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Pharmacognostic and HPTLC finger printing studies on leaf of *Oxystelma esculentum* (L.f.) Sm

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Abstract

Oxystelma esculentum (L.f.) Sm. is belonging to the family Apocynaceae, commonly known as rosy milk weed, in Telugu palateega and is found near water logged areas. The leaves are used as medicine in diuretic, galactagogue, anthelmintic, antiulcer, laxative and antiperiodic. The plant is also used in throat infections, skin diseases and jaundice. The present paper provides a detailed account of the Pharmacognostical studies. The study includes macro and micro morphological characters including powder characteristics, organoleptic characters, HPTLC finger printing and preliminary phyto-chemical aspects. The present work can serve as a useful tool in the identification, authentication and standardization of the plant material.

Keywords: *Oxystelma esculentum* r. Br, pharmacognosy, HPTLC fingerprinting, phyto-chemical, apocynaceae

Introduction

Oxystelma esculentum (L. f.) Sm. an important medicinal plant, commonly known as rosy milk weed, in Telugu palateega belonging to the family Apocynaceae, A Perennial, pretty, twining wild herb or under shrub with milky juice; roots fibrous from the lower nodes stems numerous, long, much- branched, slender, glabrous. Stem: Terete, profusely branched, branches greenish purple, thin, slender. Latex: milky. Leaves: opposite, evergreen, membranous, oblong, linear-lanceolate, 4-10.5× 0.5 -1cm, subcoriaceous, glabrous, hairs rare, venation pinnate with intramarginal vein, base rounded or obtuse, apex acute, margin entire, petiolate, petiole 0.5-1 cm long, very slender. Inflorescence: interpetiolar, drooping cymes; usually 2-4 flowered, sometimes single flowered, peduncle 6-15 cm long. Flowers: strikingly handsome, white or rose medium sized, 1.5-2.5 cm across, found throughout the plains of the Indian subcontinent near water- logged areas [1, 2, 3, 4 & 5]. (PLATE-1. A, B&C). The whole plants, especially leaves, have been reported to possess antiperiodic, anthelmintic, diuretic, laxative, antiulcer and galactagogue activity. It is used ethnomedicinally in throat infections, skin diseases and also in the treatment of jaundice [6 & 7]. The latex and roots are used in Rheumatism (La), jaundice(R) [8]. The roots are used in jaundice [9]. The leaves are used in scabies [10]. The whole plant used in gargle in aphthous ulcerations of the mouth and in sore-throat and the roots also used in jaundice [11]. Decoction of plant is used as gargle in aphthous ulcerations of mouth and in sore throat. The roots are considered specific for jaundice. The milky sap is used as a wash for ulcers [12]. In the present investigation the leaves are found folk-lore useful for jaundice Leaves are ground pasted 2 spoon fuls of paste mixed in a glass of water is administered twice daily for 21 days in the cure of jaundice. Extraction method followed by analysis with a LC tandem mass spectrometry system and applied the multivariate statistical approach to optimize the extraction conditions. The analytical method showed high extraction yields for the determination of this compound in



Plate 1: A-B Flowering twig of *O. esculentum*

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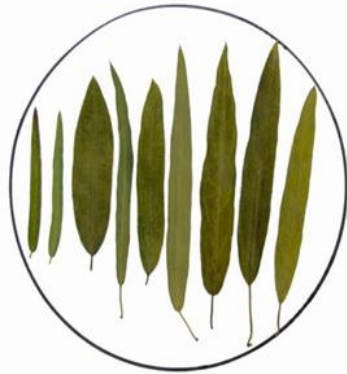


