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Pharmacognostical and phyto-chemical study of *Paribhadra twak* (*Erythrina indica* Lam.)

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Abstract
Paribhadra (*Erythrina indica* Lam.) is one of the easily available drugs in Ayurveda belonging to Leguminaceae family. It is a thorny deciduous tree growing up to 60 feet height. The present work includes an exploration of morphology, qualitative, quantitative microscopy and phytochemical screening of stem bark of *Erythrina indica* Lam. Transverse section of mature bark showed the presence of stratified and lignified cork cells; secondary cortex consists of large, tangentially elongated parenchymatous cells, calcium oxalate crystals, circular stone cells, parenchymatous cells surrounding the stone cells and large crystals of calcium oxalate. The powder microscopy revealed the presence of calcium oxalate crystals, cork cells, strongly lignified phloem fibers, stone cells, calcium oxalate crystals and starch grains.
Phytochemical analysis showed the presence of phytoconstituents like carbohydrates, flavonoids, alkaloids, tannins and phenolics. HPTLC finger printing technique was performed.

Keywords: *Erythrina indica*, paribhadra, twak, pharmacognosy, phytochemical, HPTLC etc.

Introduction
Paribhadra, widely known as "Coral tree" is one of the important drugs mentioned in Ayurveda. *Erythrina indica* Lam. is a medium-sized, spiny, deciduous tree normally reaching up to 60 feet height [1]. *Erythrina indica* Lam. is widely distributed in tropical and subtropical regions. It is well known for various medicinal properties. The description of *Paribhadra* have been described in Ayurvedic texts and various Nighantus like Saushruta Nighantu, Ashtanga Nighantu, Madhava Dravyaguna, Raja Nighantu, Kaiyadeva Nighantu, Madanapala Nighantu and Bhavaprakasha Nighantu. Sushruta mentioned the usage of *Paribhadra* in the preparation of *Kshara* [2], in the treatment of *Udakameha* [3], *Krimi* [4], *Vata* vyadhi [5] and as *Ksharagada* in *Visha* [6]. Its bark is pale brown in colour, smooth and has white or yellow lines on it. *Paribhadra* is very useful in treating the diseases like liver disorders, fever and rheumatism. It contains alkaloids and flavonoids as a major phytochemical constituent. These alkaloids possess neuromuscular blocking, smooth muscle relaxant, CNS depressant, hydrocholeretic and anticonvulsant action [7].
In traditional medicine various parts of *Erythrina indica* Lam. has been used. The bark is anti-bilious, febrifuge and useful as a collyrium in ophthalmia. Collyrium is used in watery eye, tinea-tarsi, and purulent conjunctivitis [8]. The stem bark decoction is used in dysentery and worms.

Materials and Methods
Collection of stem bark
The stem bark of *Erythrina indica* Lam. was collected from The Dakshina Kannada district of Karnataka. The barks were dried under shade, finely powdered and stored in airtight containers. This powder was used for physico-chemical study and phyto-chemical analysis.

1. Pharmacognostical study
a) **Macroscopic features**: Morphology of fresh stem bark of *Erythrina indica* Lam. was observed and organoleptic characters of stem bark were noted.
b) **Microscopic features:** Transverse sections of fresh bark were taken and stained by using appropriate stains (saffranine), observed under electronic microscope [9].
c) **Powder microscopy:** The powder microscopy was done by using glycerin, phloroglucinol and concentrated Hydrochloric acid and observed under electronic microscope.

2. Physico-chemical study
Stem bark powder was subjected to various physical and chemical analysis. Physical properties like - moisture content, p^H value, foreign matter, total ash, acid insoluble ash, water soluble ash, water soluble extractive value, aqueous extractive value, ethyl alcohol extractive value, petroleum ether extractive value, chloroform extractive values were performed.

