PHARMA SCIENCE MONITOR
AN INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF STEM BARK *ERYTHRINA INDICA* LAM.

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ABSTRACT

The present study was aimed at pharmacognostic and preliminary phytochemical evaluations of *Erythrina indica lam.* Stem bark belonging to family Leguminosae. *E. indica* is a native of costal forest communities from east Africa, through southeast to Australia. In India it is distributed in coast forests from Mumbai to Malabar and in the Andaman's and Nicobars. This tree common in Bengal and many parts of India especially in southern often grown in gardens. Bark is used in fever, liver ailment and rheumatism, nervine sedative activities. The pharmacognostic investigations were carried out in terms of organoleptic, microscopic and physical parameters. The dried root powder was subjected to successive Soxhlet extraction using petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. These solvent extracts were subjected to a preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins and phenolic compounds. The phytochemical evaluation revealed the presence of carbohydrates, alkaloids, flavonoids, Phenolic, tannins.

Keywords: Erythrina indica, Leguminosae, organoleptic, microscopic and phytochemistry.

INTRODUCTION

Erythrina indica (Leguminosae.) lam

Erythrina indica also known as Indian coral tree or tropical coral tree or tiger's clow or Moochy wood tree or variegated coral tree, Pangara (Marathi) Paribhadra (Sanskrit), Dadap (Hindi).[1,2] *Erythrina indica* is a compact shrub with knobby stems. It posses dense clusters of deep crimson flowers, that spread broadly open. *E. indica* is a medium-sized, spiny, deciduous tree normally growing to 6-9 m (occasionally 28 m) tall. Young stems and branches are thickly armed with stout conical spines up to 8 mm long, which fall off after 2-4 years rarely, a few spines persist and are retained with the corky bark. Bark smooth and green when young, exfoliating in papery flakes, becoming thick,

corky and deeply fissured with age. Leaves trifoliate, alternate, bright emerald-green, on long petioles 6-15 cm, rachis 5-30 cm long, prickly; leaflets smooth, shiny, broader than long, 8-20 by 5-15 cm, ovate to acuminate with an obtusely pointed end. Leaf Petiole and rachis are spiny.

Flowers in bright red to scarlet erect terminal racemes 15-20 cm long. Stamens slightly protruding from the flower. Fruit a cylindrical torulose pod, green, turning black and wrinkly as they ripen, thin-walled and constricted around the seeds. There are 1-8 smooth, oblong, dark red to almost black seeds per pod. Erythrina comes from the Greek word 'eruthros' meaning red, alluding to the showy red flowers of the Erythrina species.[3, 4]

An Indian preparation said to destroy pathogenic parasites and relieve joint pain. Juice from the leaves is mixed with honey and ingested to kill tapeworm, roundworm and threadworm.[5] Women take this juice to stimulate lactation and menstruation. A warm poultice of the leaves is applied externally to relieve rheumatic joints. The bark is used as a laxative, diuretic and expectorant. Different parts of plant are used in tradition medicine as nervine sedative, collyrium, in opthalmia, anti-asthmatics, and antiepileptic, antiseptic and as an astringent. Bark is used in fever, liver ailment and rheumatism. The leaf juice used to heal wounds and sores. Leaf paste applied for muscular pain in cattle. Leaf extract possess nematicidal property. The root extract possess antimicrobial activity.[6] Bark is astringent and used as febrifuge and anthelmintic.[7]

Seeds and leaves of plant contain A new 3-phenylcoumarin, indicanine A (1), has been isolated from the root bark of the African medicinal plant *Erythrina indica*, together with three known compounds, robustic acid (2), daidzein, and 8-prenyldaidzein. Alkaloids : N-norprotosinomenine (I), protosinomenine (2), erysodienone (3), /3-erythroidine, erysopine, erythraline, erythramine, erysodine, erysotrine, erythratine, N,N-dimethyltryptophan, and hyparphorine and also it contain erythrinins A, B, C. these are recently found.[8] The structure of the new compound was characterized, as 4-hydroxy-5-methoxy-3-(4'-methoxyphenyl)-2"-(1-methylethenyl) dihydrofurano [4", 5":6, 7] coumarin by means of extensive spectroscopic analyses.[9]