Plate-1: C-Leaf microscopy of *O. esculentum*

Materials and Methods

Oxystelma esculentum (L.f.) Sm was collected in the flowering and fruiting stage from Sriramagiri village, Nellikudur mandal, Warangal district, Telangana, India. Collected material was poisoned and mounted on herbarium sheets, taxonomically identified by the Botanical Survey of India (BSI) Deccan Regional Centre Hyderabad and deposited in Herbarium Hyderabadense, Department of Botany, Osmania University, Hyderabad. The leaves are boiled, fixed in F.A.A. (Formaldehyde – Acetic acid – Alcohol), dehydrated through xylene – alcohol series embedded in paraffin wax. The sections were cut at 10 – 12 μm on Optica 1090A rotary microtome, stained with crystal violet and basic fuchsin combination and mounted in canada balsam [13]. Epidermal peels were obtained by gently scraping and peeling by razor blade, double treatment method [14] was also employed. Were stained with safranin and mounted in glycerin. The powder microscopy characters were studied by boiling the drug in distilled water, stained in safranin and mounted with glycerine. The photomicrography was done on Olympus BX53 Research trinocular microscope attached with digital sony camera.

Phyto-Chemical Studies

Collected material were washed thoroughly under running tap water, for removing soil particles and dried under shade at room temperature (25°C) for ten days, until concurrent dry weights were obtained using electronic balance (Type BL-220H, NO.D427600501) and ground into fine powder by using mechanical pulverizer. The powdered material was meshed through 0.3mm mesh (Jayanth scientific IND. Mumbai.) and stored in an air tight sterile plastic container at room temperature.

Hot continuous successive extraction using Soxhlet apparatus

Successive extractions were carried out using Soxhlet apparatus, for the plant samples extraction. An earlier study on phytochemical extraction suggests that soxhlet extraction process provided standard

results. 20g finely leaf powder of plant species were packed in porous bag or a “thimble” made of strong filter paper, packed individually and placed in the extraction chamber of the soxhlet and 200 ml solvent was taken in each round bottom flask. The leaf powder were extracted successively with petroleum ether at 60°C, ethyl acetate at 77°C, chloroform at 61°C, acetone at 56°C and methanol at 65°C. Extraction temperatures were adjusted to boiling points of solvent to allow a faster rate of cycling of fresh solvent. Eight hours of duration was allocated to each solvent for hot continuous and successive extraction or till the solvent in siphon tube of an extractor become colorless. The extracts were cooled, filtered through Whatmans No.1 filter paper and extractions were carried out in the order of increasing polarity of those solvents i.e. from petroleum ether to methanol and preceded by phyto-chemical screening [15 & 16]. After the extraction the solvents were removed using rotary evaporator (Heidolph 36000130 Hei-Vap Value Collegiate Rotary Evaporator, G5B Dry Ice Condenser) and crude residues were obtained. These crude residues were kept in refrigerator when not in use.

Observations and Results

Leaf Macroscopy: Leaves are linear-lanceolate, rounded at base, pubescent A Perennial, pretty, twining wild herb or under shrub with milky juice of the *Oxystelma esculentum*- is linear-lanceolate, rounded at base, pubescent A Perennial, pretty, twining wild herb or under shrub with milky juice; Leaves: opposite, evergreen, membranous, oblong, linear- lanceolate, 4-10.5 × 0.5 - 1cm, subcoriaceous, glabrous, hairs rare, venation pinnate with intramarginal vein, base rounded or obtuse, apex acute, margin entire, petiolate, petiole 0.5-1 cm long, very slender. (PLATE-1. C)

Leaf Adaxial surface studies

Microscopic characters: Epidermal cells 5-7 sided, polygonal isodiametric, few anisodiametric, sides thick, mostly straight, few straight to curved, surfaced striated, contents dense with calcium oxalate crystals. E.C.F. 2272 per sq.mm². Dist: all over except on veins, irregularly arranged, variously oriented. Costal cells: Polygonal to linear, 5-8 sided, sides thick, straight to curved, surface striated, contents dense with calcium oxalate crystals in few. Dist: on primary and secondary veins, irregularly arranged parallelly oriented. Stomatal complex: Mostly anomocytic, few tetracytic, sunken; subsidiaries 4-5, monocyclic, mostly of a-type, few f-type, rarely c-type, guard cells linear, contents dense. Dist: near to the primary and secondary veins and at apex region. S.F. 62 per sq.mm². S.I. 2.57. Trichome complex: i). uniseriate conical hair: Foot: 1-celled, rounded, embedded, walls thin, contents slightly dense. Body: 2-8 celled cells broader than long, terminal cell longer, conical, walls thin, surface smooth, contents slightly dense. Dist: Common, all over, more on veins. (PLATE-2. A, B&C).

