

PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF *ZINGIBER ZERUMBET* (L.) SM. RHIZOME

Rout Om Prakash^{1*}, Rout Kedar Kumar³, Acharya Rabinarayan², Mishra Sagar Kumar³

¹Dept. of Dravyaguna, Rajiv Lochan Ayurvedic Medical College, Chandkhuri, Durg, Chhatisgarh, India

²Dept. of Dravyaguna, I.P.G.T & R.A., Gujarat Ayurved University, Jamnagar, Gujarat, India

³Pharmacognosy & Phytochemistry Division, University Dept. of Pharmaceutical Sciences, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India

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ABSTRACT

Zingiber zerumbet (L.) Sm. is a well known medicinal plant employed to cure various diseases. The Current study provides a detailed summary of pharmacognostical and phytochemical characters of rhizome to give clear standards for identification of the drug. The study revealed the presence of the oil cells in cortex and central cylinder region containing yellow to orange coloured oleo-resin is the main characteristic feature. The presence of globose, ovoid and irregularly rounded starch grains are the distinguishing features and can be used as anatomical markers. Rhizome powder showed some of the characteristic features such as starch grains with a distinct hilum situated at narrow end and parenchymatous cells with characteristically wrinkled wall and prismatic crystals. Preliminary phytochemical analysis of the rhizomes revealed the presence of glycosides, sterols, triterpenes, saponins, tannins, carbohydrates, proteins, amino acids and volatile oils. The present study signifies the use of TLC, HPTLC fingerprint profiles for determining the identity, purity of the drug and also for developing standards.

KEYWORDS: *Zingiber zerumbet*, indigenous medicines, Pharmacognosy, Phytochemistry, TLC/HPTLC

*Corresponding author

Dr. Om Prakash Rout, Lecturer, Deptt. of Dravyaguna, Rajiv Lochan Ayurvedic Medical College & Hospital, Chandkhuri, Gundardehi Road, Durg, Chhatisgarh, India Email: omprakash_rout2000@yahoo.com

INTRODUCTION

Zingiber zerumbet (L.) Sm. (Family: Zingiberaceae) known locally as “Kachur or narkarchur or Sthulagranthi”, is a perennial herb with leafy stems growing to about 1.2 m in height that is widely cultivated throughout the tropics including Southeast Asia, Korea, India and Bangladesh for its medicinal properties¹⁻⁴. Rhizomes are employed against cough, stomachache, asthma and also as a vermifuge. It is used in leprosy and other skin diseases. The rhizome yields an essential oil, which is used as perfume in soap and other toilet articles⁵. It is used as stimulant, carminative and flavorings agent; given in dyspepsia and flatulent colic; prescribed as an adjunct to many tonic and stimulating remedies⁶. It is used to treat fish poisoning. It is used as a cough remedy and to treat the bacterial diseases, thrush and diabetes. The rhizome is used as stimulant, antihypertensive, carminative and flavouring agent; to treat dyspepsia wounds, hemorrhoids and flatulent colic for the cure of stomach troubles and fever. It is used in

peptic ulcers and related stomach problems as well as infections⁷. Phytochemical investigations on this plant have revealed the isolation of several sesquiterpenes, flavonoids and aromatic compounds⁸⁻¹². The volatile oil of the rhizome contains zerumbone, humulene, camphrene α -caryophyllene and camphene¹³⁻¹⁵. The rhizomes of this plant are used as an anti-inflammatory agent in traditional medicine³. A monocyclic sesquiterpene, zerumbone (2*E*, 6*E*, 10*E*-humulatrien-1-one), which was found as a major component of the essential oil of *Z. zerumbet*, has been studied intensively for potential use in anti-inflammatory, chemopreventive, and chemotherapeutic strategies¹⁶⁻¹⁸. Extracts of the rhizomes are known to have anti-inflammatory, chemopreventive, chemotherapy applications¹⁹⁻²⁰ and are anti HIV¹⁶, antitumour²¹, cytotoxic²², antibacterial³ agents. It finds prominent importance not only in Ayurvedic medicine, but also in modern medicine. The pharmacognostic studies²³ including botanical description, macro- and microscopic characters of rhizome, root, physical

constants, colour reaction, powder study and fluorescent analysis have already been carried out. But the extensive pharmacognostic studies and TLC/HPTLC analysis have not been established properly. Therefore, the current study has been undertaken to carry out the detailed pharmacognostic and phytochemical studies for this species, which will be helpful for the proper identification and development of standardization protocols of commercial samples.

