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Research Article

PHYTOCHEMICAL STUDIES AND PHARMACOGNOSTICAL EVALUATION OF *ZINGIBER CASSUMUNAR* ROXB

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ABSTRACT

Objective: *Zingiber Cassumunar* Roxb is a well known medicinal plant employed to cure various diseases were reported to possess good medicinal value in traditional system of medicine. The present investigation deals with microscopic, macroscopic and preliminary phytochemical investigation of rhizome to give clear standards for identification of the drug.

Method: For the microscopic evaluation, the powder was soaked in a solution of 20% chloral hydrate and then mounted on a glass slide with the help of glycerine. The mounted slides were then observed under a photographic microscope. Microscopic sections were cut by free hand sectioning.

Result: The research paper study revealed that the yellow colour inside the rhizome is the main characteristic feature. The presence of central cylinder region containing yellow coloured oleo-resin and oil cells in cortex are the main characteristic feature. The presence irregularly rounded, ovoid starch grains and oil globulues situtated inside the parenchyma 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 rounded shape situated at narrow end and parenchymatous cells with characteristically wrinkled wall, air spaces. Cork, cortex, cork cells, and floem fibres also shows pharmacognostical characteristics of *Z. cassumunar* Roxb. Preliminary phytochemical analysis of the rhizomes revealed the presence of glycosides, sterols, triterpenes, saponins, tannins, flavonoids, amino acids and volatile oils.

Conclusion: The present study signifies the use of TLC (Thin layer chromatography) profiles for determining the identity of active chemical constituents.

Keywords: *Zingiber cassumunar* Roxb, Indigenous medicines, Oil globulues, Pharmacognostical characteristics, Thin layer chromatographic.

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INTRODUCTION

India has heritage of traditional medicine, Materia medica of India provides a lot of information on the folklore practices and traditional aspects of therapeutically important natural products. The evaluation of these drugs is mostly based on phytochemical, pharmacological, and allied approaches including various instrumental techniques such as chromatography, microscopy, and others [1]. The herbal medicine is based on traditional medicine, exists in every continent of the globe and in every cultural area of the world. Each of these traditional medicines has its own origin and an individual basic philosophy [2]. Exploration of the chemical constituents of the plants and pharmacological screening may provide us the basis for developing a lead molecule. Herbs have provided us some of the very important life-saving drugs used in the armamentarium of modern medicine.

About *Zingiber cassumunar* Roxb.

Zingiber cassumunar Roxb. (family: *Zingiberaceae*), known locally as "Phlai" in Thai, is a perennial herb, consisting of underground rhizomes. Conventionally, the rhizome of this plant has been used for treatment of inflammation, muscle and joint problems, menstrual disorders, abscesses, and skin diseases and wound healing the ethnomedical use of *Z. cassumunar* Roxb. (*Zingiberaceae*) and its frequently reported uses of the rhizome include topical treatment of sprains, contusions, abscesses, and skin diseases. The rhizome can be crushed and directly rubbed onto the afflicted area or the sliced rhizome can be fried in a pan together with coconut oil, to obtain a composite oil, which is applied to the inflamed area [3]. The findings support the use in Thai traditional medicine of Zingiberaceous plants, especially *Z. cassumunar*, for treatment of allergy and allergic-related diseases [4].

Phytochemical investigations of *Z. cassumunar* rhizomes have revealed the presence of phenylbutanoids, cyclohexene derivatives, naphthoquinones, vanillin, vanillic acid, veratric acid, terpenoids, β-sitosterol, and curcuminoids.

In combination with other medicinal plants, *Z. cassumunar* was found to be effective in relieving asthmatic symptoms in children and adults. Several isolated compounds have been found to possess anti-inflammatory activity, for example, two phenylbutanoids [5]. (*E*)-4-(3_,4_-dimethoxyphenyl)but-3-enyl acetate and (*E*)-4-(3_,4_-dimethoxyphenyl)but-1,3-diene.

Ginger has been used as a spice and as natural additives for more than 2000 years [6]. Furthermore, ginger has many medicinal properties. Studies have shown that the long-term dietary intake of ginger has hypoglycemic and hypolipidemic effect [7]. Ginger has been identified as an herbal medicinal product with pharmacological effect. Ginger suppresses prostaglandin synthesis through inhibition of cyclooxygenase - 1 and cyclooxygenase - 2. In traditional Chinese and Indian medicine, ginger has been used to treat a wide range of ailments including stomach aches, diarrhea, nausea, asthma, and respiratory disorders [8]. Ginger is widely used because it contains good medicinal properties.