Leaf Abaxial surface studies

Epidermal cell complex: As described on lamina adaxial. E.C.F. 1800 per sq.mm².

Stomatal complex: As described on lamina adaxial expect. Subsidiaries mostly of c-type, few a-type. S.F. 662 per sq.mm². S.I. 24.2.

Trichome complex: As described on lamina ataxia. (PLATE-2. D, E&F).

Abbreviations: E.C.F- epidermal cell frequency; S.F- stomatal frequency; S.I- stomatal index; Dist- Distribution

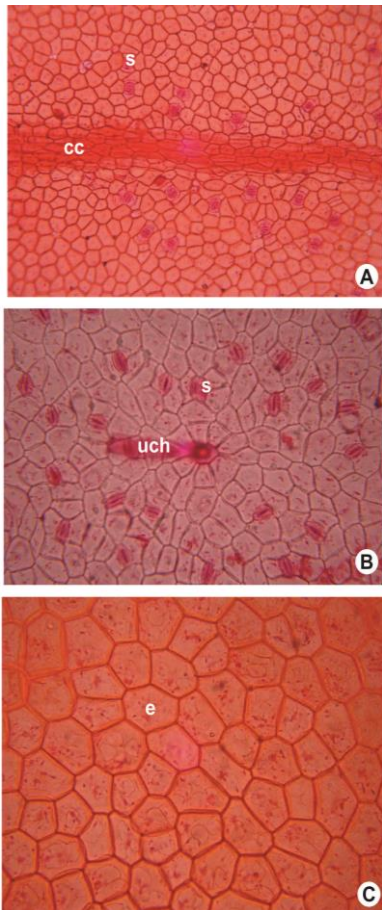


Plate 2: Adaxial A, B & C)

2A. Leaf adaxial surface with costal cells X145; 2B. Leaf adaxial surface with uch X 110; 2C. Leaf adaxial surface with Epidermal cells X 80.

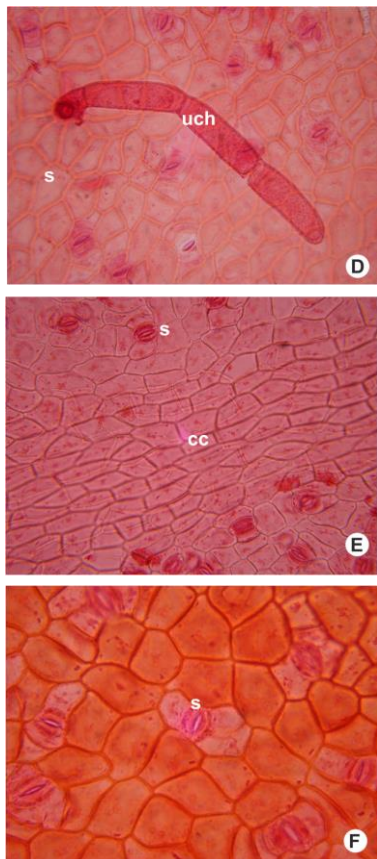


Plate 2: Abaxial. D, E & F

2D. Leaf abaxial surface with uch X 70;
2E. Leaf abaxial surface with stomata and costal cells X80;
2F. Leaf abaxial surface with stomata X 80.

Abbreviations: s-stomata; uch-unicellular conical hair; cc- costal cells; e- epidermal cells.

Transverse Section of Leaf

T.S. of leaf: in T.S. of leaf Faintly ribbed adaxially and prominently on abaxial at midvein; 670-1063(865) µm and lamina 130-261(204) µm in thickness.

