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Pharmacognostic Studies and HPTLC Fingerprint Profile of Stem of *Oroxylum indicum (L) Vent: A* Threatened and Vulnerable Medicinal Plant.

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ABSTRACT

Oroxylum indicum (L) Vent. is found both in the Himalayas and Western ghats. It is extensively used in the Indian system of medicine and is one of the important Rasayana drugs mentioned in ayurveda. Proper identification of the plant is requisite for obtaining its complete therapeutic effects. The present investigation has been carried out on stem of *Oroxylum indicum* (L) Vent. used in various ayurvedic preparations. The study deals with Pharmacognostic and development of HPTLC fingerprint of stem. The Pharmacognostic studies include macroscopic, microscopic characters and proximate analysis. HPTLC fingerprint was developed using Toluene:Ethyl Acetate:Formic Acid as solvent system. In conclusion the macroscopic and microscopic characters, Physicochemical determination and HPTLC fingerprints can be used as a diagnostic tool in the identification of *Oroxylum indicum* (L) Vent and also will be helpful for standardization and quality control of the precious indigenous drug. The study scientifically validates the use of the plant in traditional medicine.

Keywords: *Oroxylum indicum* (L) Vent., Pharmacognostic studies, HPTLC fingerprint profile.

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INTRODUCTION

Oroxylum indicum (L) Vent (Bignoniaceae) is a medicinally important medium sized deciduous tree distributed in India, Sri lanka, China, Thailand, Philippines and Indonesia. Its distribution in India is restricted to Eastern and Western Ghats and North East regions. It is a small to medium sized deciduous tree, up to 12 m in height, branched at top, bark light brown, soft and often with numerous corky lenticels. The plant has been used as a single drug or as a component of certain poly herbal drug preparations in Indian Ayurvedic system of medicine [1]. The existence of *Oroxylum indicum* (L) Vent. in natural population is highly threatened and it has been categorized as vulnerable medicinal plant by the Govt. of India [2] .Various parts of this plant are utilized for medicinal purposes. *Oroxylum indicum* (L) Vent. finds place in preparation of Dashmula which is used as an astringent, anti-inflammatory, anti-helminthic, anti-bronchitic, anti-leucodermatic, anti-rheumatic, anti anorexic and for treatment of leprosy and tuberculosis. [3,4] . The stem bark of this plant is reported to contain flavonoids namely Chrysin, Oroxylin A, Baicalein. Baicalein is reported to possess an anti inflammatory [5], anti ulcer [6], antioxidant [7] , hepatoprotective [8] and immuno modulatory [9] activity while Chrysin and Baicalein both are reported to have antibacterial, antifungal and antiviral activity [10,11]. Literature survey revealed that information on stem bark in terms of Pharmacognostic studies and phytochemical evaluation by HPTLC is minimal. With this background the current study is aimed on structured features of stem (young and mature) and also on chemical fingerprinting using HPTLC. This would help the standardization and quality control of this precious indigenous drug.

MATERIAL AND METHODS

Plant Material

The stem was collected form Dispur, Assam and was identified and authenticated at the Blatter herbarium, St. Xavier's College, Mumbai (Accession No 54436). The stem was then dried in shade, pulverized and stared in our tight container for further study.

Pharmacognostic studies

Pharmacognostic studies i.e. macroscopic and microscopic studies for evaluation of the drug for confirmation of its identity, determination of quality and purify and detection of adulteration was done using fresh material. For microscopic studies fixation was carried out in a solution of formaldehyde, glacial acetic acid and ethanol.

Physicochemical Studies

Physicochemical studies such as ash values (Total ash, Acid Insoluble ash, Water soluble ash & Sulphated ash) were carried out as per standard methods of [12]. Extractive values of different solvents were determined.

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HPTLC Analysis

HPTLC of the Ethanolic extract was carried out. Solvent system of Toluene:Ethyl Acetate:Formic Acid (15:5:1) was used for plate development and profile was obtained and Rf values were recorded. The HPTLC analysis was performed on aluminum plates pre-coated with Silica Gel 60 F 254 (Merck, Germany). Extract was applied on the plate with the help of CAMAG Linomat - IV sample applicator. The plates were developed in a CAMAG twin trough chamber, previously calibrated with a mobile phase for 20 mins. Each plate was developed up to 8 cm, air dried and scanned at wavelength of 254 & 366 nm using CAMAG TLC scanner 3. The chromatograms were recorded. The plates were then derivatized with 10% Methanolic KOH and heated at 105º C on hot plate, till the development of colour bands and observed under white light. The colour of recorded bands and Rf values were recorded [13-15].