MATERIALS AND METHODS

Collection of the plant material

Z. zerumbet rhizomes were collected from the field of Silviculture office, Ghatikia, Bhubaneswar, Odisha, identified and authenticated by Mr. Rashmi Ranjan Pani, HOD, Deptt. of Botany, Mangala Mahavidyalaya, Kakatpur, Puri, Odisha. The voucher specimen was preserved at UDPS, Utkal University for future references.

Processing of the plant material

After collection, the rhizomes were washed thoroughly, shade dried, coarsely powdered, passed through the mesh no 44 and stored in air tight container for further analysis.

Macroscopic examination

Macroscopic and organoleptic features viz. colour, odour, taste shape, sizes etc. of the rhizomes were observed.

Microscopic examination

For microscopic studies, transverse sections (TS) were prepared, stained²⁴ and observed under microscope. The microscopic character of the rhizome powder was also observed. Photomicrographs were obtained by observing under compound binocular microscope and the figures were drawn with the help of camera lucida.

Determination of physicochemical parameters

Physicochemical parameters such as foreign matter, moisture content, total ash, acid insoluble ash, water- and alcohol- soluble extractives were determined according to methods described in the Indian Pharmacopoeia²⁵.

Preliminary phytochemical analysis

For preliminary phytochemical studies, 5g powdered material was successively extracted using Soxhlet apparatus with petroleum ether, chloroform, ethanol and water. The extracts were concentrated by distilling off the solvents under reduced pressure. The presence of different phytoconstituents viz. glycosides, sterols, triterpenes, saponins, tannins, carbohydrates, proteins, amino acids and volatile oils were determined following standard procedure²⁶.

Thin layer chromatographic study

TLC study²⁷ of the chloroform extract of the rhizomes was carried out on aluminium plates precoated with

silica gel F₂₅₄ of 0.2 mm thickness using ethyl acetate: hexane (1.5:8.5) as mobile phase and observed under visible light after derivatisation with anisaldehyde sulphuric acid reagent followed by heating the plate at 110°C. The colour and R_f values of the resolved spots were noted.

High performance thin layer chromatographic study

HPTLC fingerprint pattern²⁸ of the chloroform extract was developed by using Camag HPTLC system equipped with Linomat -V applicator fitted with a 100 µL syringe, Camag TLC scanner-III with CATS 4 software for interpretation of data. Chromatography was carried out on prewashed (methanol) and preactivated (50°C for 30 min) 10 cm × 10 cm aluminum-backed plates precoated with 0.2 mm layer of silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany). The sample was applied to the plate as 6 mm band, positioned 10 mm from bottom and 10 mm from side. The rate of application was constant at 130 nL/s. The plate was developed with ethyl acetate: hexane (1.5:8.5) as mobile phase in a Camag twin-trough glass chamber previously saturated with mobile phase vapour for 6 min. The development distance was 80 mm, which required 6 min under laboratory conditions. After development, the plate was dried, derivitised with methanolic sulphuric acid reagent followed by heating at 110°C and analyzed using CAMAG TLC scanner-III with CATS 4 software. The plate was observed under UV light at 260 nm. The R_f values of the resolved spots were noted and the resulting fingerprint was observed.

RESULTS AND DISCUSSION

Macroscopic characters

Rhizomes are 7-15 cm long and 1-2.5 cm broad, irregularly branched with node and internodes. Scale leaves are present at the nodal region. The outer surface of the rhizome is smooth and light grey in colour, internally light yellow. These are hard and brittle, breaking with a short fracture, fragrant odour, aromatic, spicy and slightly bitter in taste (Figure 1).

Microscopic characters

The rhizome is circular with epidermis, cortex, endodermis and closed, collateral vascular bundle. Transverse section of the rhizome showed outer single layered epidermis having rectangular and elongated cells, followed by thin walled cork cells of 6-10 layers, irregularly elongated. Cortex consists of several layers of parenchymatous cells with intercellular air spaces and contains starch. Oil cells are present in cortex. Central cylinder region contains a yellow to orange coloured oleo-resin. Endodermis consists of single layer of cells. Stele consists of a broad central zone of ordinary parenchymatous cells. Closed, collateral vascular bundles are found in a circle in the region just inside the

epidermis. The starch grains are abundant in the cortex and mostly globose, ovoid and irregularly rounded. The tracheids are non-lignified and have reticulate, spiral or scalariform thickening on the walls (Figure 2, 3 & 4).

Powder characters

The rhizome powder was studied under different magnifications, which showed the presence of epidermal fragments, cork cell, parenchyma containing starch grains, fragments of parenchyma with oleoresin, parenchyma cells showing wrinkled walls, starch granules, co-oxalate crystals, unicellular trichomes, isolated vessels, isolated trichomes, and isolated fibers (Figure 5).