Epidermis: One layered, cells mostly barrel shaped oval to circular; about 25-50 (34) µm long and 10-25(19) µm wide and isodiametric cells 20-43(32) µm in diameter on lamina adaxial. Abaxially cells smaller, about 15-38(26) µm in long, 10-15 (12) µm wide and isodiametric cells 18-35(25) µm in diameter; cells over midvein cells mostly barrel shaped oval to circular, 18-38(27) µm long, 13-25(17) µm wide and isodiametric cells 10-30(18) µm in diameter on adaxial; abaxially barrel shaped oval to circular 20-30(24) µm in long, 10-20(13) µm wide and isodiametric cells 8-25(18) µm in diameter; walls thick, cuticle covered over the surface.

Stomata: flushed with epidermal cells on either sides, contents slightly dense. (PLATE- 3 A&B)

Mesophyll: Heterogenous, differentiated in to palisade and spongy tissues.

Palisade: 2- layered, adaxial, throughout, except on midvein and secondary veins, cells columnar, cylindrical, 28-63(45) µm long and 8-23 (14) µm wide, intercellular spaces narrow, contents slightly dense with chloroplasts, occasionally interspersed with sphaero crystal aliferous idoblasts.

Spongy tissue: 4-6 layered cells mostly oval to circular, oblong, 15-35 (24) µm in diameter, intercellular spaces large, contents dense with chloroplasts, occasionally interspersed with sphaero crystal aliferous idioblasts. (PLATE-3B).

Ground tissue: Of midvein heterogenous, differentiated into collenchyma and parenchymatous tissues.

Collenchyma: as a group of cells on adaxial and 1-2 layered abaxially; cells polygonal, oval to spherical, lamellar, 12-35(25) µm in diameter on adaxial and abaxially 25-47(36) µm in diameter, contents scanty.

Parenchyma: 5-8 celled thickness on either sides, smaller, polygonal, circular, oval to spherical, cells about 30-58(42) µm and in diameter adaxially and abaxially larger, 38-68(51) µm in diameter, walls thin, with small intercellular spaces, contents scanty; cells interspersed with sphaero crystal aliferous idioblasts. (PLATE-3A).

Vascular tissue: At midvein consists of a single arcuate vascular bundle, 400-540 (489) µm tangentially long, 173-259(220) µm radially wide, bicollateral, conjoint, endarch, xylem at the centre; tracheary elements 40-60 in number in midvein, arranged in radial rows, polygonal, lignified, about 15-40(26) µm in diameter, walls thin secondary wall thickenings of tracheary elements in L.S. mostly helical. Phloem towards abaxial and adaxial, aperiyclic, 2-3 celled vascular cambium is present. Secondary vascular bundles oval to circular. Xylem parenchyma arranged in between tracheary elements, cells polygonal, slightly thick walled contents scanty. Phloem consists of phloem parenchyma, sieve cells, companion cells. Phloem parenchyma compactly arranged without inter cellular spaces. (PLATE-3A).

Transverse section of petiole

T.S. of Petiole: In T.S. of petiole Oval shaped, in outline, 654-1193(979) µm in diameter.

Epidermis: one layered, cells mostly oval to circular few barrel shaped, elongated cells 15-30(23) μm long, 10-20(14) μm in wide, isodiametric cells 12-25(18) μm in diameter, walls thick, contents dense with few tanniniferous idioblasts and few scanty; cuticle thick over the surface, stomata and trichomes absent. (PLATE-3C).

Ground tissue: Heterogenous, consists of chlorenchyma, collenchymas, parenchyma tissues.

Chlorenchyma: 1-layered, beneath the epidermis on adaxial, cells closely packed without intercellular spaces, contents dense with chloroplasts.

Collenchyma: 1-3 layered throughout, interrupted at adaxial side, cells polygonal oval to circular, lamellar, 17-45(30) μm in diameter, contents scanty.