Other *Zingiber zerumbet* (L.) Sm. (family: *Zingiberaceae*) have revealed the isolation of flavonoids, sesquiterpenes, and aromatic compounds. The volatile oil of the rhizome contains zerumbone, humulene, camprene α-caryophyllene, and camphene. The rhizomes of this plant are used as an anti-inflammatory agent in traditional medicine. 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 strategies. Extracts of the rhizomes are known to have anti-inflammatory, chemopreventive, and chemotherapy applications and are anti-HIV, antitumor, cytotoxic, and antibacterial agents [9].

Thus, *Z. cassumunar* Roxb. also finds prominent importance not only in Ayurvedic medicine but also in modern medicine. However, the extensive pharmacognostic studies and thin-layer chromatographic (TLC) 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.

MATERIAL AND METHODS

Plant material

Z. cassumunar Roxb. [10] Plant species along with leaves, flowers, roots, and rhizomes were collected from the Bhimashankar region. Then, this plant was identified and authenticated by Dr. G. G. Podar, HOD, Department of Botany, Y. C. College of Science, Karad and Voucher specimen was deposited at the same college as number AAK2.

Processing of plant material

After collection, rhizomes were washed thoroughly and were separated from other parts, cleaned and dried for further use.

Macroscopic examination

The detail macroscopic characters of fresh rhizomes were noted including special features such as color, odor, shape, and size [6].

Microscopic examination

For the microscopic evaluation, the powder was soaked in a solution of 20% chloral hydrate and then mounted on a glass slide with the help of glycerine. The mounted slides were then observed under a photographic microscope [11]. Microscopic sections were cut by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the rhizomes were made and examined microscopically. Histochemical reactions were observed with different staining agent for the general and specific microscopic characteristic of tubers. Photomicrographs of the microscopical sections were taken with the help of motic microscope [12,13].

Powder characteristics

Preliminary examination like behavior of powder with different chemical reagents and microscopically examination was carried out according to the method given in Khandelwal and Kokate [6,13].

Fluorescence analysis of tuber powder

Powder material was analyzed under visible light, short UV light (254 nm) after treatment with various organic/inorganic solvents/ reagents such as petroleum ether, methanol, water, 10% aqueous NaOH, 50%HCl, 50% $H2SO_4$, acetic acid, 50% HNO_3, etc. [14].

Physicochemical parameters

Physicochemical parameters, such as percentage of total ash, extractive values and moisture content loss on drying (LOD), and swelling index, were determined as per official method of the Indian pharmacopoeia and the WHO guidelines on the quality control methods of medicinal plant materials [15,16].

Extraction and preliminary phytochemical screening

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plants [17].

Powdered drug was extracted with n-hexane, dichloroform, chloroform, acetone, ethanol, and water successively by soxhlet extraction and microwave. Microwave extraction can be the better alternative to conventional extraction.

The extracts were dried and weighed. The presence or absence of different phytoconstituents, namely, triterpenoid, steroids, alkaloids, vitamins, tannins, glycosides and flavonoids, etc., were detected by usual given method [6].

Thin-layer chromatographic studies

TLC studies [18] of dichloromethane extract of *Z. cassumunar* Roxb. were studied. It was carried out on aluminum plates precoated with silica gel F254 of 0.2 mm thickness and mobile phase was developed with trial and error basis. Finalized mobile phase for dichloromethane extract was chloroform:dichloromethane (5.4:11.2) and for alcoholic extract toluene:ethyl acetate:methanol:formic acid (6:6:0.4:1.6). Plate was observed in visible light and it was also sprayed with anisaldehyde sulfuric acid spray reagent followed by heating the plate at 110°C. The color and Rf value of resolved spots were noted.