MATERIAL AND METHODS

Procurement of Plant Material

The Bark of *Erythrina indica* have been collected from the Satpuda Platue of Nandurbar (Maharashtra). The plant is authentificated by Dr. Santosh Tayade, Dept. of Botany, Art's, Science and Commerce College, Lonkheda, Shahada, Dist-Nandurbar (M. S). The voucher specimen has been preserved in the laboratory for future reference in the Dept. of Pharmacognosy and Phytochemistry, College of Pharmacy, Shahada. The collected plant material was air dried and used for the study of macroscopic and microscopical characters. Finally dried pieces of bark were subjected to size reduction to get coarse powder and then passed through sieve no. 40 to get uniform powder. Then uniform powder was subjected for the determination of ash values, extractive values, and loss on drying, fluorescence analysis and phytochemical constituents.

PHARMACOGNOSTIC EVALUATION

Organoleptic Evaluation

In organoleptic evaluation, various sensory parameters of the plant material, such as color, odour, taste, shape and texture of the stem bark were recorded and shown in table no.1.

Microscopical investigation

Transverse Section of *Erythrina indica* stem bark

The transverse section of the bark was taken to observe microscopic characteristics like cork, phelloderm, phloem, xylem etc. The characteristic are shown in microscopic pictures as fig. no.1.

Powder Analysis

To a little quantity of powder taken onto a microscopic slide, 1–2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added, mounted in glycerol, covered with a cover slip and observed under microscope with 10 × 10 magnification. The characteristic features of the powder viz., vascular tissues, xylem fibers, calcium oxalate crystals, starch grains, stone cells etc. were recorded using standard techniques.[10,11,12,13,14] Lignified cells, fibers and stone cells appear pink in color. Presence of starch grains was detected by the formation of blue color on addition of 2 - 3 drops of 0.01M iodine solution.

PHYSICAL EVALUATION

In physical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were determined. [10,11,12,13,14,15]

DETERMINATION OF ASH VALUES [16]

A) Determination Total Ash Value

Accurately weighed (2 gm) of air-dried bark powder of *Erythrina indica* was taken in a silica dish and incinerated at a temperature not exceeding 450°C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

B) Determination of Acid Insoluble Ash

The total ash obtained from 2g of bark powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

C) Determination of Water Soluble Ash

The total ash obtained from 2g of bark powder was boiled for 5 minutes with 25 ml of water; the insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450ºC. The weight of insoluble matter was subtracted from the weight of the ash, the difference in weight represent the water-soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug. The results were given in table no.3.

EXTRACTIVE VALUES [17]

A) Water-soluble extractive value

Accurately weighed (5 gm) of stem bark powder of *Erythrina indica* was added to 50mlof boiled water at 80°C in a stoppered flask. It was then shaken well and allowed to stand for 10 minutes so as to cool it and filtered. 5ml of filtrate was transferred to an evaporating dish, which was 7.5 cm in diameter, the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven for 2 hours at 100°C and residue was weighed. Percentage of water-soluble extractive was calculated with reference to the air-dried drug.

B) Determination of Alcohol Soluble Extractive Value

Accurately weighed powder (5 g) of stem bark powder of *E. indica* was taken and macerated with 100 ml of 95% alcohol for 24 h. The contents were frequently shaken during the first 6 h and allowed to remain for 18 h. After 24 h, the extract was filtered and 25 ml of the filtrate was evaporated. The extract was dried at 105°C to a constant weight.

C) Chloroform soluble extractive value

Accurately weighed (5 gm) of stem bark powder of *E. indica* was macerated with 100 ml of chloroform in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precaution against loss of Chloroform. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage chloroform soluble extractive was calculated with reference to the air-dried drug.