Parenchyma: Rest of the ground tissue is parenchymatous, cells polygonal, oval to circular 33-73(51) μm in diameter, walls thin, intercellular spaces narrow, often interspersed with sphaerocrystalliferous idioblasts, 12-35(28) μm in diameter, contents dense with tanniniferous idioblasts, 10-30(22) μm in diameter, mostly on adaxial. (PLATE-3C).

Vascular Tissue : Consisting of a large arc shaped vascular bundle at centre with a 2 small spherical vascular bundles in adaxial ridges; large vascular bundle laterally 490-817(631) μm long and vertically 163-327(232) μm wide, conjoint, bicollateral, aperiocyclic; xylem consists of numerous tracheary elements arranged in radial rows, few laterally aligned; adaxial bundles small, spherical, consisting of few tracheary elements surrounded by phloem; tracheary elements polygonal, oval to circular 15-33(25) μm in diameter, walls thick; secondary wall thickenings of tracheary elements in L.S. consist mostly helical, annular and scalariform and few bordered pitted; helices double and single, annular rings free. Xylem parenchyma is present in between tracheary elements, cells polygonal, walls slightly thick, intercellular spaces absent, contents scanty; a 2-3 layered cambium is present in between xylem and phloem. Phloem consists of sieve tubes with companion cells, phloem fibers and phloem parenchyma; phloem parenchyma cells compactly arranged, cells polygonal, walls thin, intercellular spaces absent, contents scanty, occasionally interspersed with sphaerocrystalliferous cells. (Plate-3C).

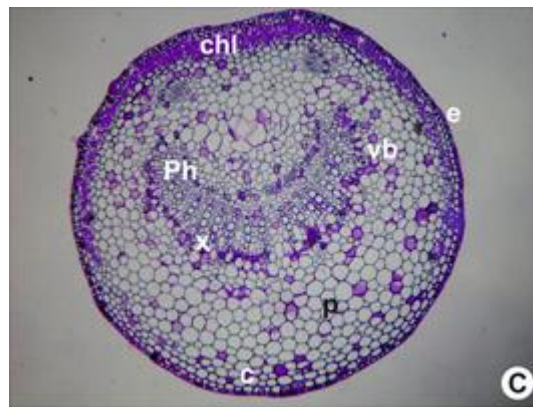


Plate 3: 3A. T.S. of leaf midvein X60; 3B.T.S. of leaf lamina X50; 3C.T.S. of petioleX40.

Abbreviations: e-epidermis; ade-adaxial epidermis; abe- abaxial epidermis; pl- palisade; sp- spongy tissue; c-collenchyma; p- parenchyma; chl- chlorenchyma; vb-vascular bundle; ph-phloem; x- xylem; cr-crystal.

Powder microscopy

1. Pieces of epidermis with anomocytic and tetracytic stomata;
2. Pieces of epidermis with straight to curved sides with stomata;
- 3.Xylem vessels with helical thickenings;
- 4.Pieces of powder consists annual rings;
5. Powder consists of sphaero crystals.

Organoleptic characters

Colour-Green; Touch-coarse; Odour-fragrant; Taste-no characteristic.

