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PHARMACOGNOSTICAL EVALUATION AND PHYTOCHEMICAL CHARACTERISATION OF *VETIVERIA ZIZANIOIDES* (L.) NASH ROOTS

S. Dutta^{*}, S. Mondal, K. Hazra, A. K. Mangal, R. Bolleddu, J. Dahiya, S. Kharwar and P. V. V. Prasad

Central Ayurveda Research Institute, 4-CN Block, Sector-V, Bidhannagar, Kolkata - 700091, West Bengal, India.

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Correspondence to Author:

S. Dutta

Central Ayurveda Research Institute,
4-CN Block, Sector-V, Bidhannagar,
Kolkata - 700091, West Bengal,
India.

E-mail: mailtosreya.27@gmail.com

ABSTRACT: *Vetiveria zizanioides* (L.) Nash has been suggested in the Indian system of medicine as the roots of *Usira* for a number of diseases such as fever, dysuria, fatigue syndrome, wound healing, skin disorders, etc. (*Jvara*, *Trishna*, *Mutrakrachhra*, *Vrana*). This will be created helpfulness towards this medicinal plant and for preparation of different Ayurvedic formulations *i.e* *Usira sava*, *Yogarajaguggulu*, *Sadanga Kvatha Curna*. The objective of the present study is to develop the pharmacognostic and phytochemical profiles of *Vetiveria zizanioides* (L.) Nash root. The present study considers the detail investigation of macroscopy, microscopy, preliminary qualitative phytochemical analysis, physicochemical evaluations and HPTLC chromatography profiling. Microscopical investigation shows the presence of rectangular cells of epidermis, elongated and polygonal parenchyma groups, oval aerenchymatous cells, cork cells, elongated cells sclereids, sclerenchymatous stone cells, groups of sclerenchymatous fiber, prismatic crystals of calcium oxalate and starch grains. The presence of various secondary metabolites was confirmed by phytochemical screening and HPTLC profiling. Identification and authentication of roots may be helpful from botanical and chemical screening data for developing pharmacopoeial standards.

INTRODUCTION: *Vetiveria zizanioides* (L.) Nash syn. *Chrysopogon zizanioides* (L.) Roberty or Vetiver (Family Poaceae) is a fast-growing perennial grass, commonly known as *Virana*, *Adhaya*, *Sevya* (Sanskrit), *Khasa*, *Bena*, *Khas* (Hindi), *Khas-khas*, *Vennaramula* (Bengali), *Sugandhivalo*, *Valo* (Gujrati), *Bala*, *Vala* (Marathi), *Ramacham*, *Lamajja* (Malayalam), *Vetiver*, *Vilamichaver* (Tamil), *Vetivelu*, *Vettiveru* (Telugu). The plant belongs to the.

The highly valued thick fibrous adventitious roots are aromatic in nature. This tufted grass grows throughout India's plains, ascending up to an elevation of 1200 m. Though it originated in India, Vetiver is widely cultivated in tropical regions. It has been reported that almost all Vetiver grown worldwide are a single clone (same DNA)¹.

It is known to improve a person's breathing, lower their heart rate, and be a relaxant for the nervous system. It has also exhibited anti-inflammatory properties, controls diabetes, and is known to cure skin diseases². The plant's root is also known as traditional herb for their cooling and diuretic activity. The root decoction is used as an analgesic, Anthelmintic³, antipyretic⁴, antioxidant⁵ and antituberculosis agent⁶.



Aqueous extract from whole plant of *Vetiveria* exhibits antioxidant activity⁷ and its root extract exhibits anticancer activity against breast cancer cell lines⁸. Several studies show that this plant contains in the plant are khusimol, khusimone, vetivene, khositone, vetiverol, vetivone, terpenes, benzoic acid *etc*^{9,10}. The volatile oil extracted from the root of VZ contains monoterpenes, sesquiterpene and triterpenes¹¹. Vetiver oil is commonly used as a main odor contributor in the perfume industry and as a flavor agent for food in the food industry¹². This work aims to analyze the pharmacognostical study, including powder microscopy and transverse section. The chemical study includes phytochemical screening, physico-chemical parameters, and HPTLC fingerprint profile

MATERIALS AND METHODS:

Solvents and Chemicals: The chemicals, reagents, solvents of Emplura grade, and Aluminium supported Thin Layer Chromatography plates were purchased from E. Merck Ltd., Mumbai, India.

