied using a Linomat 5 applicator in different concentrations (2–10 µL). The mobile phase consisted of toluene:ethyl acetate:methanol (9:1:0.5, v/v/v). The chromatographic plates were developed in a saturated chamber for 20 minutes and scanned at 254nm and 366nm using a TLC Scanner 4.

The HPTLC fingerprint profile of *Curcuma zedoaria* extract revealed the presence of several phytoconstituents when analysed at both 254nm and 366nm, indicating the chemical complexity of the rhizome extract. At 254nm, the chromatographic profile exhibited five distinct peaks (bands) at the following R_f values: 0.255, 0.416, 0.597, 0.905, and 0.940. Among these, the peak R_f 0.255 was the most prominent, indicating the major UV-absorbing phytoconstituent. At 366 nm, the profile displayed six distinct peaks at R_f values of 0.206, 0.253, 0.269, 0.597, 0.647, and 0.905. with the peak at 0.255 representing the predominant fluorescent constituent.

The systematic increase in detectable bands across tracks validates the sensitivity and reproducibility of the HPTLC method for the phytochemical authentication of *Curcuma zedoaria*.

The pictorial representation of the developed plate of rhizome methanolic extracts are given in Fig.5. Densitometric scanned pictures at 254nm and 366nm of the developed plates as fingerprint profile are shown in Fig.6 and 7.

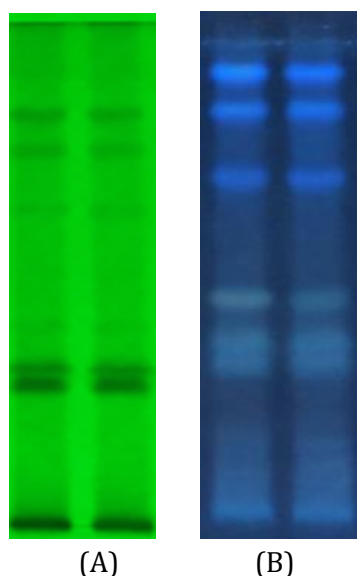


Figure 5: HPTLC chromatogram of *Curcuma zedoaria* rhizome extract developed using Toluene: Ethyl acetate: Methanol (9:1:0.5, v/v/v) as the mobile phase. (A) Plate viewed under UV 254nm showing prominent UV-quenching bands, with a major band at R_f ~0.30–0.32 (B) Plate viewed under UV 366nm displaying a characteristic fluorescent profile with distinct blue fluorescent bands distributed across the chromatogram, representing the presence of diverse UV-active secondary metabolites in the rhizome extract.

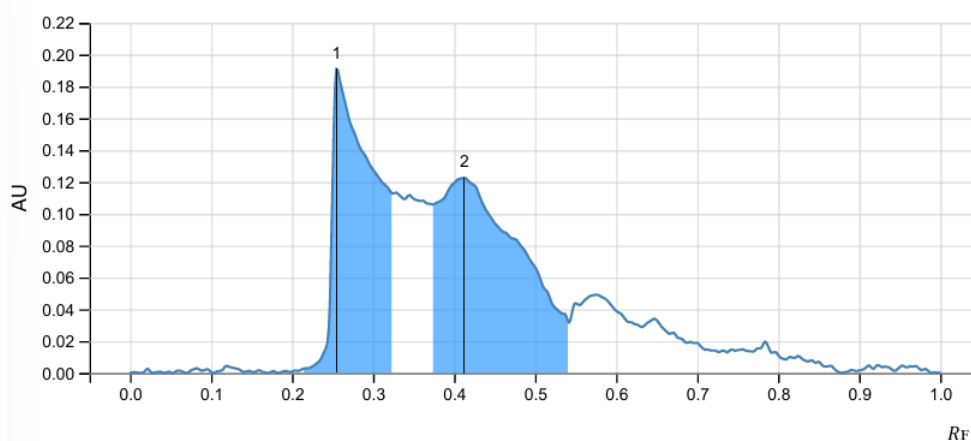


Figure 6: HPTLC densitogram of *Curcuma zedoaria* rhizome extract (3.0 μL application) recorded at 254 nm. The chromatographic profile exhibited two prominent peaks at Rf values of 0.26 and 0.41.

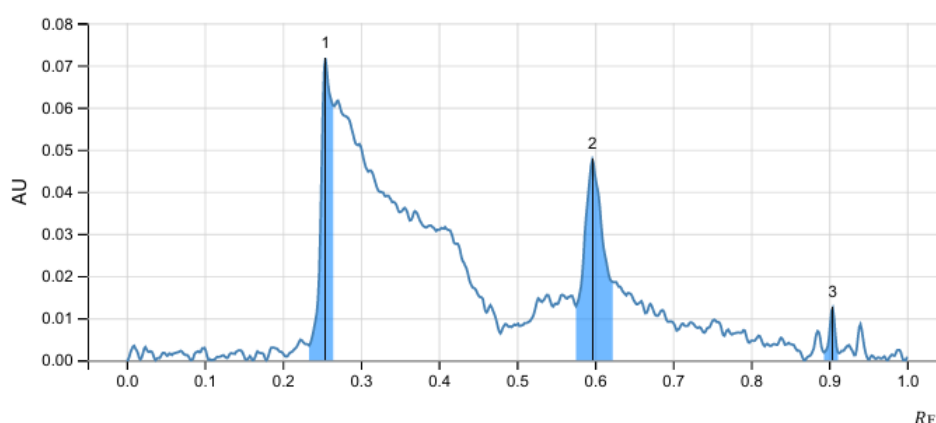


Figure 7: 3D densitogram of *Curcuma zedoaria* rhizome extract recorded at 366 nm for the 7.0 μL application volume. The chromatogram exhibits fingerprint profile with three distinct peaks corresponding to Rf values of approximately 0.26, 0.61, and 0.91.

CONCLUSION

The present study establishes comprehensive pharmacognostic, physicochemical, and HPTLC fingerprint data for the dried roots of *Karchur* (*Curcuma zedoaria* (Christm.) Roscoe). Microscopic characteristics and physicochemical parameters provide reliable diagnostic criteria for the development of a reference monograph.

Preliminary phytochemical screening of aqueous and ethanolic extracts revealed the presence of steroids, saponins, tannins, reducing sugar, alkaloids, flavonoids, and terpenoids, while only steroids, alkaloids and terpenoids were detected only in the petroleum ether extract as indicated by characteristic reactions with specific reagents. These secondary metabolites are recognized for their medicinal value and distinct physiological activities. Moreover, the phytochemical profile highlights the potential of *Curcuma zedoaria* rhizome extracts for further pharmacological evaluation to explore their therapeutic efficacy. The chromatographic fingerprint developed in this work may be employed as a

reference tool for ensuring quality, identity, and authenticity of herbal preparations formulated with *Karchur*. This study strengthens the scientific basis for the standardization and quality assurance of *Curcuma zedoaria* rhizome, thereby supporting their safe and effective use in herbal medicine.

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