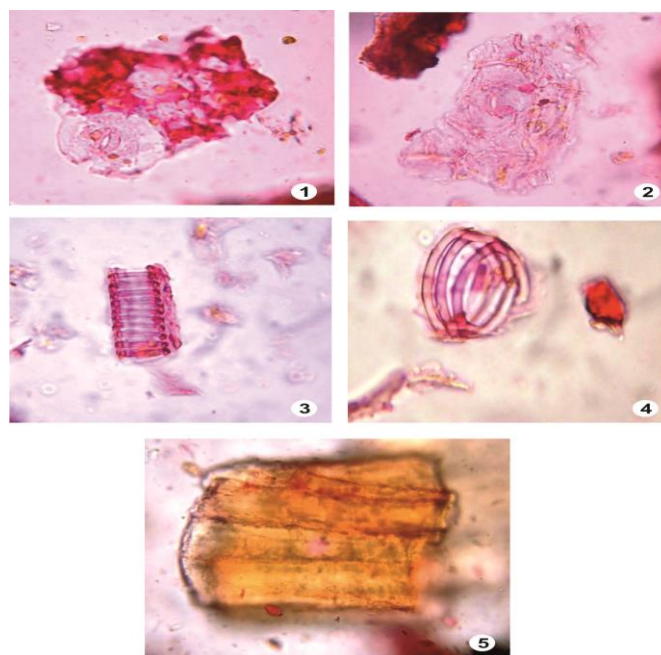
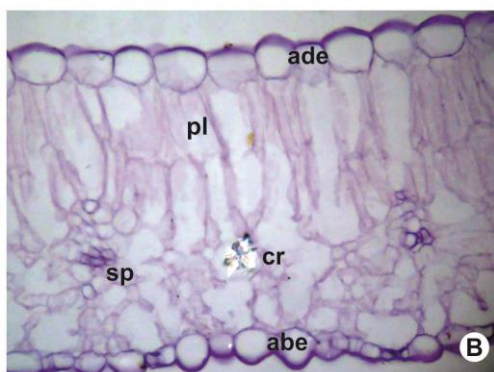
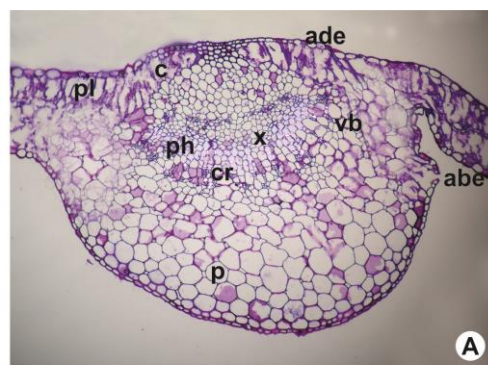


Plate 3: 1.stomata; 2.epidermis with stomata; 3. Helical thickenings; 4.annual rings; 5.crystals.

Preliminary phytochemical analysis of *Oxystelma esculentum*

Preliminary phyto-chemical screening revealed presence of alkaloids and tri terpinoids in chloroform, acetone and methanol extracts, where as carbohydrates found in chloroform and ethyl acetate extracts, while proteins, aminoacids, quinones and leucoanthocyanins, were absent in all extracts, flavonoids and saponins were indentified in acetone and methanol extracts, while phytosterols and glycosides were positive in all extracts, while phenols and tannins were identified in petroleum ether, ethyl acetate and methanol extracts, cardiac glycosides were found in all extracts but except acetone exatract, while steroids were positive in petroleum ether and ethyl acetate extracts, where as comarins were present in petroleum ether, acetone and methanol extracts,

anthraquinones were identified only in methanol extract, terpenoids were found in all extracts, but except petroleum ether extract, diterpenoids and resins were identified in petroleum ether, ethyl

acetate, acetone and methanol extracts, while gums and carboxylic acid were present only in chloroform extract respectively.^[17, 18, 19, 20] (Table-1)

Table 1: Preliminary phytochemical analysis of *Oxystelma esculentum*:

S. No	Pyto. Name	PET. Ether	Chloroform	Ethyl Acetate	Acetone	MEOH
1	Alkaloids	-	+	-	+	+
2	Carbohydrates	-	+	+	-	-
3	Proteins	-	-	-	-	-
4	Aminoacids	-	-	-	-	-
5	Flavonoids	-	-	-	+	+
6	Saponins	-	-	-	+	+
7	Phytosterols	+	+	+	+	+
8	Phenols	+	-	+	-	+
9	Tanins	+	-	+	-	+
10	Glycosides	+	+	+	+	+
11	Cardioglycosides	+	+	+	-	+
12	Steroids	+	-	+	-	-
13	Coumarins	+	-	-	+	+
14	Anthra Quinones	-	-	-	-	+
15	Quinones	-	-	-	-	-
16	Terpinoids	-	+	+	+	+
17	Di Terpinoids	+	-	+	+	+
18	Tri Terpinoids	-	+	-	+	+
19	Resins	+	-	+	+	+
20	Gums	-	+	-	-	-
21	Carboxylic Acid	-	+	-	-	-
22	Leucoanthocyanins	-	-	-	-	-