RESULT AND DISCUSSION

Microscopic features

The anatomy of *Oroxylum indicum* (L) Vent. stem shows normal development (Plate 1). Young stem reveals primary structure consisting of single layered epidermis followed by 2-3 layered collenchymatous hypodermis (Plate 2). Cortex shows development of patches of stone cells. Eustele shows conjoint, collateral, open and exarch vascular bundles. The central region is occupied by parenchymatous pith. Normal secondary growth is evident in mature stem. The inter fascicular cambium joins with fascicular cambium to form a ring of cambium which gives rise to secondary phloem and xylem. Traces of rushed primary phloem are clearly seen under the stone cells.

Physicochemical properties

Physicochemical constants like moisture content, Total ash, insoluble ash, water soluble extractive and alcohol soluble extractive determined and summarized in Table 1 and 2. This would help in substantiate standardization of *Oroxylum indicum* (L) Vent.

Table 1.Quantitative Standards of stem of *Oroxylum indicum* (L) Vent.

Sr.no	Parameter	Value %
1.	**Ash Values**	
	a)Total ash	18.00
	b)Acid insoluble ash	2.5
	c)Water soluble ash	5.11
	d)Sulphated ash	19.02
2.	**Extractive values**	
	a)Alcohol soluble extractive	12.45
	b)Petroleum ether soluble extractive	1.05
	c)Water soluble extractive	27.48
3.	**Loss on Drying**	20.02
4.	**Crude Fibre content**	27.30

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Plate 1: Transverse section of stem of *Oroxylum indicum* (L) Vent.

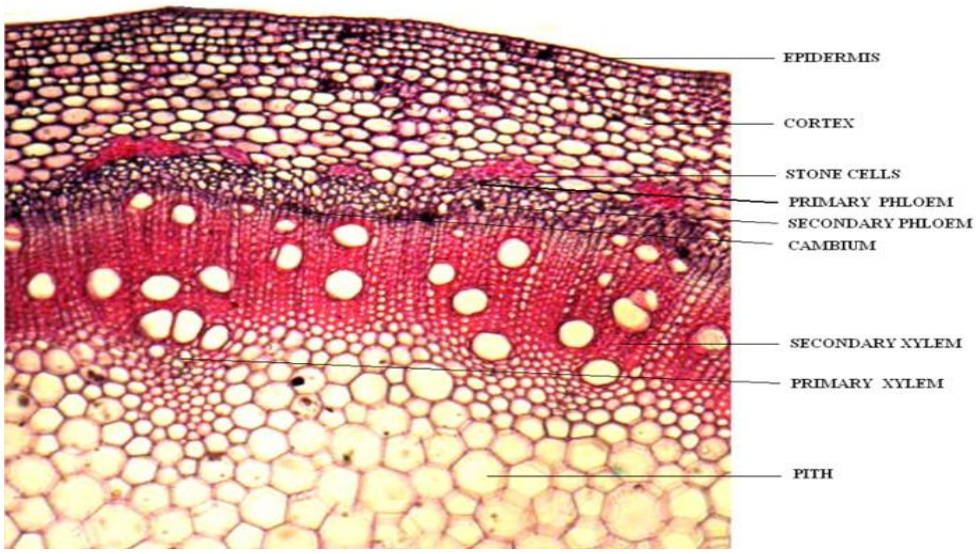
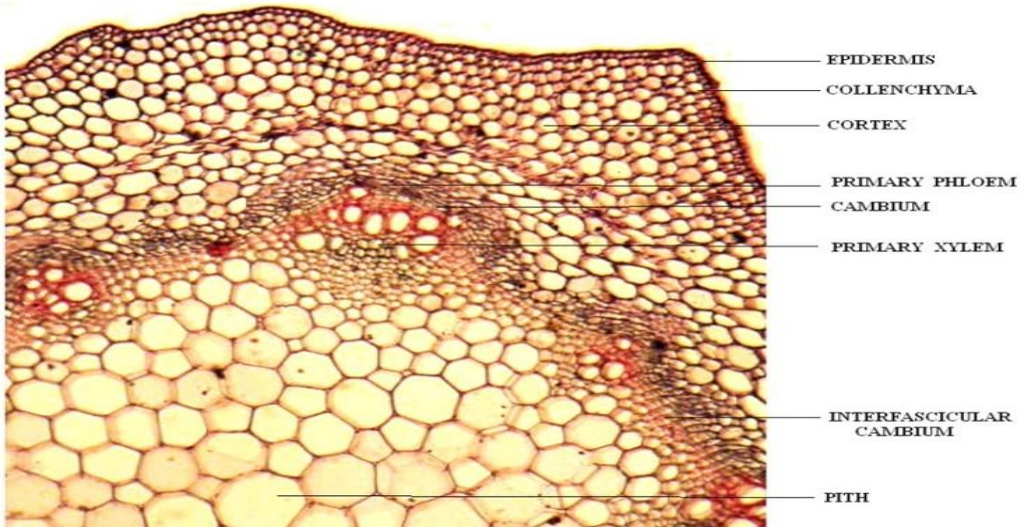