Plant Materials Collection and Authentication: Matured roots of *Vetiveria zizanioides* (L.) Nash syn. *Chrysopogon zizanioides* (L.) Roberty was collected from natural habitat of Baruipur, South 24 pgs, West Bengal (22.3508°N 88.4171°E) on September 2021. Underground root portions (in tuft of bunch) from the peripheral shoot region were separated and authenticated (Voucher specimen no. CARI/Kol/RT-18) by Dr. M. N. Das, ex-scientist, Dept. of Pharmacognosy, Central Ayurveda Research Institute for Drug Development, Kolkata, West Bengal.

Plant Sample Processing: The plant materials *i.e.*, root were washed with aqueous 70% (v/v) ethanol, and dried at an ambient temperature (24-27 °C). A small portion of the air-dried plant sample was used for macroscopic, organoleptic and anatomical (transverse section) studies, while the rest of the plant materials were shade dried and pulverized with a grinder (National SM 2000). The whole and powdered plant samples were stored at room temperature in airtight, light-resistant containers as per guidelines of Ayurvedic Pharmacopoeia of India¹³. The finely (sieved in 60# mesh) and coarsely powdered samples were used for powder analysis, physicochemical, phytochemical and

chromatographical examinations according to the standard method.

Macroscopy of Plant Material: The morphological parameters *viz.* structure, shape, size, texture of the plant material were noted by naked eye observation with a simple microscope (Olympus OIC DM), and organoleptic parameters *viz.* colour, odour, taste were noted by sense organs *etc*^{13,14}.

Cytomorphology of Plant Material: The fresh root samples were transversely sectioned with a clean, sharp diamond edge blade. The selected sections were treated with a graded ethanol procedure (starting from 30%, towards absolute grade along with safranin stain), mounted on slides in Canada balsam, and observed under a digital trinocular compound microscope (Olympus CX21i attached with Magcam DC14 camera) at 10X and 40X magnifications and finally, the photomicrographs were taken^{13,14}.

Powder Microscopy of Plant Material: Fine (sieved in 60# mesh) dried powdered sample *i.e.* root (~2 g) was separately treated with different solutions *i.e.*, aqueous saturated chloral hydrate (for maceration), 50% glycerin, phloroglucinol in conc. HCl (for staining lignified tissues) and 0.02 N iodine reagent (for starch grains), mounted on slides with 50% glycerine following a standard protocol and observed under the binocular compound microscope (Olympus OIC-07964) at 10X and 40X magnifications. The photomicrographs of different cellular structures and inclusions were taken using Magcam DC14 camera attached to an Olympus CX21i trinocular compound microscope^{13,14}.

Physicochemical Evaluation: The physico-chemical parameters like a loss on drying, ash values, and extractive values of the root of the plant were determined by standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/WHO protocols^{15,16}. Extractability was studied with different solvents like hexanes, chloroform, acetone, Ethyl acetate, methanol, ethanol, and water. Conventional methods performed extractions.

Preliminary Phytochemical Analysis: 1 g of the coarse powder was taken in a round bottom flask,

and 20 ml of CH₃OH was added. The mixture was refluxed for one hour and filtered (Whatman No. 40 filter paper). The filtrate was taken screening of phytochemicals by using standard chemical tests^{17, 18}.

Fingerprint Analysis by High-Performance Thin Layer Chromatography (HPTLC): 1 g of the sample was subjected to reflux with methanol (20 ml) for 1 h and the extract was filtered through filter paper (Whatman No. 40).

The filtrate was concentrated ($c = 1 \text{ g}/10 \text{ mL}$) and taken for HPTLC profiling. The extract (4 μL) was applied in the form of 6 mm band, 15 mm from the bottom of a 5 × 10 cm pre-activated aluminum supported precoated silica gel 60F254 plate (Mfg. by Merck, India; 22.01.2020, Batch No. 1.05563.0003), with the help of ATS-4 applicator attached to a CAMAG HPTLC system. The plate was developed in a pre-saturated twin trough chamber using the mobile phase as Hexane: Ethyl acetate: Methanol: Formic acid = 5: 5: 0.5: 1 (v/v) to a distance of 90 mm, dried for 5 min in ambient air. Images of the developed plate were captured under 254 nm and 366 nm UV light.

