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Pharmacognostic and Hptlc Studies on *Oldenlandia Umbellata*

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Abstract

Oldenlandia umbellata belongs to family Rubiaceae has been traditionally used as remedy against asthma and bronchitis. It is a small annual plant native to India and grows in tropical climate. Pharmacognostic studies and HPTLC profiles were carried out in the present study to ensure the authenticity and quality of *oldenlandia umbellata*. Microscopic observation of leaf of *Oldenlandia umbellata* revealed that the leaf is provided with the paracytic type of stomata. There are more number of stomata are present (46.3 ± 2.5) in the lower surface of the leaf whereas, no stomata were present on the upper surface. Transverse section of stem of *Oldenlandia umbellata* consist of outer most layers called Epidermis. Epidermis bears unicellular epidermal hairs. The transverse section of root consists of outer most layer of cork cells (Bark) followed by cork cambium. The HPTLC separation was achieved using N Hexane: Ethyl Acetate: Acetic Acid: formic acid (60:40:2.5:2.5) as the mobile phase. The ethanolic extract of *oldenlandia umbellata* showed sixteen different Rf values, of which the compound having Rf value 0.43 constitute more than 16% of total extract.

Keywords: *oldenlandia umbellata*, Rubiaceae, HPTLC, stomata

Introduction

Plants have been used in treating human diseases for thousands of years. The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines. Healing with medicinal plants is as old as mankind itself [1]. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today, for our various ailments. [2]

The drugs are derived either from the whole plant or from different parts like leaves, stem, bark, root, flower and seed. Some drugs are prepared from excretory plant products such as gums, resins and latex. World Health Organization has shown great interest in documenting the use of medicinal plant used by tribes from different part of the world [3]. The world is endowed with a rich wealth of medicinal plants. These plants are a local heritage with global importance. [4] The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, terpenoids, phenolic compounds and glycosides. [5]

With the emerging worldwide interest, in adopting traditional practices, in the healthcare systems by exploiting their potential, the evaluation of the botanicals in these systems of medicine in India is utmost important. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage but also to rationalize the use of natural products in the healthcare. Standardization is system to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect [6].

The genus *Oldenlandia* (family Rubiaceae) consists of different species, many of which are used in traditional medicine. *Oldenlandia umbellata* is commonly known as Indian madder. It is a low growing plant native to India and commonly found in parts of India (Coromandel coast), Burma, Sri Lanka, Cambodia and Indonesia. The plant is well-known in Siddha Medicine for its styptic property. It is also a drug that can be administered for bronchial asthma, as a decoction of the entire plant [7] The leaves are reported to act as febrifuge, expectorant, and also used in consumptive, and asthmatic affections [8] The aerial parts of the plant is claimed to possess antibacterial activity. It inhibits tumour growth, modulates immune activity, stimulates reticulo endothelial system, phagocytosis and augment macrophage oxidative burst [9] The present research work is focused with microscopical evaluation and HPTLC profiles studies.

Materials and methods

Collection and identification of plant material: The plant *oldenlandia umbellate* was collected in fresh condition as the healthy plant from Tirunelveli District of Tamilnadu and was identified and authenticated by Dr. V. Chelladurai (Research Officer–Botany, Tirunelveli, Tamilnadu). The voucher specimen of the plant was deposited in the Institute's herbarium for further reference.

The fresh plant parts (leaf, stem and root) were used for the study of macroscopic and microscopic characters. Macroscopic study was performed for various parameters. Free hand transverse sections of leaf, stem and root were studied for different microscopic characters. The fresh plant material was air dried at room temperature and were pulverized into coarse powder for easy extraction and great penetration of solvents to dissolve the active constituents present inside the cell.^[10] The powdered material was extracted with ethanol by using soxhlet apparatus and the extract was concentrated to dryness and stored in dessicator for further studies. TLC and HPTLC studies were carried out with the extract. HPTLC studies were carried out in CAMAG Automatic TLC Sampler 4 (ATS4). Stationary phase used was TLC plates silica gel 60 F 254 with plate size 5 x 10 cm (E.