3. Preliminary phyto-chemical study
The powder was extracted with 50ml each of water, ethyl alcohol, petroleum ether and chloroform for 30 minutes at 50°C. Various phytoconstituents present in the bark were detected by their respective chemical tests using the appropriate extracts. Preliminary phytochemical screening was done for the evaluation of organic compounds like carbohydrates, starch, proteins, amino acid, steroids, flavonoids, glycosides, saponins, alkaloids, tannins and phenolics. The powdered drug was made into ash and diluted with distilled water, boiled and filtered. The solution was tested to know the presence of inorganic compounds like calcium, magnesium, sodium, potassium, iron, sulphates, phosphates chlorides and carbonates.

4. Chromatographic study
Using H.P.T.L.C finger print profile - 1g of powder was extracted with 10 ml of alcohol. 5, 10µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate 7:1. The developed plates were visualized in UV 254, 366, under white light and then derivatized with vanillin Sulphuric acid and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded.

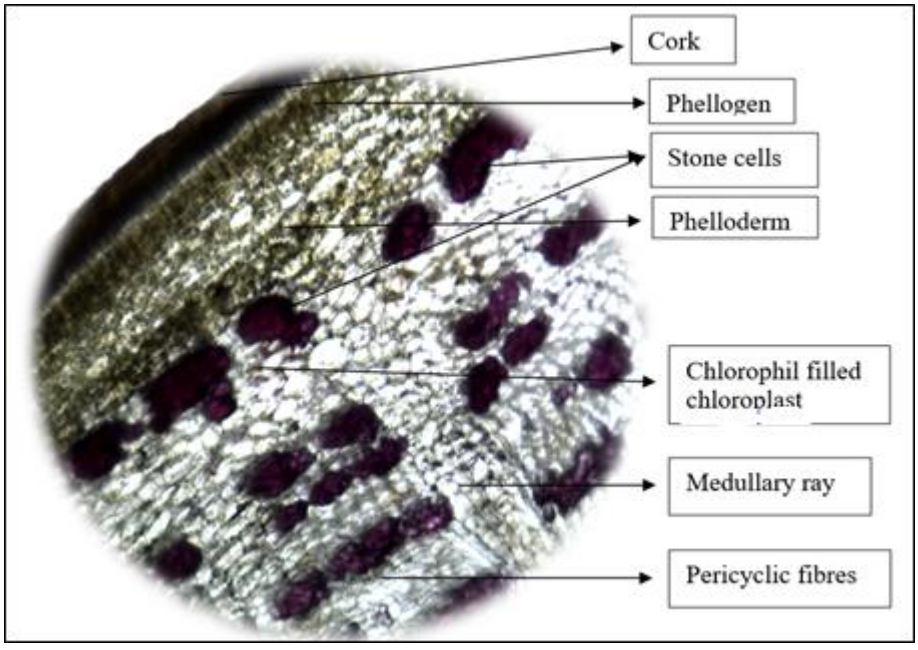
Results
1. Pharmacognostical study
a) Macroscopic study

Bark - outer view

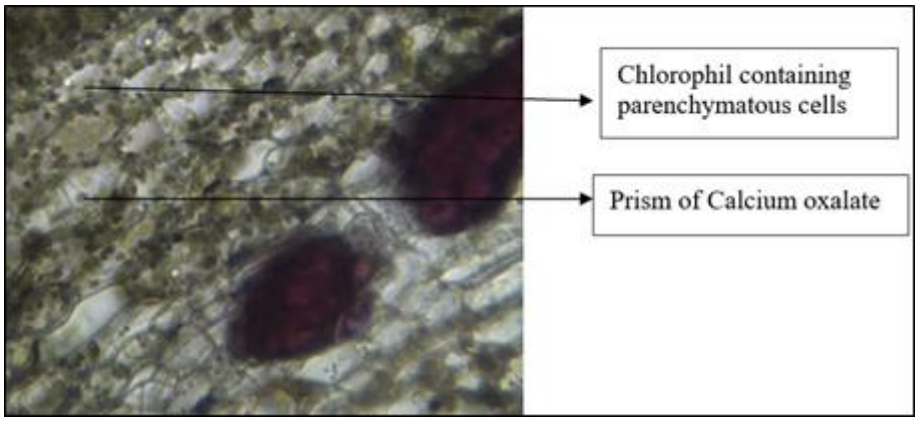
Bark - inner view

The stem bark of *Erythrina indica* Lam. was studied macroscopically in the Dravyaguna laboratory of KVGAMC, Sullia. Mature dried stem bark about 0.5-2.0 cm thick, smooth, exfoliating in narrow strips; outer surface yellowish to yellowish-grey, lenticels found at short intervals, longitudinal lines on the outer surface, yellowish to cream coloured; whole bark differentiated into outer non-fibrous and inner fibrous zones, outer bark breaks readily with a short fracture, inner bark fibrous [9].