An image was also captured using visible light after derivatizing the plate with anisaldehyde-sulphuric acid reagent¹⁹. Densitometric scanning²⁰ of the developed plate at 254 nm, 366 nm, and 556 nm were performed.

RESULTS & DISCUSSION:

Macroscopic Characters: Root has neither stolon nor rhizome, present in tuft or clusters, grow downward finely structured and very strong, fragrant wiry, fibrous up to 2 mm in

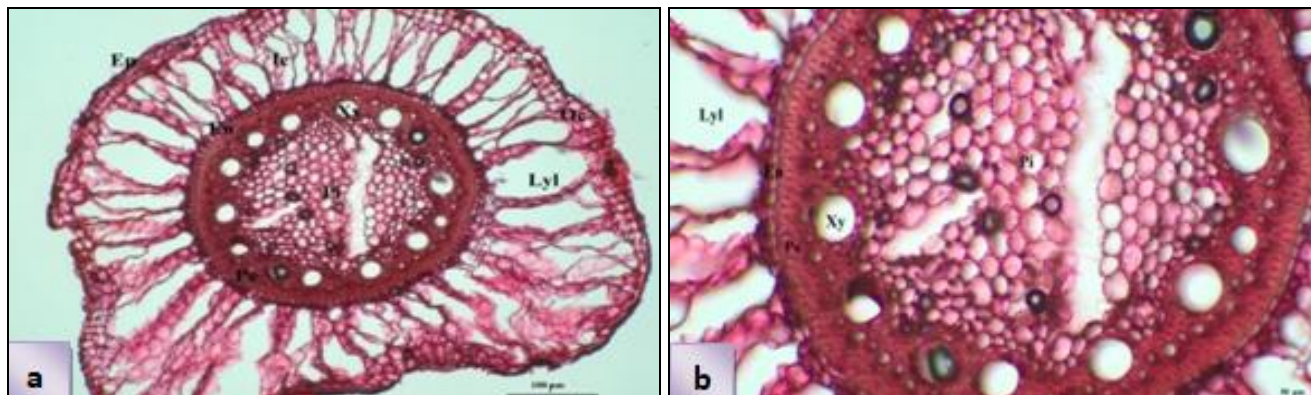
diameter, minute, longitudinally grooved; colour creamish to light yellow, short and splintery fracture, with strong aromatic odour and slightly bitter taste.



FIG. 1: VETIVERIA ZIZANIOIDES (L.) NASH ROOTS IN TUFT

Cytomorphological Characters: The transverse sections (T.S.) of roots show from the periphery towards centre, the epidermis, the cortex, the vascular region, and central pith. Epidermis single layered, cortex parenchymatous, divided in outer and inner distinguished zone, outer cortex if 3 to 4 cell layered and inner cortex 5 to 9 thin cell layered, aerenchymatous interrupted by large lysigenous lacunae. Endodermis represented by inner cortical single parenchymatous layer with tangential and radial band like casparian strip. Few dark coloured oil cells are adjacent to the endodermis facing the inner cortex. The vascular region ring like comprising of metaxylem vessels and 4 to 6 cell layered pericycle consisting of sclereids and thick pericyclic fibres. The central pith region is parenchymatous with lots of starch grains and few cystolith **Fig. 2A to 2I**.