Physico-chemical parameters

The physico-chemical parameters of the rhizomes are presented in Table 1, which are important diagnostic features of the plant. The percentage water-soluble extractive was found to be significantly higher than that of the alcohol-soluble extractive (Table 1).

Preliminary phytochemical analysis

Rhizome extracts were subjected to qualitative phytochemical screening for the identification of chemical constituents and the results are summarised in Table 2.

Thin layer chromatographic study

Thin Layer Chromatogram of the chloroform extract under UV 254 nm showed five fluorescent spots at R_f 0.06, 0.18, 0.24, 0.33 & 0.63 (fluorescent green). After derivitisation with anisaldehyde sulphuric acid reagent, it showed seven major spots at R_f 0.06 (blue), 0.18 (brown), 0.24 (green), 0.33 (pink), 0.52 (orange) and 0.63 (violet) (Figure 6).

High performance thin layer chromatographic study

The HPTLC fingerprinting patterns of the chloroform extract of the rhizomes was developed at 260 nm. The binary solvent system, ethyl acetate: Hexanes (1.5:8.5) efficiently resolved the components present in the crude extract. In total five peaks (R_f 0.18, 0.24, 0.33, 0.40, 0.53) were observed in the chromatogram and the components at R_f 0.33, 0.40 were present in significant level (Figure 7). The developed TLC/HPTLC chromatogram of chloroform extract of the rhizomes of *Z. zerumbet* may be treated as chromatographic fingerprint and could be used efficiently for identification, and quality assessment of this plant drug.

CONCLUSION

The plant *Zingiber zerumbet* (L.) Sm. Finds its application in Ayurveda and other traditional systems of medicines. The macroscopic and microscopic characters of the rhizome and its powder revealed the presence of different diagnostic structures, which will help for proper identification of the plant. The different physico-

chemical parameters of the rhizomes were observed for future references. The preliminary phytochemical test of the crude extracts indicated the presence of different phytochemical constituents. The developed TLC/HPTLC chromatogram of the chloroform extract indicated the chemical profile of the rhizomes. All these parameters can act as diagnostic tool for identification and authentication of raw drug samples and play an important role in quality control and detection of adulteration.

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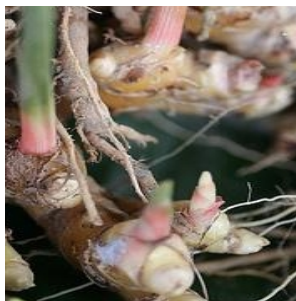
Table1: Physicochemical parameters of the rhizomes of *Zingiber zerumbet* (L.) Sm.

S. No.	Parameters studied	Observed values (% w/w)
1	Foreign matter	1.24
2	Loss on drying	11.2
3	Total ash	07.30
4	Acid-insoluble ash	0.82
5	Alcohol-soluble extractive	9.45
6	Water-soluble extractive	24.21

Table 2: Preliminary Phytochemical analysis of extracts of *Zingiber zerumbet* (L.) Sm.

S.No.	Test for	Petroleum ether extract	Chloroform extract	Ethanollic extract	Aqueous extract
1	Alkaloids	-	-	-	-
2	Glycosides	-	-	+	+
3	Flavonoids	-	-	-	-
4	Sterols	+	+	-	-
5	Triterpenes	-	+	+	+
6	Saponins	-	-	+	+
7	Tannins	-	-	+	+
8	Carbohydrates	-	-	+	+
9	Proteins	-	-	+	+
10	Amino acids	-	-	+	+
11	Volatile oils	+	-	-	-