b) Microscopic study [10]
The transverse sections (T.S) of bark of *Erythrina indica* Lam. were taken and photomicrography was done after proper mounting and staining.



Photograph No. 01



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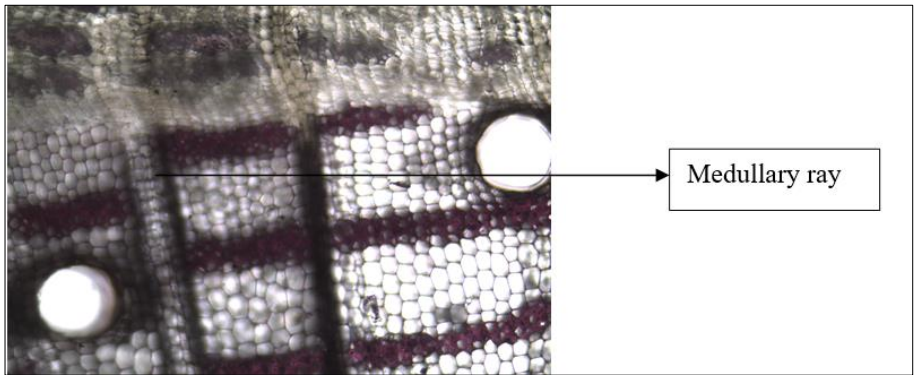

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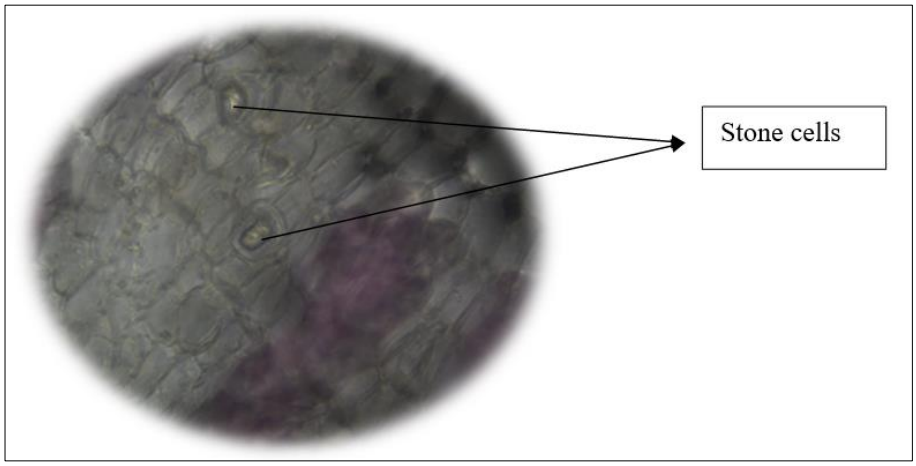


Photograph No. 04

The outermost tissue is the cork, which is light cream in colour, is composed of about 4-5 narrow bands of tangentially elongated compressed cells that alternate with single layer of tangentially elongated wider cells. The narrower cells are slightly lignified and extend up to 3-4 layers. The radially broader cells often contain a single prism of calcium oxalate and are rectangular to polygonal in shape & often contain some brownish material.

Inner to cork is phellogen consisting of a single row of thin walled, somewhat rectangular cells. This is followed by the phelloderm which is composed of 5-6 rows of thin-walled chlorophyll containing parenchymatous cells. Some of the phelloderm cells contain prismatic calcium oxalate crystals.