RESULTS

Macroscopic characters

Green color leaves are present at the nodal region with 5–8 cm long rhizomes are 10–14 cm long and 2.5 cm broad, branched irregularly with node and internodes. White flowers are developed 30 cm above the ground part. The outer surface of the rhizome is smooth and light grey, internally dark yellow which is the identification characteristic of *Z. cassumunar* Roxb. These are hard and brittle at outer region, easily

Fig. 1: Green leaves

Fig. 2: White flowers

Fig. 3: Whole plant

Fig. 6: Rhizomes with dark yellow inside

Fig. 4: Rhizomes with dark yellow inside

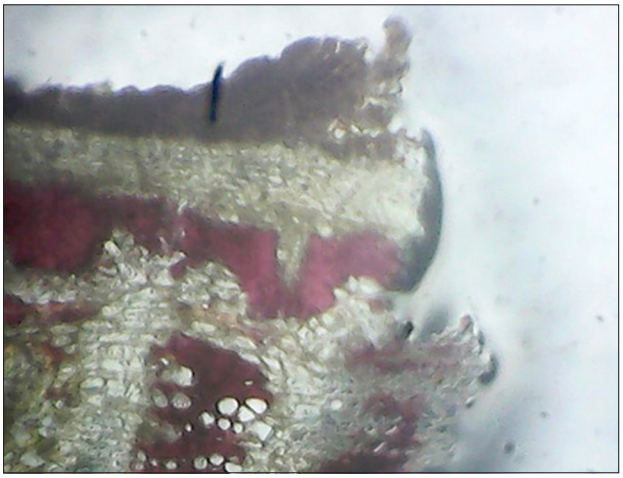

Fig. 7: Transverse section

Fig. 5: Rhizomes with dark yellow inside

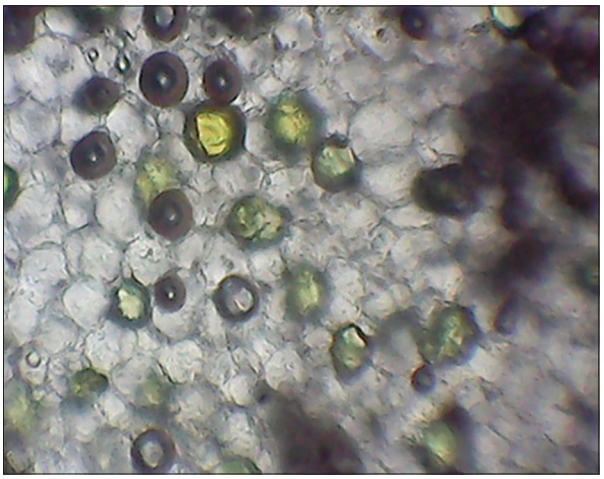

Fig. 8: Parenchyma with yellow color oil globules

breaking, fragrant odor, aromatic, and slightly bitter-sweet in taste (Fig. 1-6).

Microscopic characters

Transverse section of the rhizome showed outer single layered epidermis having rectangular and elongated cells and it is followed by thin-walled cork cells of 6–10 layers, irregularly elongated. The rhizome showed cork cells, cortex, and collateral vascular bundle. Cortex consists of several layers of parenchymatous cells with intercellular air spaces and contains starch. Oil cells are present in cortex as well as parenchyma cells. Yellow colored oleoresin is present at central cylinder region. Closed, collateral vascular bundles are found in a circle in the region just inside the epidermis. The starch grains are abundant in cortex with ovoid irregular shape (Figs. 7-10).

Powder characteristics

Macroscopic and microscopic

The rhizome powder is light brown, slightly rough in touch with slight aromatic odor. Pressing a little amount of powder between filter paper, greasy stain was found, indicating presence of oil cells. Starch grains were present when stained with iodine solution. Microscopical examination the powder showed cork cells, isolated fibers, yellow colored oil globules, pholem fiber surrounded with cork cells, and parencymatoues cells. Behavior of powder with different chemical reagents is shown in Table 1. The fluorescence