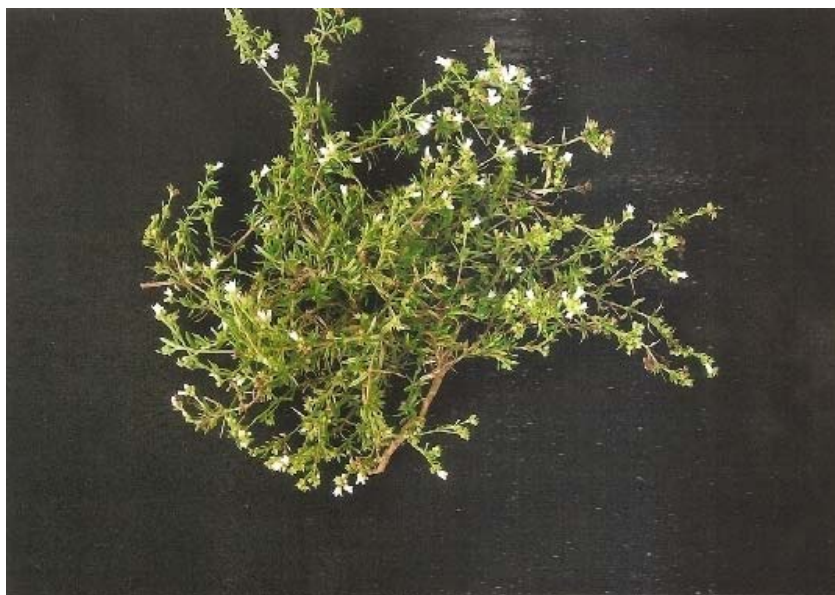
MERCK KGaA) The mobile phase used to perform this studies is N Hexane: Ethyl Acetate: Acetic Acid: formic acid (60:40:2.5:2.5) The samples (4, 6, 8 mcg/L) were applied to the plate 8mm band length using Camag 100mcg/L sample syringe (Hamilton, Bonaduz, Switzerland), with an automated camag TLC applicator Linomet 5 with N₂ flow. CAMAG twin trough glass tank (20 x 10cm) was used for the HPTLC plates development. The plate was scanned using Camag scanner TLC scanner 3 with scan speed 10mm/sec.

Result and discussion

Plant morphology

Macroscopy: Leaves are simple, entire and opposite, lanceolate, base decurrent, subsessile, stipules hairy, flowers 3-4mm long bisexual and actinomorphic, white 3-12 in umbellate cymes, petals, minutely hairy stamens 4, capsules 2-3mm across globose. Calyx is mostly somewhat reduced and 4-5 lobed. Sympetalous corolla is mostly 4-5 lobed occasionally with 3 or upto 10 lobes. The androecium consists of as many stamens as corolla lobes and is alternate to corolla tube.

Plant profiles



Oldenlandia Umbellata

Microscopic observation of leaf of *Oldenlandia umbellata* revealed that the leaf provided with the paracytic type of stomata. There are more number of stomata are present (46.3 ± 2.5) in the lower surface of the leaf whereas, no stomata were present on the upper surface. The stomata measures about $32.0 \pm 0\mu\text{m}$ in length and $18.25 \pm 1.25\mu\text{m}$ in breadth. Transverse section of the leaf revealed that the epidermal cells consist of straight anticlinal walls.

Unicellular trichomes are also present. Beneath every epidermal cell there are about 4.6 ± 0.1 palisade parenchyma cells are present, they are dark green and tightly packed and are multi layered. Below the palisade parenchyma there are loosely arranged spongy parenchyma cells are present. Vascular bundle cells are present in the midrib region of the leaf. The epidermal cells present in the lower surface are smaller when compare to the epidermal cells present on the upper surface. The epidermal cells of upper surface are very larger than any another cells. Some needle crystals were also noticed in leaf. The quantitative leaf parameters listed in table no 1 and T.S. was shown in figure 1 &2.