Secondary cortex consists of large, tangentially elongated to polygonal, parenchymatous cells. A few cells contain prismatic crystals of calcium oxalate, stone cells occur in singles or in groups which are circular, elongated or rectangular in shape. Parenchymatous cells surrounding stone cells groups contain large crystals of calcium oxalate.

Secondary phloem consisting of sieve tubes with their companion cells, phloem fibres and phloem parenchyma traversed by phloem rays. Phloem fibers arranged in tangential strips alternating with the regular thin-walled phloem elements, sieve elements in outer and middle regions of phloem mostly get collapsed and crushed and form many tangential strips of ceratenchyma between the tangential groups of phloem fibers; fibers are large, thick-walled with narrow lumen; crystal fibers numerous, septate and each chamber contains a single prismatic crystals of calcium oxalate; phloem parenchyma thin-walled, a few of them contains crystals of calcium oxalate similar to those found in the secondary cortex and crystal fibres; phloem rays numerous and mostly multiseriate running almost straight in the inner phloem region but bent towards left or right in the

outer phloem region; ray cells thin-walled, radially elongated in the inner region and slightly tangentially elongated towards outer region.

c) Powder microscopy [11]

Powder microscopy of *Erythrina indica* Lam. was done in Care Keralam, Thrissur.

Photograph 5: Powder microscopy

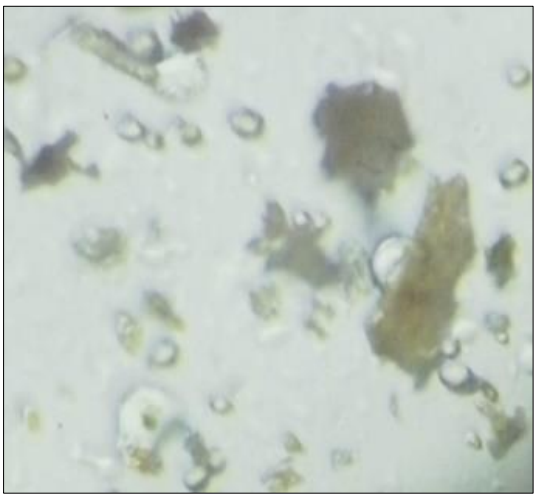


Fig 1: Calcium oxalate crystals

Fig 2: Cork cells of polygonal shape

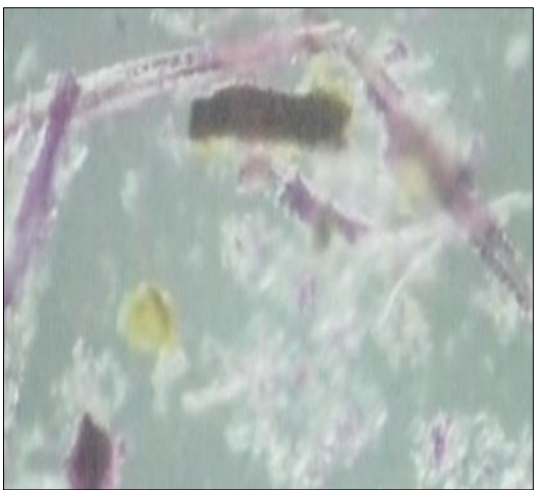


Fig 3: Phloem fibers strongly lignified and with simple pores, stone cells in groups

Fig 4: Prismatic crystals of calcium oxalate

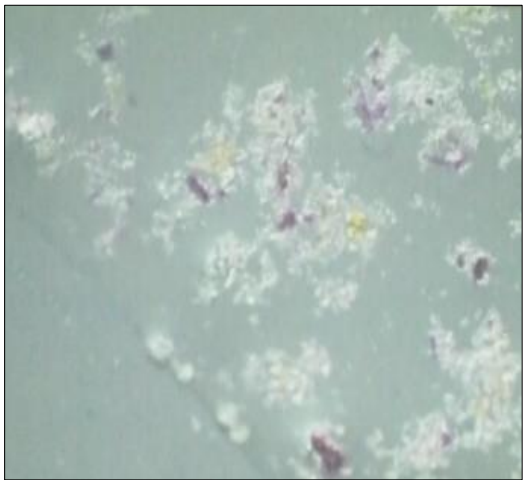


Fig 5: Starch grains

2. Physico-chemical study

The powder was extracted with 50ml each of methanol and water for 30 minutes at 50°C. The following phytoconstituents were detected in the stem bark powder:

1) Organoleptic evaluation [12]

Organoleptic characteristics of stem bark

- Color: Pale brown
- Odour: Characteristic
- Taste: Astringent
- Texture: Rough
- Shape: Recurved

2) Physical evaluation [13-17]

Table 1: Showing the results of physical analysis of stem bark

Parameters	Values
Moisture content % at 105° C	8.7%
Total Ash value	5.2%
Acid insoluble ash	2.7%
Water soluble ash	1.8%
Water soluble extractive	10.8%
Alcohol soluble extractive	7.6%
Petroleum ether Extractive value	1.1%
Chloroform extractive value	3.2%
Total % of foreign matter	Nil
p^H	5.12
Loss on drying	0.85%

3) Chemical evaluation

Preliminary phytochemical screening [18, 19]

The results obtained after the phytochemical analysis of the aqueous, petroleum ether, ethyl alcohol and Chloroform extracts of *Erythrina indica* Lam. bark were as follows:

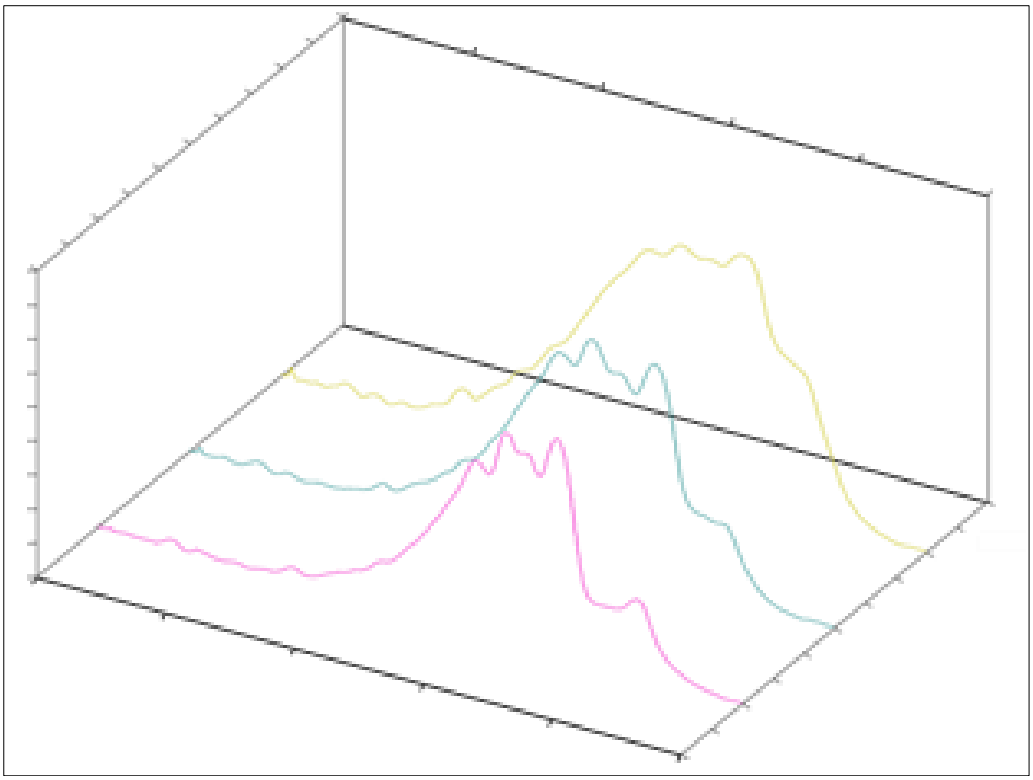
Table 2: Showing results of chemical analysis of stem bark powder: Organic compounds.