Photomicrographs:



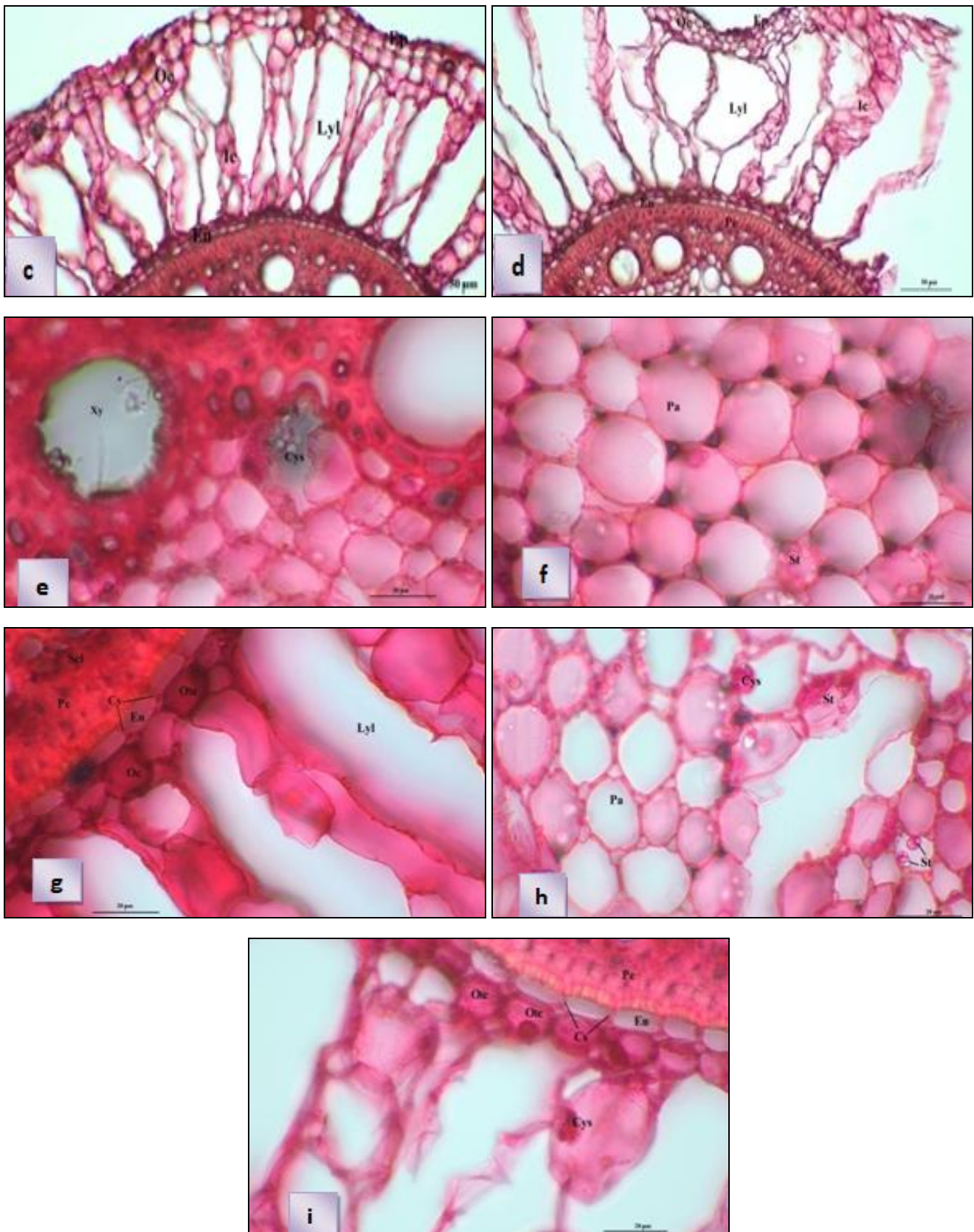