“+” = present; “-” = absent

Profile of HPTLC finger printing of *Oxystelma esculentum*

The profile of chromatographic separation of leaf methanol extract scanned at 254 nm, reveals eleven spots (Fig.1) out of which spots 5 and 11 possess maximum composition with R_f at 0.41 and 0.92. While, densitogram scanned at 366 nm, revealed eleven spots with spots 1 and 4 showing maximum composition at R_f 0.16 and 0.40 respectively (Fig.3). It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the drug. This can be considered as valuable standards in pharmacopoeia. At 254 nm, eleven spots appear at R_f 0.12, 0.16, 0.24, 0.31, 0.41, 0.52, 0.66, 0.71, 0.74, 0.79 and 0.92 (All brown) (Fig.2) with various concentrations while at 366 nm, eleven spots appears at R_f , 0.16 (blue), 0.23 (yellow), 0.24 (blue), 0.40 (yellow), 0.62 (yellow), 0.63 (blue), 0.65 (light green), 0.71 (yellow), 0.73, (blue) 0.76 (blue) and 0.91 (blue) (Fig.4). This is a vital finger print parameter to ensure the reliability and reproducibility of drug.

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.10	106.0	0.12	9.5	4.39	0.13	111.4	214.9	1.78
2	0.13	111.7	0.16	36.3	16.78	0.19	97.1	1580.8	13.11
3	0.23	98.2	0.24	8.1	3.76	0.26	95.7	257.7	2.14
4	0.29	100.1	0.31	16.6	7.69	0.32	108.7	462.0	3.83
5	0.35	112.1	0.41	63.7	29.49	0.46	89.2	6120.1	50.76
6	0.51	93.8	0.52	11.0	5.09	0.53	97.3	303.7	2.52
7	0.64	96.4	0.66	7.4	3.41	0.66	98.4	160.5	1.33
8	0.70	103.4	0.71	11.7	5.41	0.72	104.5	361.5	3.00
9	0.73	105.8	0.74	9.2	4.27	0.75	101.3	167.9	1.39
10	0.79	99.6	0.79	4.7	2.20	0.80	101.4	51.1	0.42
11	0.85	104.2	0.92	37.8	17.51	0.94	136.8	2376.7	19.71

Fig 1: HPTLC densitogram of methanolic extract of *Oxystelma esculentum* scanned at 254 nm by using chloroform: methanol: butanol (7: 2: 1 v/v)

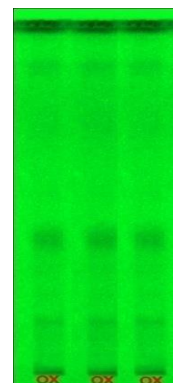
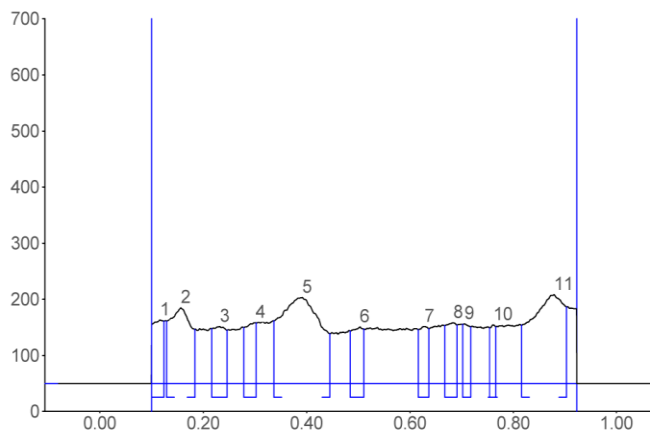
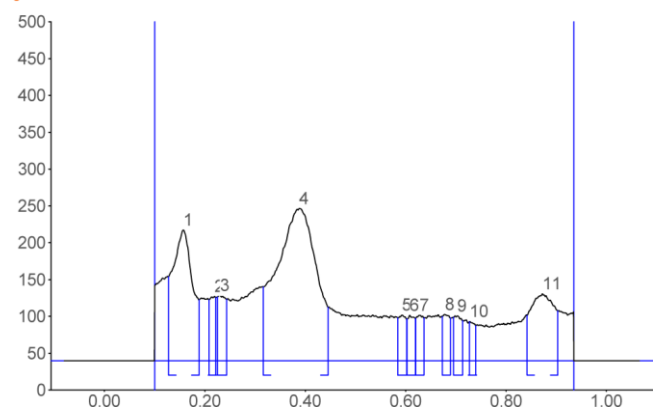