Transverse section of stem of *Oldenlandia umbellata* consist of outer most layers called Epidermis. Epidermis bears unicellular epidermal hairs. Epidermis is followed by hypodermis and parenchymatous cortex, these cells arranged without any intercellular spaces. Cortex is followed by single layer of endodermis. Phloem cells followed this endodermis, then xylem consisting of multi layer of cells and can be distinguished into proto-xylem (towards periphery) and meta-xylem (towards center). At the center, pith is present. Some needle like structures were present in the pith. The T.S. was shown in figure 3.

The transverse section of root of *Oldenlandia umbellata* consists of outer most layer of cork cells (Bark) followed by cork cambium. Epiblema is followed the cork cambium and is consists of parenchyma cells tightly packed without intercellular spaces. Epiblema followed by hypodermis and cortex. Cortex cells consists of parenchyma cells, which are arranged without cellular spaces, and these cells consists of chloroplasts so they appeared as green. Endodermis encloses the vascular bundles and is represented by a single layer of

cells. Phloem surrounded the xylem. Phloem represented by 5-10 layers of closely packed cells which is followed by the xylem consisting of multi layer of cells and can be distinguished into proto-xylem (towards periphery) and meta-

xylem (towards center). At the center, ground tissue is present. Ground tissue consists of some circular and needle like granules. The T.S. was shown in figure 4.

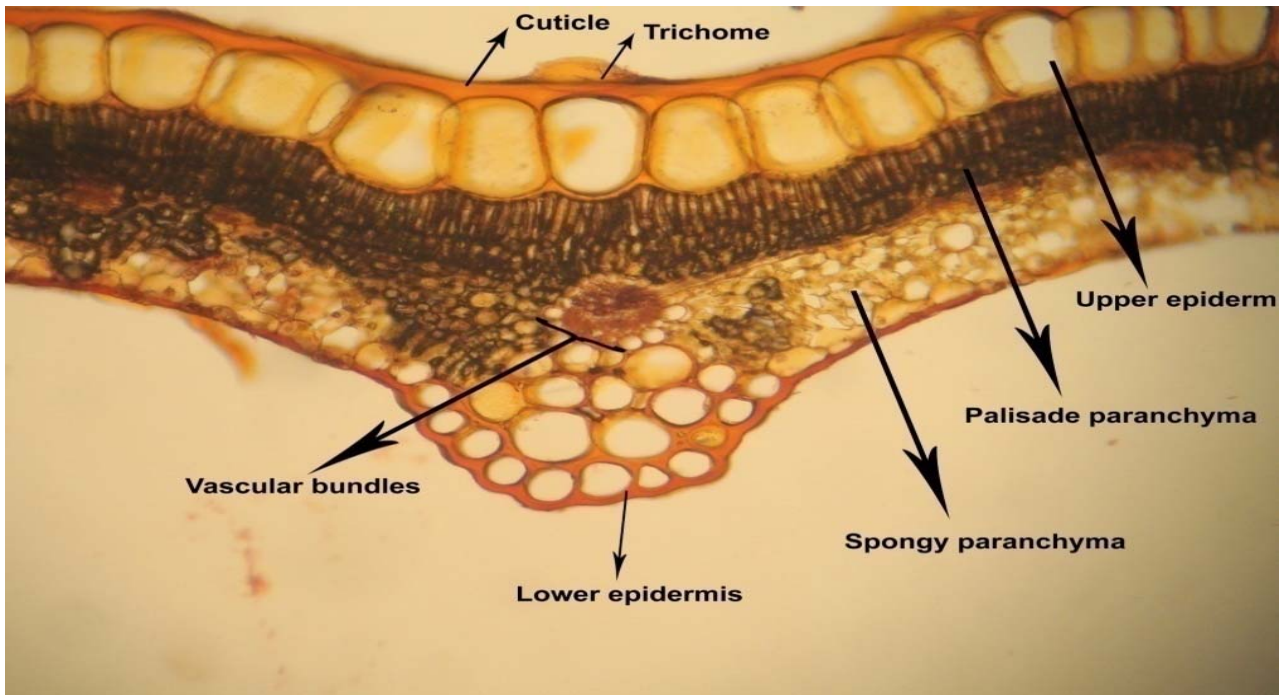


Fig 1: T.S. Of *Oldenlandia Umbellata* (Leaf)