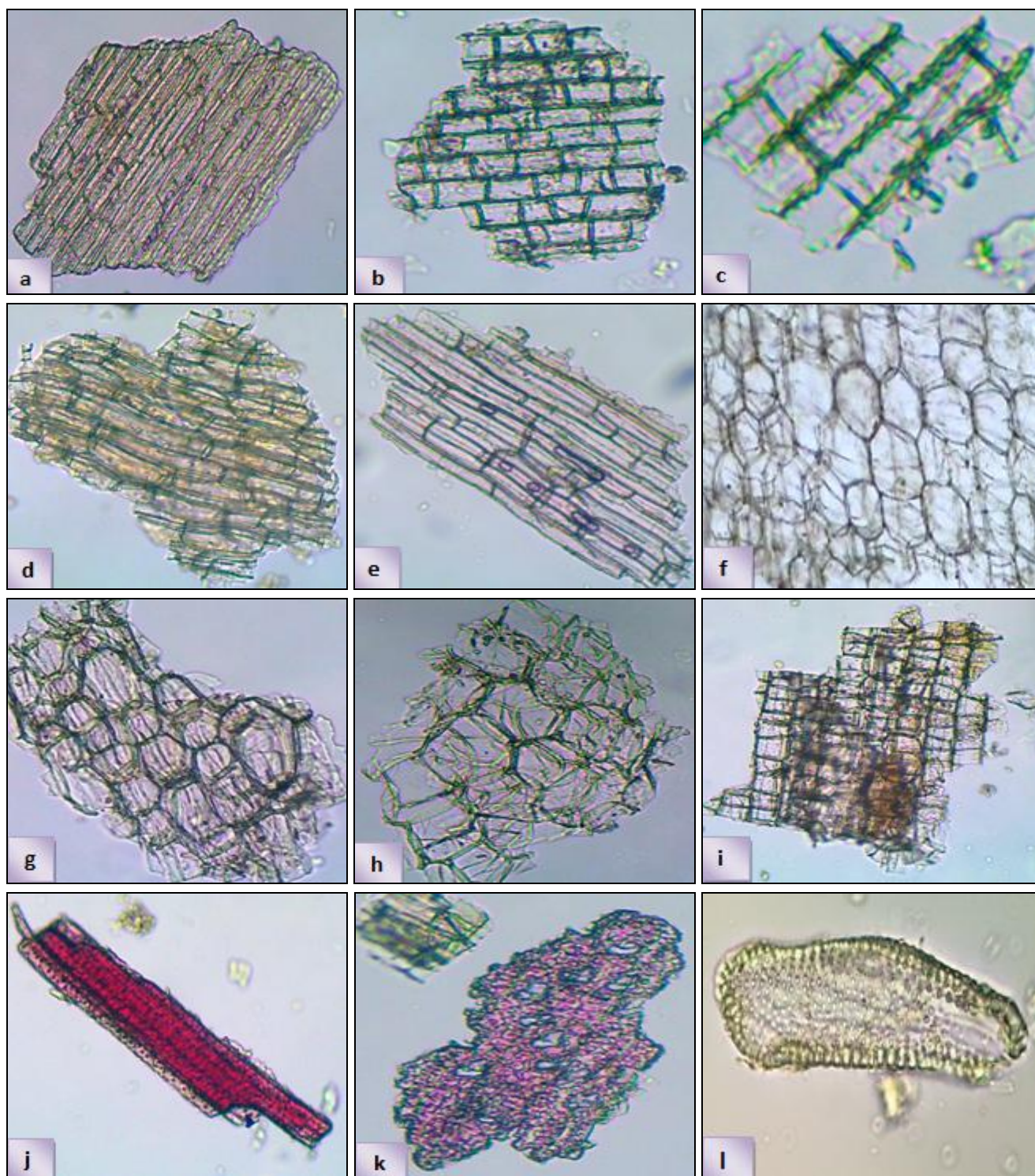