D) Petroleum ether (40-60°C) soluble extractive value

Accurately weighed (5 gm) of stem bark powder of *E. indica* was macerated with 100 ml of Petroleum ether in a closed flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precaution against loss of Petroleum ether. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105°C and weighed. Percentage Petroleum ether soluble extractive was calculated with reference to the bark. The results of extractive values are given in table no. 3.

LOSS ON DRYING [18]

Accurately weighed (2 gm) quantity of bark powder was taken in a tarred glass bottle and initial weight was taken. The sample was heated at 105°C in an oven and weighed. This procedure was repeated until a constant weight was obtained. The moisture content of the sample was calculated with reference to air-dried drug and the results are in table no. 3.

FLUORESCENCE ANALYSIS [19]

The stem bark of *E. indica* (entire and powder) are examined in short and long ultraviolet radiation to detect the fluorescent compounds and report its authenticity. The results are given in table no.3.

PRELIMINARY PHYTOCHEMICAL SCREENING [20]

The stem bark powder was subjected to successive extraction in a Soxhlet apparatus using water, alcohol, chloroform and petroleum ether (40-60°C) the extracts were evaporated to dryness. The dried extracts were weighed, and percentage yields were calculated. The results are given in table no. 4. The extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., Molisch's, Fehling's, Benedict's and Barfoed's tests for carbohydrates; Biuret and Millon's tests for proteins; Ninhydrin's test for amino acids; Salkowski and Liebermann-Burchard's reactions for steroids; Borntrager's test for anthraquinone glycosides; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoid glycosides; Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids; and ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics.

RESULTS:

In the present study the stem bark of *Erythrina indica* Linn was evaluated for its pharmacognostic, and phytochemical aspects which revealed the following results.

A. Pharmacognostic Evaluation

1. Organoleptic Evaluation

The results of organoleptic evaluations were given in table 1.

Table no.1 Organoleptic/Macroscopic characteristics of stem bark of *Erythrina indica* lam.

Sr. No.	Parameters	Observation of Bark
1.	Colour	Dark Brownish outside Yellowish inside
2.	Odor	Characteristic
3.	Taste	Astringent
4.	Shape	Recurved
5.	Texture	Rough

2. Microscopical Investigation:

(i) Transverse Section:

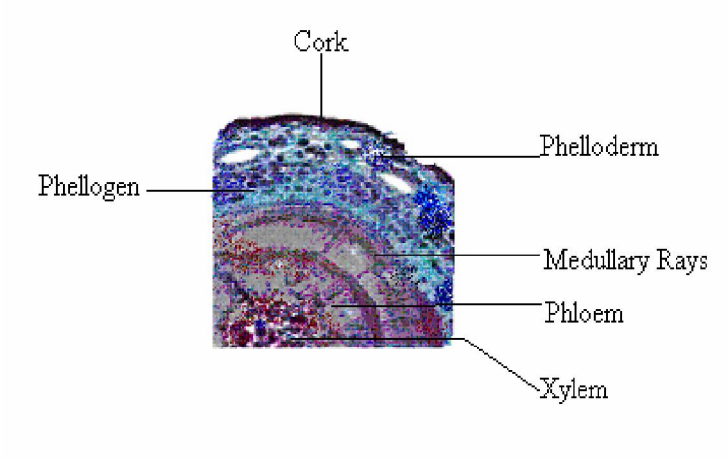

Fig.1. Transverse section of the stem bark of Erythrina Indica

The transverse section of the bark shows the typical microscopic characteristics like cork, phellogen, phelloderm, cambium, phloem, xylem etc. The characteristic are shown table no. 2.

(ii) Powder Analysis:

TABLE 2: MICROSCOPIC CHARACTERISTIC OF STEM BARK POWDER OF *ERYTHRINA INDICA* LAM.

Sr. No.	Observation of Bark powder
1.	Beaded walled epidermal cells
2.	Sclerenchymatous fibers
3.	Stone cells
4.	Calcium oxalate crystals
5.	Starch grains
6.	Lignified vessels

3. Physical Evaluation

(i) Ash values: The total ash value, acid insoluble ash value and water soluble ash value was found to be 5.2 %w/w, 2.7 %w/w and 1.8 %w/w respectively as shown in table no.3.