SL No	Tests		Aqueous	Ethyl Alcohol	Petroleum Ether	Chloroform
1	Carbohydrate	Molisch's	-ve	+ve	+ve	-ve
		Fehling's	-ve	+ve	-ve	-ve
		Benedict's	-ve	+ve	+ve	-ve
2	Starch	-ve		-ve	-ve	-ve
3	Proteins	Biuret test	-ve	_ve	-ve	-ve
4	Amino-acids	Ninhydrin test	-ve	-ve	-ve	-ve
5	Steroids	Salkowski's reaction	-ve	-ve	-ve	-ve
6	Flavanoids	Shinoda test	-ve	-ve	-ve	-ve
		Alkaline reagent test	+ve	-ve	-ve	-ve
		Lead acetate test	+ve	-ve	+ve	-ve
7	Glycosides	Keller killiani test	-ve	-ve	-ve	-ve
8	Saponins	Foam test	-ve	-ve	+ve	-ve
9	Alkaloids	Mayer's test	-ve	-ve	-ve	+ve
		Hager's test	-ve	-ve	-ve	+ve
		Wagner's test	-ve	-ve	-ve	-ve
		Dragendroff's test	-ve	-ve	-ve	+ve
10	Tannins and Phenolics	5% $FeCl_3$	+ve	+ve	-ve	-ve
		Lead acetate	+ve	+ve	-ve	-ve

Table 3: Showing results of chemical analysis of bark powder: Inorganic compounds:

Inorganic constituents	Result
Calcium	-
Magnesium	-
Sodium	+
Potassium	-
Iron	-
Sulphates	+
Phosphates	+
Chlorides	-
Carbonates	+

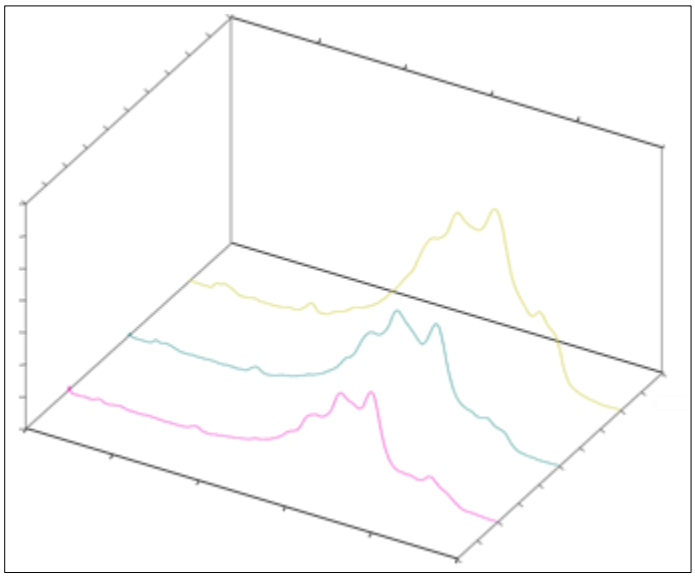
High performance thin layer chromatography of *Erythrina indica* lam. Stem bark [20]