FIG. 2: TRANSVERSE SECTIONS (T.S.) OF ROOTS OF *VETIVERIA ZIZANOIODES* (L.) NASH. a. T.S. of root; b. T.S. through central zone of root; c. & d. T.S. through peripheral or cortical zone (enlarged view); e. & f. T.S. through vascular and central pith zone(enlarged view); g, h. & i. T.S. through Endodermis, inner cortex and pith (enlarged view); Ep: epidermis, Oc: outer cortex, Ic: inner cortex, Lyl: lysigenous lacunae, En: endodermis, Cs: casparian strip, Pc: pericycle, Pa: parenchyma, Xy: xylem, Pi: pith, St: starch, Cys: cystolith, Scl: sclereid, Olc: oil containing cell.

Powder Microscopy: Fine powder of root brownish yellow in color having slightly bitter taste and strongly aromatic odour, shows the presence of rectangular cells of epidermis with wavy wall, groups of elongated and polygonal compactly arranged parenchyma with cell content, groups of oval aerenchymatous cells, cork cells, elongated

thick-walled sclereids, sclerechymatous pitted stone cells, groups of sclerenchymatous fiber, pitted and reticulate vessels sometimes associated with fibres, prismatic crystals of calcium oxalate measuring 20 to 40 μ in diameter and starch grains measuring 10-12 μ in diameter **Fig. 3A to 3R**.

Photomicrographs:



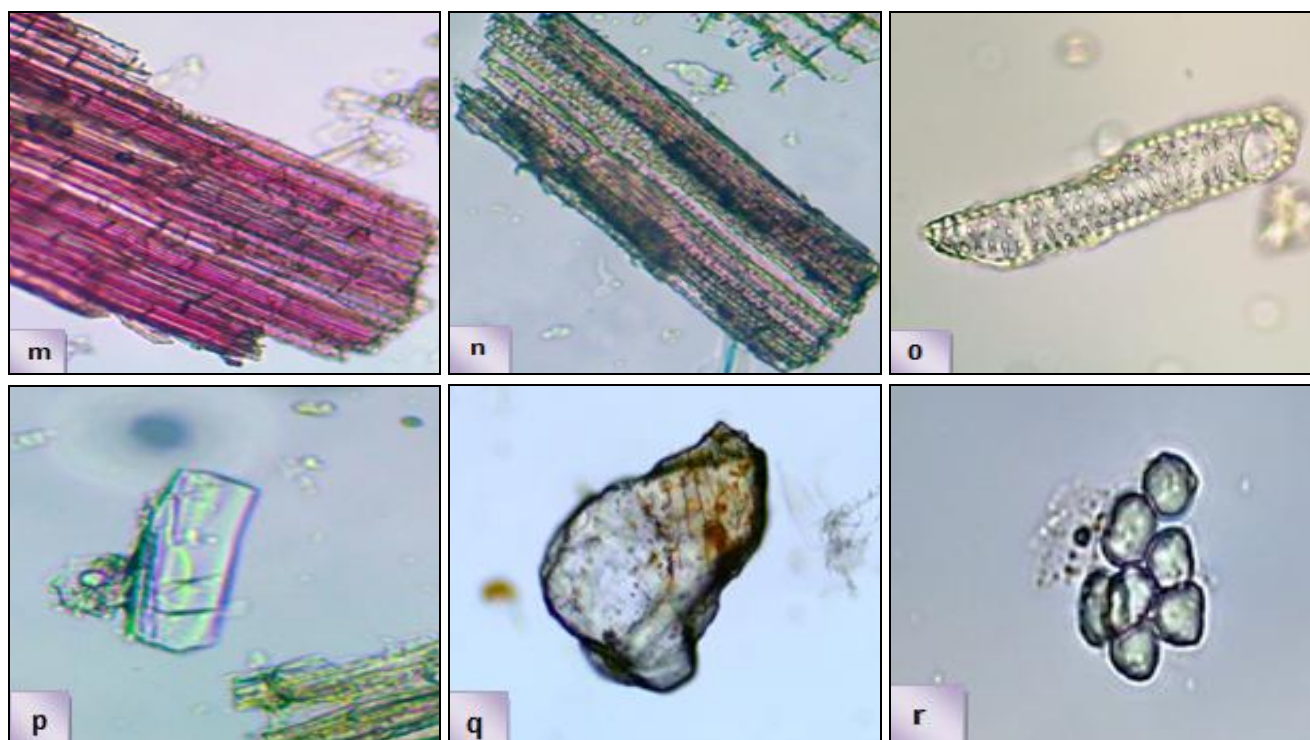


FIG. 3: COMPONENTS OF ROOT POWDER OF *VETIVERIA ZIZANIOIDES* (L.) NASH. a, b, c: Groups of rectangular cells of epidermis with wavy wall (40X, 100X,400X); d,e: Groups of elongated compactly arranged parenchymatous cells with cell content(100X); f: Groups of polygonal parenchymatous cells (100X); g, h: Groups of oval aerenchyma cells(100X); i: Groups of cork cells (100X); j:Groups of long sclereid (100X); k: Groups of stone cells(100X); l:Pitted stone cell (400X); m: Groups of sclerenchymatous fiber (100X); n: Groups of reticulate vessels with fibre (100X); o: Vessel with pitted thickenings (400X); p,q: Prismatic crystals of calcium oxalate(400X); r: Starch grains (400X).