'+' indicates present; '-' indicates absent

Figure 1. Rhizome of *Zingiber zerumbet* (L.) Sm

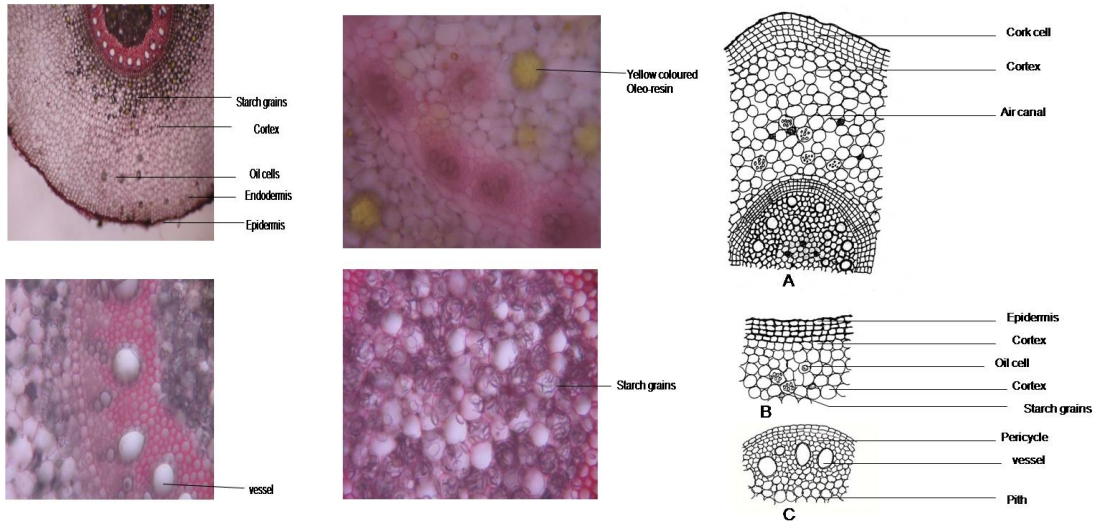


Figure 2

Figure 3

Figure 4

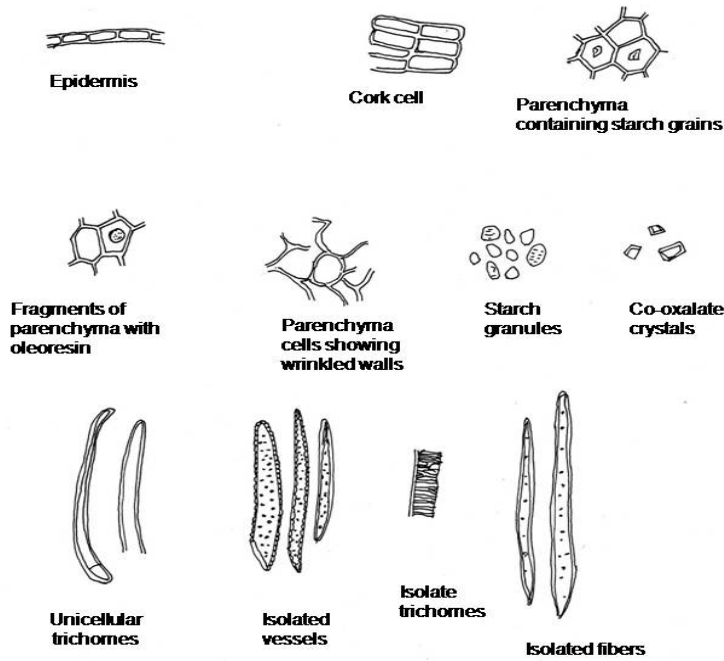


Figure 5. Powder characters of *Zingiber zerumbet* (L.) Sm

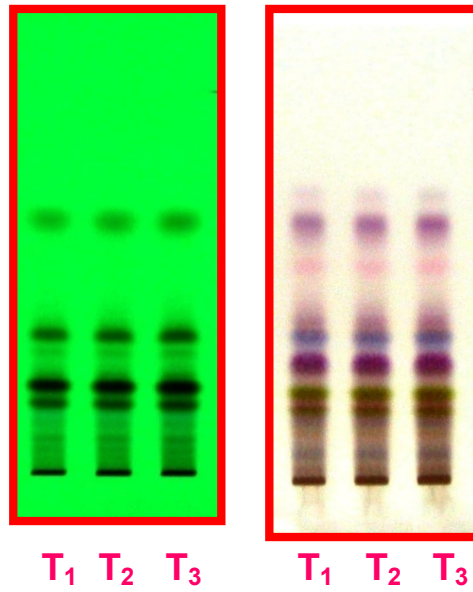


Figure 6 . TLC studies of *Zingiber zerumbet* (L.) Sm

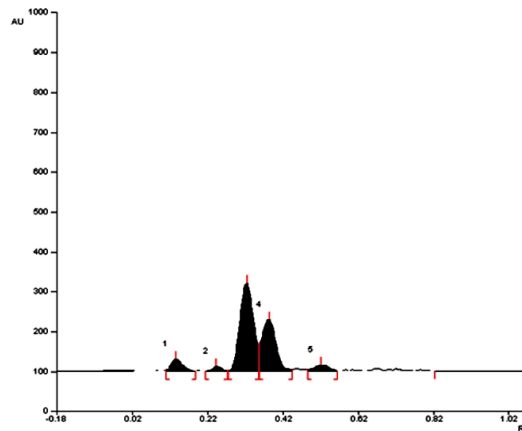


Figure 7. HPTLC fingerprinting of *Z. zerumbet*

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