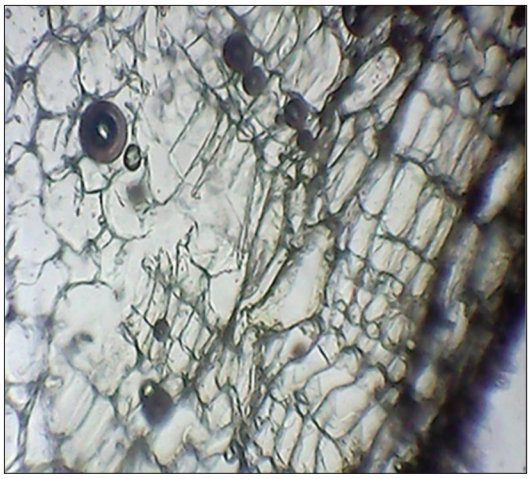

Fig. 9: Cork, cortex parenchyma with air spaces

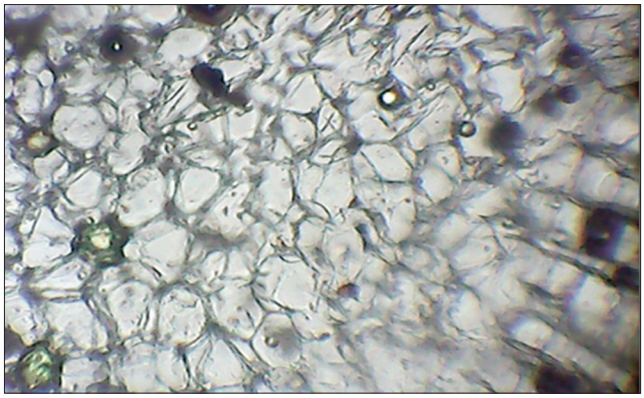

Fig. 10: Parenchyma cells

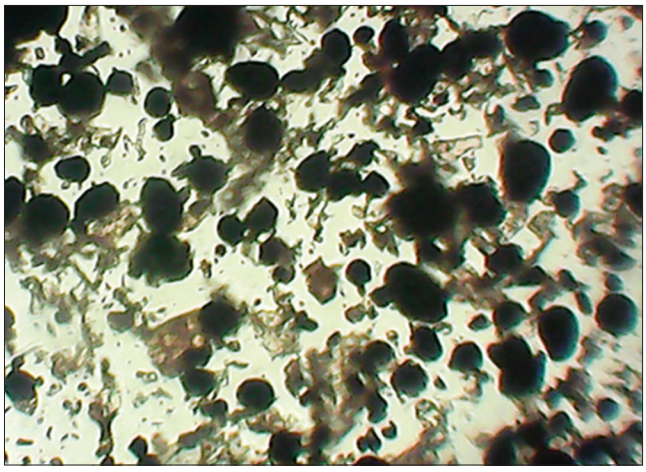

Fig. 11: Starch granules

Table 1: Behavior of *Zingiber cassumunar* Roxb. rhizome powder with different chemical reagent

Reagent	Color/ precipitate	Constituent
Concentrated sulfuric acid	Reddish	Steroid present
Picric acid solution	No yellow ppt	Alkaloid absent
Aqueous silver nitrate solution	No ppt	Protein absent
Aqueous ferric chloride (5%)	Black color	Tannin present
Aqueous mercuric chloride solution	No brown color	Alkaloid absent
Ammonical solution	No change	Anthraquinone glycoside absent
Iodine solution	Blue	Starch present
Ruthenium red	No red color	Mucilage absent
Magnesium-HCl	No change	Flavonoid present

Table 2: Fluorescence analysis of powdered rhizome *Zingiber cassumunar* Roxb.

Treatment	Visible light	Short UV (254 nm)
Powder as such	Light brown	Brown
Powder + 10% NaOH	Green	Light green
Powder + ammonia	Greenish brown	Light brown
Powder + acetic acid	Yellowish green	Green
Powder + HNO_3	Light yellow	Yellowish green
Powder + 50% HCl	Green	Slight brown turbid
Powder + iodine light green	Yellow	Light green
Powder + $FeCl_3$	Dark green	Dark green

UV: Ultra violet

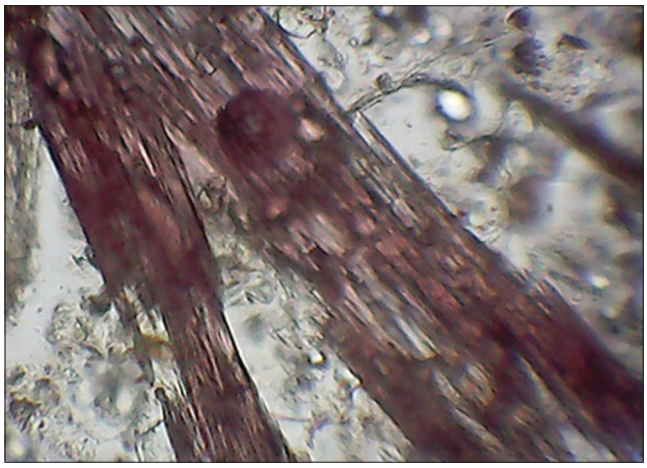

Fig. 12: Phloem fiber (closed view)