Physicochemical Parameters: Determination of the following physicochemical parameters were carried out - loss on drying, total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, alcohol-soluble extractive, hexane-soluble extractive, chloroform-soluble extractive, ethyl acetate-soluble extractive, acetone-soluble extractive **Table 1**. These parameters were determined according to the standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/WHO^{15, 16}.

TABLE 1: PHYSICOCHEMICAL PARAMETERS

Parameters	Results (% , w/w)
Loss on drying (at 105°C)	10.8 ± 0.05
Total ash	1.76 ± 0.03
Acid-insoluble ash	0.55 ± 0.01
Water-soluble ash	0.31 ± 0.01
Water-soluble extractive	8.45 ± 0.07
Alcohol-soluble extractive	6.53 ± 0.02
Hexane-soluble extractive	0.20 ± 0.01
Chloroform-soluble extractive	1.12 ± 0.02
Ethylacetate-soluble extractive	0.8 ± 0.03
Methanol-soluble extractive	1.8 ± 0.03

Preliminary Phytochemical Analysis: The methanolic extract, obtained as described in the

previous section was utilized for preliminary phytochemical testing ~2 ml of the extract being taken for each test. The results are given in **Table 2**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL TESTS OF METHANOLIC EXTRACTS OF DRIED ROOT OF *VETIVERIA ZIZANIOIDES*

S. no.	Test/Reagent used	Observation
1a	Alkaloids - Mayer's test (in dil. HCl)	Positive
1b	Alkaloids - Dragendorff's test (in dil. HCl)	Positive
2a	Carbohydrates – Molisch's test	Negative
2b	Reducing sugars - Fehling's test	Negative
3	Flavonoids - Shinoda test	Positive
4	Terpenoids/ Steroids - (Liebermann-Burchardt Test)	Positive
5	FeCl ₃ test for Phenolic compounds/ tannins	Positive
6	Glycoside (Acetic acid, FeCl ₃ , H ₂ SO ₄)	Positive
7	Test for Saponines (Foam test)	Positive

HPTLC Finger-printing Profile of Root of *Vetiveria zizanioides*: The HPTLC experimental condition was optimized by using pre-activated and precoated TLC silica gel 60 F254 plates and

different combinations of polar and non-polar solvents as the mobile phases. Best result was obtained with Hexane: Ethyl acetate: Methanol: Formic acid = 5: 5: 0.5: 1 (v/v) as the mobile phase, which showed seven bands at R_f values of 0.73, 0.65, 0.34, 0.3, 0.27, 0.22 and 0.12 and seven bands at R_f values of 0.69, 0.61, 0.46, 0.34, 0.31, 0.23 and 0.16 and eight bands at R_f values of 0.87, 0.63, 0.51, 0.43, 0.40, 0.35, 0.32 and 0.24 for roots of VZ when visualized under UV at 254 nm, 366 nm and white light after derivatization respectively.

The pictorial representation of the developed plate of root methanolic extracts are given in Figure 4. Densitometric scanned pictures at 254 nm, 366 nm and 556 nm of the developed plates as fingerprint profile are shown in Fig. 5.

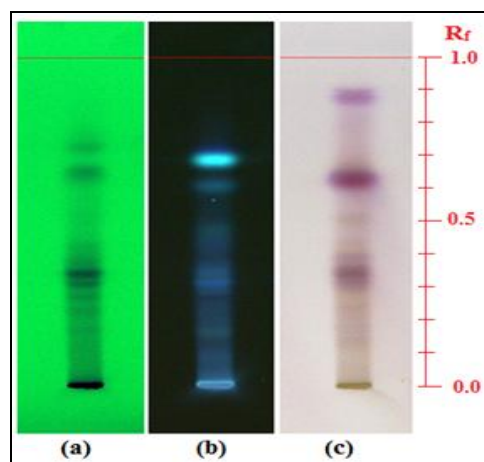


FIG. 4: PHOTOGRAPHY OF HPTLC PLATE – (A) VISUALIZATION AT 254 NM; (B) VISUALIZATION AT 366 NM AND (C) VISUALIZATION AT WHITE LIGHT AFTER DERIVATIZED WITH ANISAL-DEHYDE-H₂SO₄ SOLUTION