Table 2: Percentage yield in successive solvent extraction of stem of *Oroxylum indicum* (L) Vent.

Sr.no	Extract	% Yield
1.	Petroleum ether extract	0.65%
2.	Ethyl Acetate extract	3.15%
3.	Methanol extract	3.54%
4.	Aqueous extract	20.30%

Plate 2 : Transeverse section of young stem of *Oroxylum indicum* (L) Vent.



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HPTLC Fingerprint profile

HPTLC fingerprint profile of stem of *Oroxylum indicum* (L) Vent. in ethanolic extract is seen in Plate no. 3, densitometric scan (Figure 1 and 2) and Rf values in Table 3. HPTLC fingerprint shows seperation of 10 different bands. Bands are better visualised after derivatisation with 10% Methanolic KOH.

Table 3: Rf values of HPTLC fingerprint of stem of *Oroxylum indicum* (L) Vent.

Track 5, ID: 5

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.04 Rf	3.2 AU	-0.02 Rf	767.3 AU	22.43 %	-0.01 Rf	18.4 AU	12789.7 AU	9.53 %
2	-0.01 Rf	723.1 AU	-0.00 Rf	772.6 AU	22.59 %	0.03 Rf	44.6 AU	21505.8 AU	16.02 %
3	0.03 Rf	544.8 AU	0.04 Rf	558.8 AU	16.34 %	0.14 Rf	44.6 AU	28423.2 AU	21.17 %
4	0.15 Rf	144.7 AU	0.20 Rf	283.6 AU	8.29 %	0.27 Rf	23.8 AU	17025.3 AU	12.68 %
5	0.28 Rf	123.9 AU	0.33 Rf	212.3 AU	6.21 %	0.42 Rf	33.6 AU	14461.0 AU	10.77 %
6	0.51 Rf	42.2 AU	0.55 Rf	60.2 AU	1.76 %	0.56 Rf	57.6 AU	1828.4 AU	1.36 %
7	0.59 Rf	71.0 AU	0.66 Rf	115.8 AU	3.39 %	0.71 Rf	55.4 AU	7890.4 AU	5.88 %
8	0.72 Rf	56.0 AU	0.77 Rf	85.1 AU	2.49 %	0.79 Rf	31.3 AU	3715.2 AU	2.77 %
9	0.84 Rf	114.0 AU	0.92 Rf	564.8 AU	16.51 %	1.00 Rf	4.3 AU	26595.8 AU	19.81 %

Figure 1: Densitometric scan of stem of *Oroxylum indicum* (L) Vent.