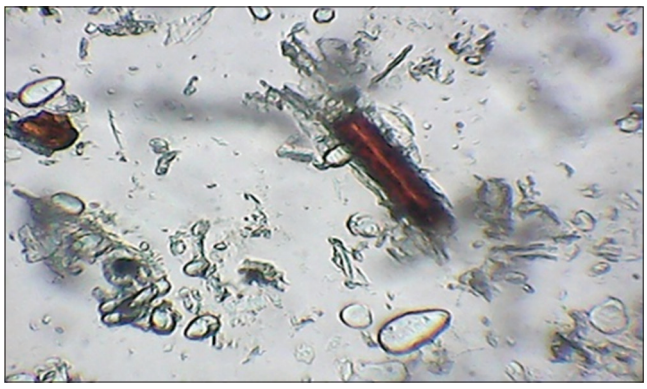

Fig. 13: Phloem fiber

Table 3: Preliminary phytochemical investigation of rhizome extracts

Test	Aqueous extract	Hexane extract	DCM extract	Chloroform extract	Acetone extract	Ethanol extract
Test for carbohydrates	+	+	+	-	-	-
Test for proteins	-	+	+	-	-	-
Test for alkaloids	+	+	+	+	-	-
Test for glycosides	+	-	-	-	-	-
Test for saponins		-	-	+	-	-
Test for flavonoids	-	+	+	+	+	+
Test for tannins and phenolic	-	+	+	+	-	-
Test for amino acids	+	-	-	+	-	+
Test for steroids	+	+	+	+	-	+
Test for fat and oil	-	+	+	+	-	+
Test for mucilage	-	-	-	-	-	-

DCM: Dichloromethane

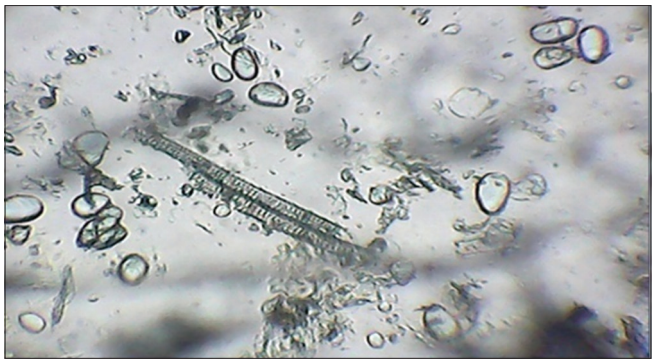

Fig. 14: Isolated fiber

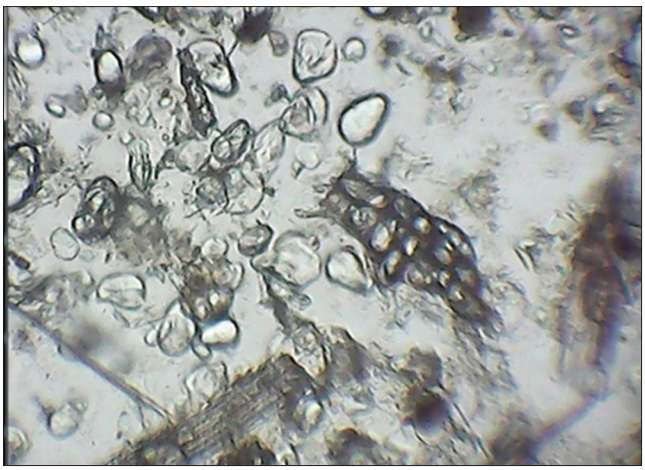

Fig. 15: Isolated parenchyma

Fig. 16: Vessels

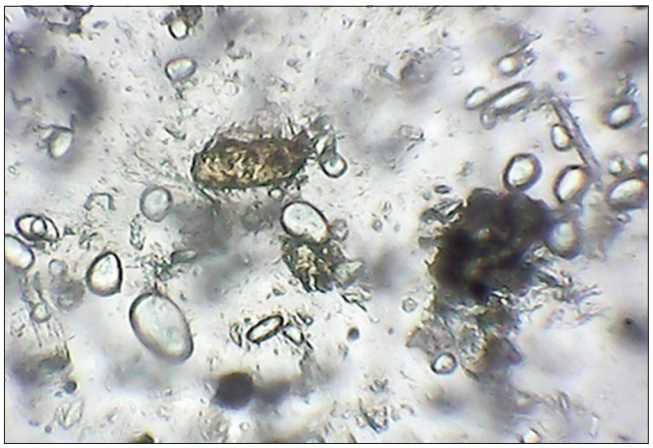

Fig. 17: Yellow colored oil globules

analysis observed in visible, short, and long ultraviolet is depicted in Table 2 (Figs. 11-17).