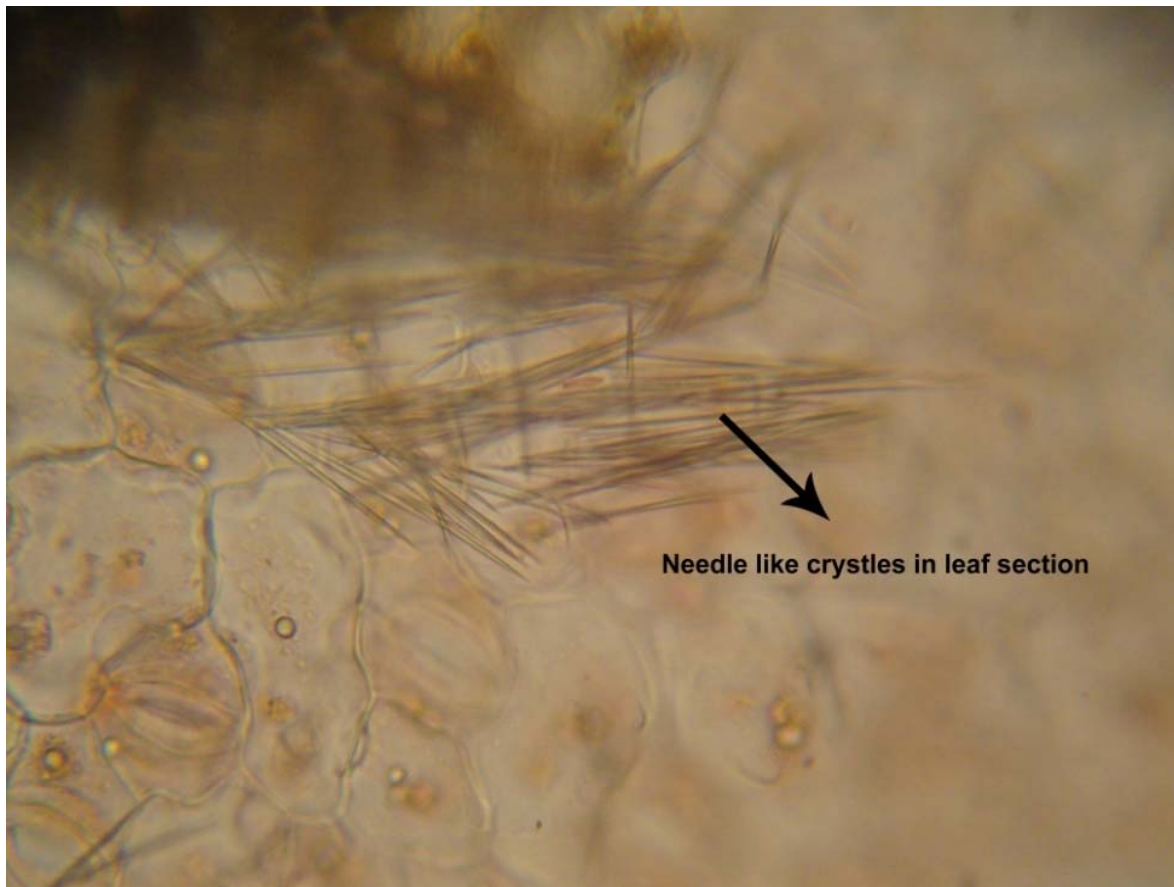


Fig 2: Needle like Crystals in Leaf Section of *Oldenlandia Umbellata*

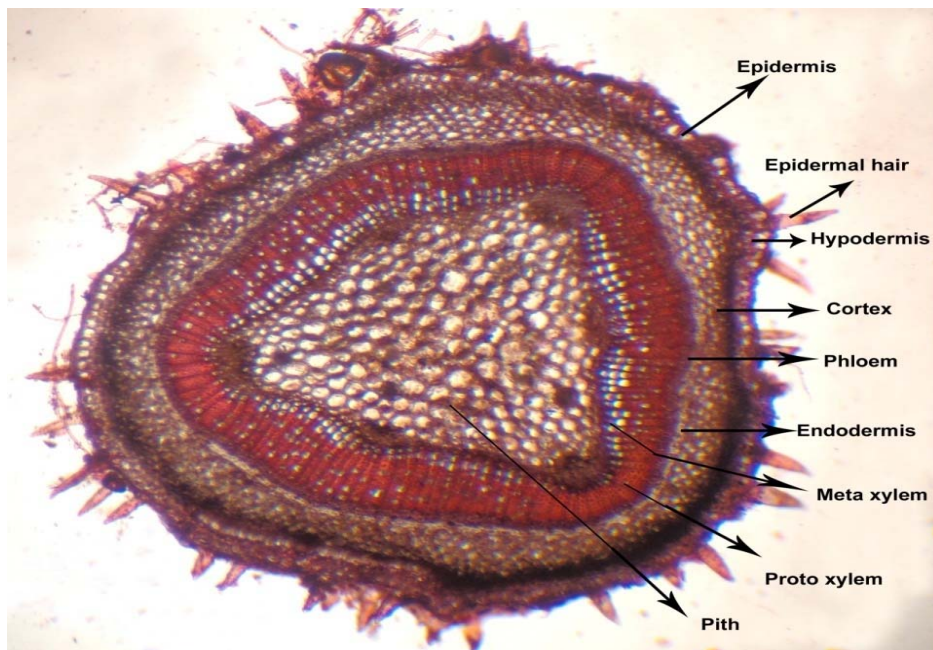