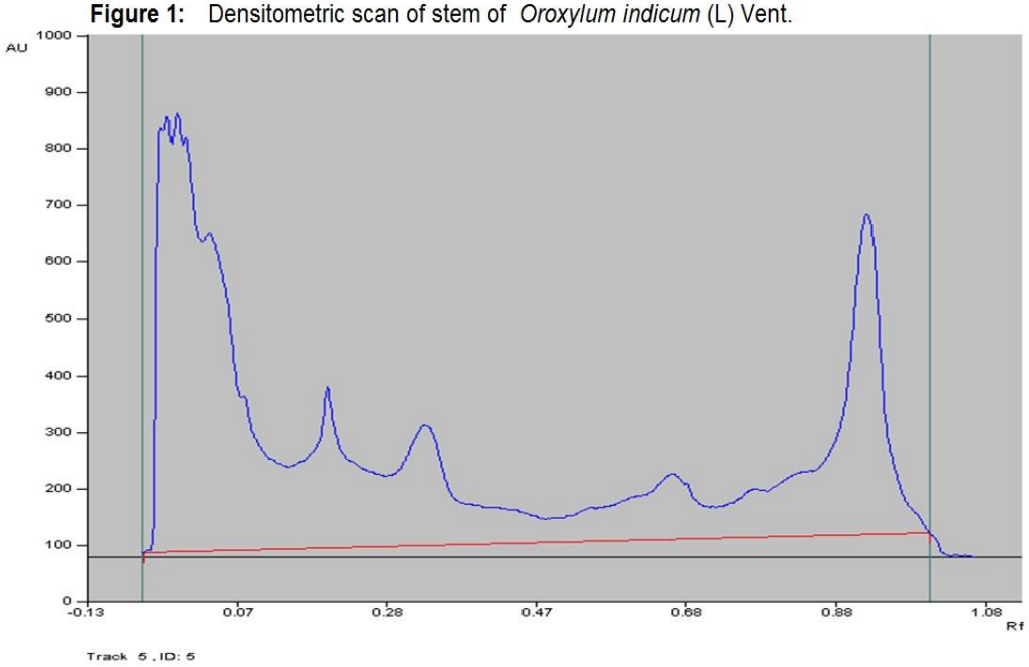

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Figure 2: Densitometric scan of stem of *Oroxylum indicum* (L) Vent.

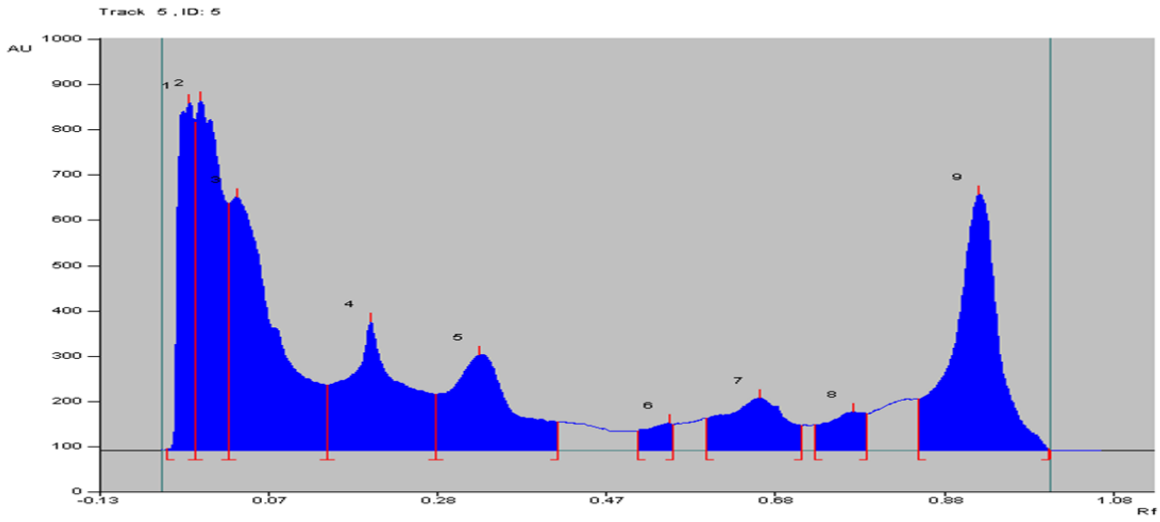

Plate 3: HPTLC fingerprint profile of ethanolic extract of *Oroxylum indicum* (L) Vent.

CONCLUSION

The present study attempts a comprehensive investigation of stem bark of *Oroxylum indicum* (L) Vent. Since the whole plant of *Oroxylum indicum* (L) Vent. is utilised for various medicinal purposes it is of utmost importance to distinguish the various plant parts, hence standardisation in terms of pharmacognistic and phytochemical studies is the need of the hour. HPTLC fingerprint developed under the above mentioned conditions can be used for authentication and standardisation of the stem of *Oroxylum indicum* (L) Vent. collected from Eastern Himalayas. The seperated bands can be further used for structural elucidation and

serve as new lead molecules for drug development. Development of such a monograph would pave the way for standardization of formulations of *Oroxylum indicum* (L) Vent.

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