Photograph 6: HPTLC Densitometric scan at UV 254 nm

Table 4: RF table 254nm

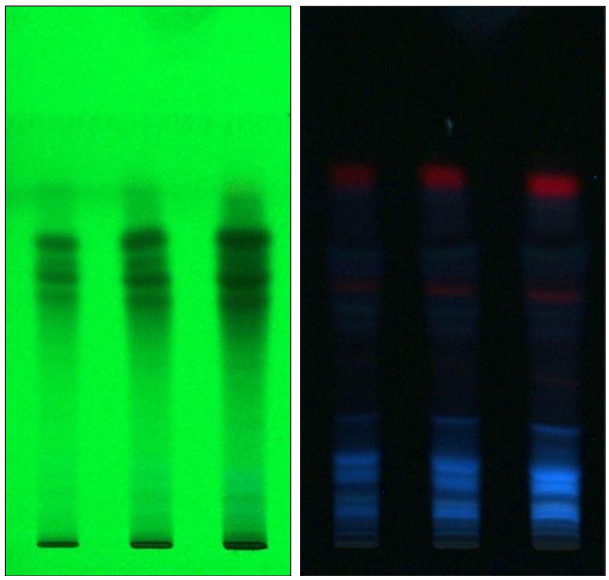
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	0.3	0.11	20.7	0.80	0.14	0.0	356.4	0.34	Unknown *
2	0.14	0.2	0.15	12.9	0.50	0.18	2.8	180.4	0.17	Unknown *
3	0.24	0.2	0.26	10.3	0.40	0.27	7.9	153.2	0.15	Unknown *
4	0.27	8.1	0.30	38.1	1.47	0.32	23.0	891.5	0.85	Unknown *
5	0.44	127.4	0.59	497.1	19.20	0.60	466.6	34763.1	33.10	Unknown *
6	0.61	466.7	0.63	603.4	23.30	0.66	553.1	19956.6	19.00	Unknown *
7	0.66	553.4	0.66	559.0	21.59	0.69	502.1	11299.8	10.76	Unknown *
8	0.69	504.5	0.71	626.9	24.21	0.78	172.9	26587.8	25.32	Unknown *
9	0.80	174.8	0.84	220.9	8.53	0.94	8.6	10837.4	10.32	Unknown *



Photograph 6: HPTLC Densitometric scan at UV 366 nm

Table 5: RF table 366 nm

Peak	Rf	Height	Rf	Height	%	Rf	Height	Area	%	Assigned substance
1	0.05	0.0	0.07	14.4	1.38	0.08	8.8	175.5	0.30	Unknown *
2	0.08	8.8	0.09	13.1	1.26	0.11	4.1	236.0	0.41	Unknown *
3	0.26	3.1	0.30	25.2	2.43	0.32	10.6	562.5	0.98	Unknown *
4	0.36	24.0	0.57	241.9	23.34	0.58	237.9	16609.8	28.82	Unknown *
5	0.58	238.2	0.62	332.1	32.04	0.68	250.5	20519.4	35.60	Unknown *
6	0.68	250.9	0.71	326.7	31.52	0.79	89.6	15899.4	27.59	Unknown *
7	0.81	75.8	0.83	83.2	8.03	0.93	1.7	3629.9	6.30	Unknown *



254 nm 366 nm

Photograph 7: High Performance Thin Layer Chromatography.

Discussion

Macroscopic study of the fresh stem bark of *Erythrina indica* Lam. is greyish in colour externally and on exfoliating it is greenish in colour. Dried stem bark is pale brown in colour. It is 0.5-2.0 cm in thickness. Stem bark has longitudinal wrinkles externally. It has characteristic pleasant odour.

Microscopic studies showed the presence of outer cork, which is light cream in colour. It is composed of alternate bands of tangentially elongated compressed cells and wider cells. Compressed cells are lignified, where as broader cells contain prism of calcium oxalate. Inner cork is having layers of phellogen and phelloderm. Phelloderm composed of chlorophyll containing parenchymatous cells, which contains prismatic calcium oxalate crystals. Secondary cortex consists of prismatic crystals calcium oxalate, stone cells, phloem fibers and phloem parenchyma.

The preliminary phytochemical screening of stem bark showed the presence of organic compounds like carbohydrates, flavonoids, saponins, alkaloids, tannins and phenolics.

Flavonoids, saponins and tannins are having antioxidant, anti-inflammatory actions and helps to prevent cell damage. Saponins are having immunostimulant, hypocholesterolemia and anticarcinogenic properties. Alkaloids are having anti-inflammatory and cardioprotective actions in the body. Inorganic compounds like sodium, sulphates, phosphates and carbonates are present.

Various studies have proven as *Erythrina indica* Lam. is having anti-microbial, analgesic, anti-inflammatory, anti-bacterial, anti-oxidant, anti-helmintic properties.

Conclusion

The present work deals with the organoleptic, macroscopical, microscopical and phytochemical evaluation of the stem bark of *Erythrina indica* lam. The morphological features of bark were observed and noted. Microscopy, powder microscopy, physical analysis, preliminary phyto-chemical analysis, HPTLC of stem bark were assessed for the proper identification of the sample.

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