Physiochemical parameters

The physiochemical parameters such as total ash value were found to be 7.4%, water soluble ash 2.85%, acid insoluble ash 1.85%, and moisture content % LOD is 8%. The extractive values are mainly useful for the determination of the exhausted or adulterated drug. Water soluble extractive values and alcohol soluble extractive values were found to be 13.4% and 10.24% w/w respectively.

Preliminary phytochemical examination

Preliminary phytochemical investigation of rhizome extracts as shown in Table 3.

Thin-layer chromatographic analysis of extract

TLC analysis of extracts gives the idea about the presence of chemical compounds. Fig. 18 shows TLC of DCM extract and Fig. 19 shows TLC of alcoholic extract. The spot color and Rf values are informed in Table 4.

CONCLUSION

The plant *Z. cassumunar* Roxb. find application in Ayurvedic and other traditional system of medicine and have some important characteristic features in rhizomes in order to identifying the plant material. The macroscopic and microscopic characters reveal the presence of yellow colour inside the rhizomes, which is important diagnostic characters that help in identification of plant material. The physicochemical

Table 4: Rf values of DCM extract and alcoholic extract of *Zingiber cassumunar* Roxb

Extract name	Rf	Values	Spot color	Mobile phase
DCM extract	Rfa	0.9	Light yellow	Chloroform:DCM (5.4:11.2)
	Rfb	0.7	Reddish yellow	
	Rfc	0.43	Brownish	
	Rfd	0.18	Reddish brown	
Alcoholic extract	Rfa	0.73	Yellowish brown	Toluene:ethylacetate:methanol:formic acid (6:6:0.4:1.6)
	Rfb	0.57	Greenish yellow	
	Rfc	0.47	Brownish	

DCM: Dichloromethane

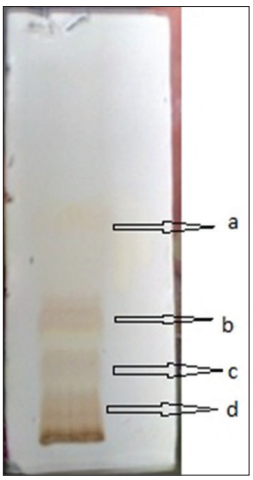

Fig.18: DCM Extract

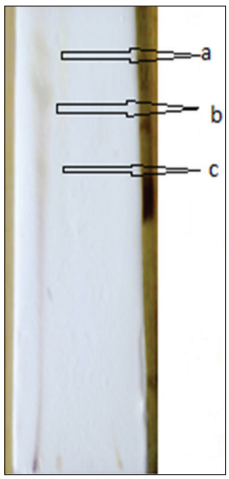

Fig. 19: Alcoholic Extract.

studies are carried out on herbal plant powder sample to establish appropriate data that may be utilized for identification and establish the purity, standard of plant sample, and those supplied in powder form [19]. The estimation of ash value commonly applied parameter for the identification, which establishes the quality and the purity of the drug. Ash value can also detect the nature of the material added to the drug for the purpose of adulteration [20]. The moisture content of the material can be identified by percentage weight of LOD. The phytochemical screening of the drug is very important to identify the different phytoconstituents present in plant materials such as steroids, terpenoids, and flavonoids. It is a very important in the process of standardization and quality control because the constituent vary from plant to plant and also in different samples of the same species depending on various atmospheric factors and storage conditions. TLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs. TLC mobile phase detection and separation of active constituents has found a variety of analytical uses in the Pharmaceutical industries. Chromatographic analysis is the first step toward understanding the nature of active principles and their detailed phytochemistry [21]. The reported pharmacognostic parameters can be considered as distinctive enough for authentification of this drug in herbal industry and can be included as microscopic standards in Indian herbal pharmacopoeia [22].

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