Fig 3: T.S. of *oldenlandia umbellata* (STEM)

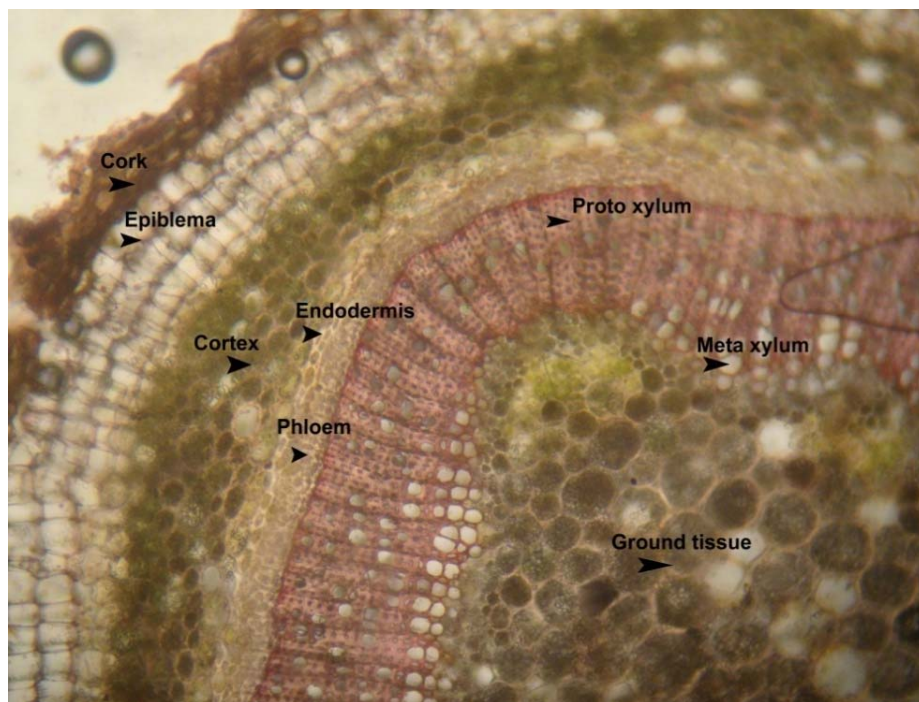


Fig 4: T.S. of *oldenlandia umbellata* (root)