(ii) Extractive Values: The water soluble, Alcohol soluble, Chloroform soluble and Petroleum ether soluble extractive values were found to be 10.8 %w/w, 7.6 %w/w, 3.20 %w/w and 1.1 %w/w respectively as shown in table no.3.

(iii) Loss on drying: The loss on drying was found to be 0.85 %w/w as shown in table no.3.

(iv) Fluorescence analysis: The stem bark powder has shown Yellowish brown and dark brown fluorescence under short and long wave lengths of UV light. Shown in table no. 3

TABLE 3: PHYSICAL CONSTANTS FOR STEM BARK OF *ERYTHRINA INDICA* LAM.

Sr. No.	Physical Constants	Results
1.	Ash Values A) Total Ash B) Acid Insoluble Ash C) Water Soluble Ash	 5.2 %w/w 2.7 %w/w 1.8 %w/w
2.	Extractive Values A) Water soluble extractive B) Alcohol soluble extractive C) Chloroform soluble extractive D) Petroleum ether soluble extractive	 10.8 %w/w 7.60 %w/w 3.20 %w/w 1.1 %w/w
3.	Loss on Drying	0.85 %w/w
4.	Fluorescence Analysis A) At 254 nm (Short wave length) B) At 366 nm (Long wave length)	 Yellowish brown Dark brown

4. Preliminary Phytochemical Screening: The percentage yield of stem bark of *E. Indica* after Soxhlet extraction was found to be maximum of 8.84 % (Aqueous extract) as compared to other extracts as shown in table no. 4.

TABLE 4: PERCENTAGE YIELD OF STEM BARK OF *ERYTHRINA INDICA* LAM AFTER SOXHLET EXTRACTION

Sr. No.	Extracts	% Yield
1.	Aqueous extract	8.84
2.	Alcoholic extract	6.2
3.	Chloroform extract	2.9
4.	Petroleum ether (40-60°C) extract	0.95

Phytoconstituents present in various extracts of *Erythrina indica* stem bark.

In the present investigation all the extracts of plant was analysed for the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, sterols, phenolic compounds, tannins and flavanoids using standard procedures. The preliminary phytochemical investigation showed the presence of alkaloids, carbohydrates, amino acids, tannins, steroids, flavonoids as shown in table no.5.

TABLE 5: DATA SHOWING THE PRESENCE OF PHYTOCONSTITUENTS PRESENT IN VARIOUS EXTRACTS OF *ERYTHRINA INDICA* STEM BARK.

Sr. No	Phyto constituents	Aqueous extract	Petroleum ether extract	Chloroform extract	Ethanolic extract
1	**Alkaloids**	Absent	Absent	Present	Absent
A	Dragendroff's test	-	-	+	-
B	Wagner's test	-	-	-	-
C	Hager's test	-	-	+	-
D	Mayer's test	-	-	+	-
2	**Saponins**	Absent	Absent	Absent	Absent
A	Foam test	-	-	-	-
3	**Carbohydrates**	Present	Present	Absent	Present
A	Molish's test	-	+	-	+
B	Benedict's test	-	+	-	+
C	Fehling's test	-	-	-	+
4	**Phenolic compounds**	Present	Absent	Absent	Present
A	Ferric chloride test	+	-	-	+
B	Lead acetate test	+	-	-	+
C	Gelatin test	+	-	-	+
5	**Amino acids and Proteins**	Absent	Absent	Absent	Absent
A	Ninhydrin test	-	-	-	-
B	Millon's test	-	-	-	-
C	Biuret test	-	-	-	-
6	**Flavonoids**	Present	Absent	Absent	Absent
A	Shinoda test	-	-	-	-
B	Alkaline reagent test	+	-	-	-
C	Lead acetate test	+	+	-	-
7	**Coumarine Glycosides**	Absent	Absent	Absent	Absent

+ = Present; – = Absent.