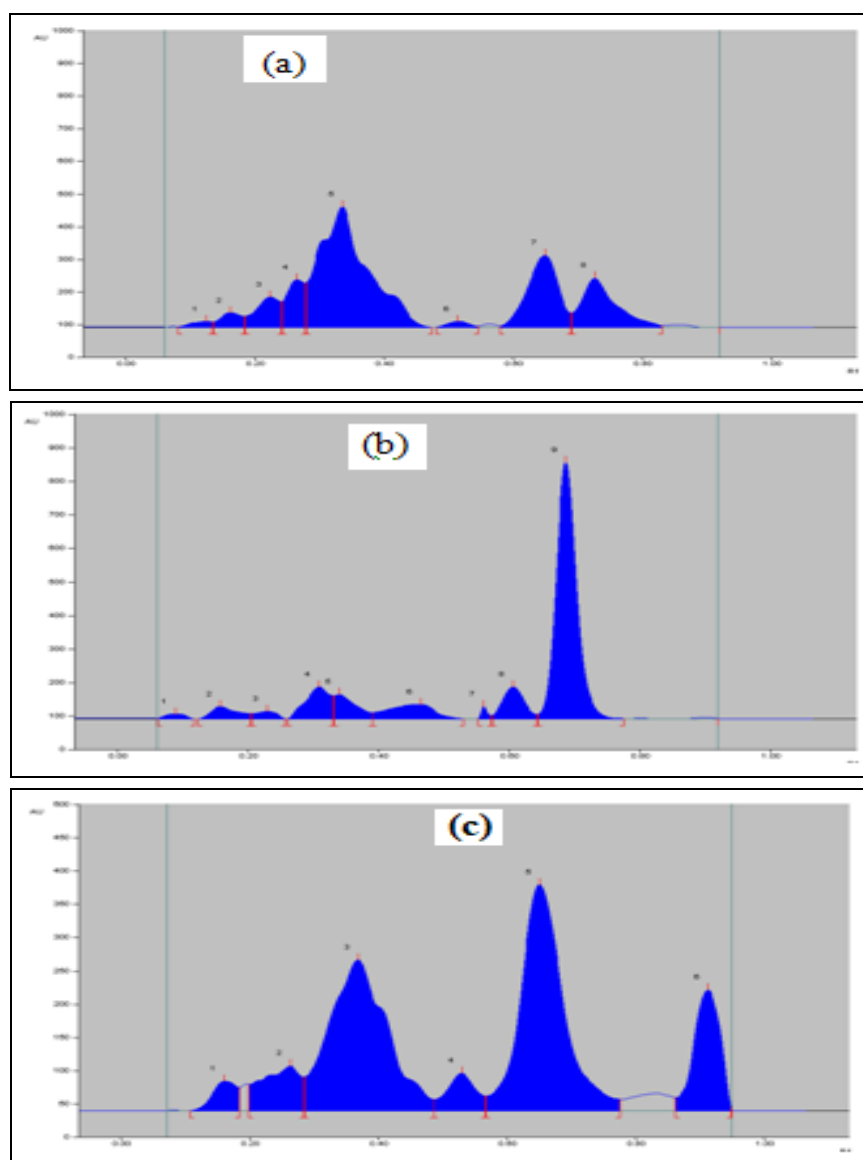


FIG. 5: DENSITOMETRIC FINGER PRINT PROFILES AT (A) 254 NM, (B) 366 NM AND (C) 556 NM OF VETIVERIA ZIZANIOIDES (ROOT)

CONCLUSION: The present investigations furnished Pharmacognostical profiling and HPTLC fingerprinting profile of dried root of *Vetiveria zizanioides*. Phytochemical screening of methanolic extract exposed the presence of alkaloids, flavonoids, saponin, tannins, and steroids by positive reaction with the relevant reagent. The data obtained in the present study may be considered diagnostic tools for authentication and identification of the plant and be the standard for future studies.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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