Table 1: Quantitative leaf parameters of *Oldenlandia umbellata*

Parameters	Range	Mean
Palisade ratio	3.38 - 5.81	4.6 ± 0.1
Stomata number Upper surface	0	0
Stomata number lower surface	43.63 - 48.96	46.3 ± 2.5
Stomata index upper surface	0	0
Stomata index lower surface	45.58 - 49.28	47.43 ± 0.67
Vein islet number	0	0
Vein termination number	0	0
Epidermal cells Upper surface	50.32 - 57.47	53.9 ± 1.5
Epidermal cells lower surface	48.54 - 54.05	51.3 ± 1.5
Stomata length	24.65 - 39.34	32.0 ± 0.5
Stomata Breadth	15.12 - 21.37	18.25 ± 1.25

Note: Stomatal no. Epidermal cells, vein islet, termination counts per 1 mm square area (1mm²), Stomata length in μm.

The coarse powder was extracted with ethanol by continuous hot percolation using soxhlet apparatus until the extraction was completed. Then the extract was filtered and solvent was removed by distillation under reduced pressure. [11, 12]

HPTLC Profiles: Determination of various phytoconstituents were carried out by HPTLC is summarized in fig 5 at 254nm and 366nm. The R_f value of constituent present in this extract is tabulated in table no 2.

The ethanolic extract of *oldenlandia umbellata* shows the presence of sixteen different compounds, of which the compound having R_f value 0.43 constitute more than 16% of total extract.