Fig 2: High performance thin layer chromatography image of *Oxystelma esculentum* at 254 nm in chloroform: methanol: butanol (7: 2: 1 v/v)

Track 7, ID: 7_OE



Track 7, ID: 7_OE



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.13	114.0	0.16	88.0	26.50	0.20	82.8	3965.7	17.49
2	0.22	82.9	0.23	7.0	2.10	0.23	86.0	105.2	0.46
3	0.23	86.8	0.24	9.4	2.82	0.25	83.6	217.4	0.96
4	0.33	100.3	0.40	139.4	41.96	0.46	72.8	15155.0	66.83
5	0.61	58.3	0.62	7.4	2.24	0.63	56.6	142.1	0.63
6	0.63	56.8	0.63	7.8	2.36	0.65	57.4	136.8	0.60
7	0.65	57.6	0.65	9.0	2.72	0.66	58.1	188.0	0.83
8	0.70	60.3	0.71	12.6	3.80	0.72	58.1	276.2	1.22
9	0.73	59.3	0.73	11.8	3.56	0.74	55.0	276.8	1.22
10	0.76	53.1	0.76	5.5	1.65	0.77	47.7	75.7	0.33
11	0.88	62.0	0.91	34.2	10.31	0.94	67.9	2138.0	9.43

Fig 3: HPTLC densitogram of methanolic extract of *Oxystelma esculentum* scanned at 366 nm by using chloroform: methanol: butanol (7: 2: 1 v/v)



Fig 4: High performance thin layer chromatography image of *Oxystelma esculentum* at 366 nm in chloroform: methanol: butanol (7:2:1 v/v)

Discussion

According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.^[21] *Oxystelma esculentum* (L.f.) Sm. is an important medicinal plant belonging to the family Apocynaceae. Stomata are amphistomatic with anomocytic type were recorded^[22, 23]. Besides tetracytic have also been presently observed. Knee-shaped covering trichome and collapsed-cell covering trichome reported^[24]. But presently unicellular conical hair observed both surfaces Mesophyll differentiated into palisade and spongy tissues. Palisade parenchyma present below the upper epidermis consists of continuous single layered cells^[22]. But presently 2 layered palisade observed beneath the upper epidermis; cells columnar, cylindrical arranged with small intercellular spaces and interspersed with sphaero crystalliferous idioblasts^[22]. Spongy

parenchyma loosely arranged with crystalliferous idioblasts This is presently confirmed. The midrib consists of a single layered collenchymatous hypodermis and bicollateral vascular bundle was reported earlier^[24]. But presently parenchyma 5-8 celled thickness which is also observed on either sides with small intercellular spaces and interspersed with sphaerocoryne stelliferous idioblasts. petiole epidermis is single layered and consists of rectangular cells followed by two layers of collenchyma and two layers of chlorenchyma cells^[22]. But presently one layered chlorenchyma confirmed^[24]. Review of earlier literature reveals that, there is very less information on the leaf, petiole anatomy and preliminary phyto-chemical and HPTLC finger printing of *O. esculentum*.

Conclusion

The powder microscopic features and organoleptic characters along with the anatomical and HPTLC fingerprinting are the diagnostic to establish the pharmacopoeial standards for the drug

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