DISCUSSION

Erythrina Indica is widely used in the traditional system of medicine for the treatment of number of diseases. The results of these investigations would helpful for proper identification, collection and investigation of the plant. The parameters determined in quantitative microscopy can be useful to differentiate closely related species. The physical constants are important parameters in detecting of drugs. The presence of various phytoconstituents can serve to treat diseases by using various pharmacological

activities. Physical standards may be used to determine the quality this plant material in future investigation. It will also helpful to carry out further research and revalidation of its use in traditional system of medicine.

CONCLUSION

The pharmacognostic parameters, which are being reported for the first time, could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in various pharmacopoeias.

ACKNOWLEDGEMENT

Authors are thankful to P. S. G. V. P. Mandal's College of Pharmacy, Shahada, Maharashtra, India, for providing all necessary facilities to carry out this research work and to Dr S. K. Tayde for authentification of plant.

REFERENCES

1. Vaidyaratnam PS: Varies, Indian Medicinal Plants A compendium of 500 species, vol II, 2006 edition, Published by Orient Longman pvt. ltd, Kotakkal, 2003, 378-381.
2. Kapoor LD: Ayurvedic Medicinal Plants, 2005 edition, published by CRC press, Delhi, 177-178.
3. Nadkarni KM: Indian Medicinal Plants and Drugs with Their Medicinal Properties and Uses, 2006 edition, published by Srishthi book distributors, Delhi, 153-154.
4. Sharma R: Medicinal plants of India An Encyclopaedia, Daya Publishing House, Delhi, 2003, 99-100.
5. Hegde N: Cultivation and uses of Erythrina variegata in western India in Westley SB and Powell MH: eds. Erythrina in the new old worlds Palahi (USA): NFRA, 11993, 77-84.
6. The Wealth of India, a dictionary of Indian raw materials and industrial products, First supplement series, National institute of science, communication and Information resources, CSIR, New Delhi, 1959, Vol I; D-I, 92-95.
7. Pullaiah T: Medicinal plants in India, Regency publication, New Delhi, Vol. I, 239.

8. Nadkarni AK: Indian Materia Medica, Vol I, 2002 edition, published by Popular Prakashan, Bombay, 508-509.
9. Nkengfack AE., Waffo AK, Azebaze GA, Fomum ZT, Meyer M, Bodo B, Van Heerden FR. Indicanine A, a new 3- phenyloumarin from root bark of *Erythrina indica*, J NAT Prod.2000; June 63(6): 855-6.
10. Ansari SH: Essentials of Pharmacognosy, (Birla Publications, Delhi, 2005–06) 207–594.
11. Goyal RK and Shah BS: Practicals in Pharmacognosy, (Nirali Prakashan, Pune, 2005) 128–155.
12. Heinrich M and Barnes J: Fundamentals of Pharmacognosy and Phytotherapy, (Churchill Livingstone, Pune, 2004) 24–29.
13. Khandelwal KR: Practical Pharmacognosy, (Nirali Prakashan, Pune, 2003) 38–161.
14. Kokate CK: Practical Pharmacognosy, (Vallabh Prakashan, Pune, 2002) 107–129.
15. Alabi DA and Alausa AA: Evaluation of the mineral nutrients and organic food contents of the seeds of Lablab purpurus, Laucaena leucocephala and Mucua utilis for domestic consumption and industrial utilization, World J Agric Sci. 2: 115–118 (2006).
16. Indian Pharmacopoeia. Vol. II, Delhi: Controller of Publication, 1996; A3: 38.
17. Indian Pharmacopoeia, Vol. I, Controller of Publication, Delhi. 1996; 209-10.
18. Indian Pharmacopoeia, Vol. I, Controller of Publication, Delhi. 1996; 651.
19. Kokate CK: Practical Pharmacognosy, New Delhi: Vallabh Prakashan; 1994: 112.
20. Khandelwal KR: Practical Pharmacognosy, Techniques and Experiments. 9th ed. Pune: Nirali Prakashan; 2002: 149-59.

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