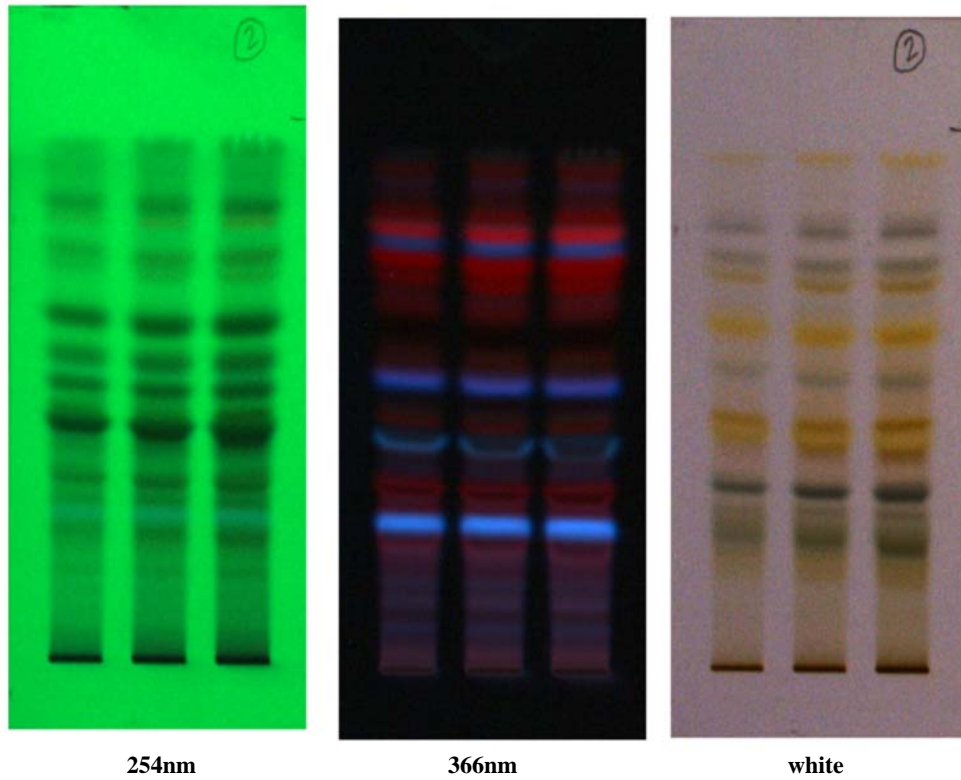
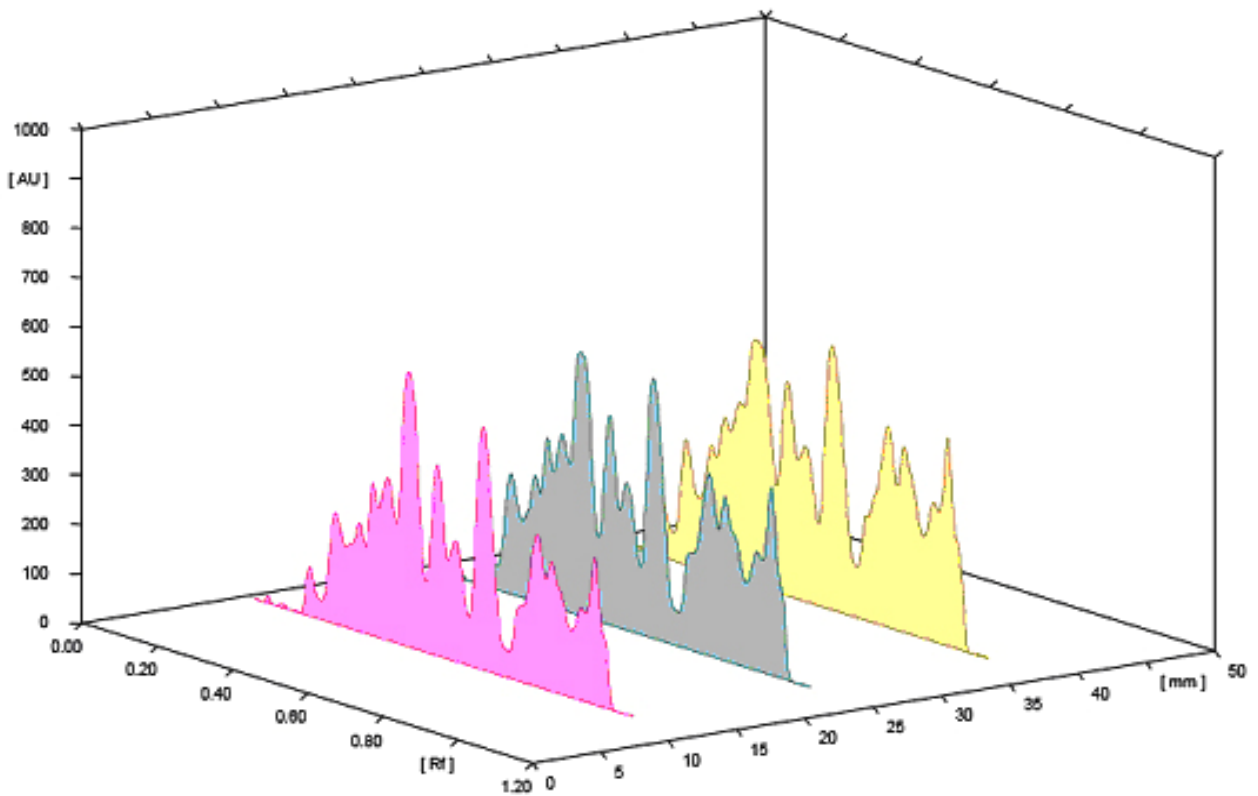


Fig 5: HPTLC finger print of EEOU

EEOU--- Ethanolic extract of *oldenlandia umbellata*



Spectra 2: Spectral representation of HPTLC finger print of EEOU

EEOU---Ethanolic extract of *oldenlandia umbellata*

Track 1, ID:

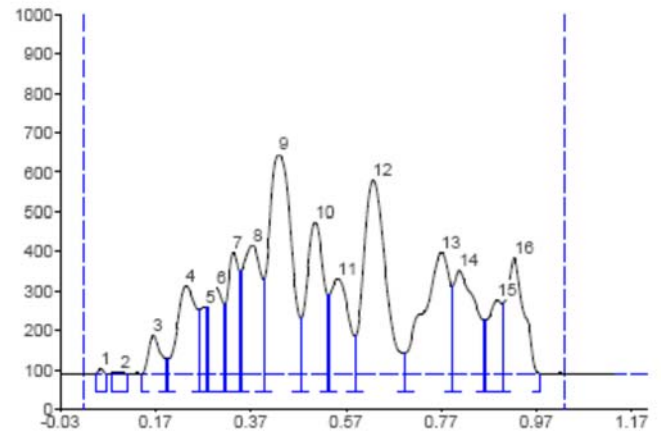
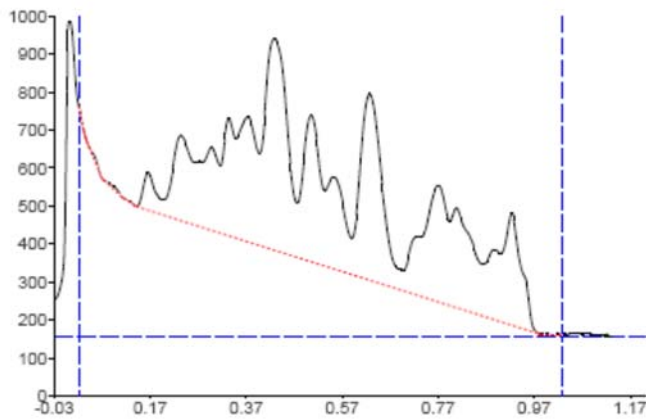


Table 2: Tabular representation of HPTLC finger print of EEOU Track 1

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	0.1	0.06	15.1	0.37	0.07	0.5	128.1	0.10
2	0.08	0.5	0.10	6.7	0.16	0.11	0.1	91.6	0.07
3	0.15	0.2	0.17	99.8	2.43	0.20	38.2	2143.5	1.61
4	0.20	38.4	0.24	224.4	5.47	0.27	163.3	7483.7	5.63
5	0.27	163.8	0.28	172.2	4.19	0.28	172.0	2033.3	1.53
6	0.28	172.4	0.30	219.5	5.35	0.32	175.4	5303.9	3.99
7	0.32	176.6	0.34	308.3	7.51	0.35	259.9	6307.8	4.74
8	0.35	261.7	0.38	328.2	7.99	0.40	241.3	10512.1	7.90
9	0.40	243.5	0.43	556.3	13.55	0.48	142.5	22107.5	16.62
10	0.48	143.3	0.51	385.9	9.40	0.54	198.6	11779.9	8.86
11	0.54	199.3	0.56	243.1	5.92	0.59	96.3	7828.4	5.89
12	0.59	96.6	0.63	491.7	11.98	0.70	53.1	19234.5	14.46
13	0.70	53.5	0.78	308.1	7.50	0.80	223.1	14216.2	10.69
14	0.80	224.1	0.81	263.2	6.41	0.87	135.8	10082.4	7.58
15	0.87	136.0	0.89	186.8	4.55	0.90	179.0	4485.9	3.37
16	0.90	179.1	0.93	296.2	7.21	0.98	0.6	9263.4	6.96

Conclusion

In the present study, the pharmacognostic characters and HPTLC profiles of *oldenlandia umbellata* were carried out, which are beneficial for the better assessment of authentic and purity of the drug. As per HPTLC studies there are sixteen different compounds present in the ethanolic extract of *oldenlandia umbellata*. Further investigations are necessary for the identification and isolation of individual compound.

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