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PHARMACOGNOSTICAL EVALUATION AND PHYTOCHEMICAL ANALYSIS OF *DELONIX REGIA* RAFIN. STEM BARK

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ABSTRACT: *Delonix regia* Rafin. belonging to family Fabaceae and subfamily Caesalpinioideae is flowering plant native to Madagascar and East Africa. *Delonix regia* reported to have anti-diarrhoeal, anti-inflammatory, antioxidant, hepatoprotective and antimicrobial activity. The present study was carried out to establish the pharmacognostical studies, physico-chemical parameters along with preliminary phytochemical screening of petroleum ether, chloroform, ethylacetate, acetone, methanol and aqueous extracts of *Delonix regia* Rafin. stem bark. The macroscopical and microscopical characteristics of drug powder were studied. The transverse section of stem bark indicated the arrangement of various cells in cork, cortex, phelloderm and pith region. The preliminary phytochemical screening of various extracts revealed the presence of carbohydrate, protein, glycosides, flavonoids, sterols, phenolic and tannin compounds. The physico-chemical parameters such as total ash, acid insoluble ash, water insoluble ash and sulphated ash (8, 2.005, 3 and 1.4 %w/w respectively), loss on drying (45 %w/w) extractive values, foaming index, swelling index and fluorescence analysis of stem bark powder were studied. These studies will be helpful to establish standards for quality, purity and sample identification of *Delonix regia* Rafin..

INTRODUCTION: *Delonix* is a genus of flowering plants in the pea family, Fabaceae and subfamily Caesalpinioideae. The name of the genus is derived from the Greek words '*delos*' meaning 'evident,' and '*onyx*' meaning "claw," that refers to the petal [1]. *Delonix*, a genus consists of two species growing in India *Delonix elata* and *Delonix regia* [2].

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Delonix regia Rafin. with an impressive range of medicinal and biological properties, has been used in the folk medicine systems of several civilizations like for the treatment of constipation, inflammation, arthritis, hemiplagia, leucorrhoea and rheumatism [3, 4]. *Delonix regia* Rafin. flower were used in dysmenorrhoea, as antibacterial, anti-inflammatory, broad spectrum antibacterial, analgesic, antimicrobial and antifungal [5, 6, 7, 8, 9].

The flowers have been used as traditional herbal remedies for gynecological disorders and they are also used as tablet binder. Seeds of *Delonix regia* contain flavonoids are used as wound healing agent in households [10, 11, 12]. For the standardization and

quality assurance purpose, the following three attributes must be verified: authenticity, purity and assays. Hence, in this work we make an attempt for the standardization of *Delonix regia* Rafin. stem bark by carrying out its pharmacognostical studies, physico-chemical parameters and preliminary phytochemical screening.

MATERIALS AND METHODS:

Plant Material: The sample of *Delonix regia* Rafin. was collected from Pehowa, District Kurukshetra Haryana in month of August, 2011. The herbarium of this plant was identified by Dr. H.B. Singh, Chief Scientist & Head, Raw Material Herbarium and Museum (RHMD), National Institute of Science Communication & Information Resources (NISCIR) New Delhi, Vide Reference no. NISCAIR/RHMD/Consult/-2011-12/1834/134 Dated - Sep 28, 2011. The bark of plant was selected for the present study. A voucher specimen of the same has been retained in the Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science & Technology, Hisar for future reference. Shade dried and pulverized bark of the plant was used for the various studies.

Chemical and Reagent: All the chemical and solvents used for the study were of analytical grade and all methods were taken from official methods.

Macroscopical Characters: The fresh and dried stem bark of *Delonix regia* were studied for their macroscopical characters such as colour, odour, taste, shape, size and texture.

Microscopical Characters: Microscopical study of *Delonix regia* stem bark in entire form and in powdered form was performed for histological evaluation. Thin transverse sections soaked in water were cut and stained with phloroglucinol and hydrochloric acid and observed under compound microscope. Photography was done by using Carl Zeiss Primostar trinocular microimaging GmbH Hingen, Germany microscope with canon photomicrograph unit[13].

Histochemical Colour Reactions: The histochemical colour reactions on the transverse section of *Delonix regia* stem bark were performed according to standard procedures reported [14, 15, 16]. The colour tests were performed for the identification of the major cell components.

Physicochemical Parameters: The physic-chemical parameters such as percentage of total ash, acid-insoluble, water soluble and sulphated ash, loss on drying, extractive values, foaming index, swelling index, crude fiber content, hemolytic activity, foreign organic matter and bitterness value were determined according to official methods for quality control of medicinal plant [17, 18, 19, 20, 21, 22].

Fluorescence Analysis: A small quantity of dried and finely powdered of *Delonix regia* stem bark was placed on grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 1-2 minutes. Then the slide was viewed in day light and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded [23, 24]. The plant material was subjected to fluorescence analysis in visible/ daylight and UV light (254 nm & 365 nm).

Preliminary Phytochemical Screening: Preliminary phytochemical screening was carried out on various extracts of *Delonix regia* stem bark powder like petroleum ether, chloroform, ethylacetate, acetone, methanol and aqueous extracts. The dried extracts were subjected to qualitative tests for identification of various plant constituents such as carbohydrates, alkaloids, glycosides, saponins, sterol and triterpenoids, flavonoids, tannins and phenolic compounds and proteins and amino acids [13, 25, 26, 27].

RESULTS AND DISCUSSION:

Macroscopical Studies: The colour of fresh bark at inner side was cream and from outer side grayish brown. The colour of dried bark from inner side was light brown and at outer side brown.

Outer bark **Inner bark**

FIG. 1: BARK OF *DELONIX REGIA* RAFIN.

The shape of fresh bark was flat and of dried bark curved. The wrinkles were present and furrows were absent. The fracture was granular shown in **Fig. 1**. Bark was tasteless and odourless.

Microscopical Characters: The transverse section of stem bark consist of cork cells and numerous layers of thin walled flat polygonal cells. Few cells were filled with reddish brown masses. The parenchyma were observed in the cortex region. Occcasional cells were filled with microcrystal of calcium oxalate and starch. Secondary cortex was composed of wide zone of compact lignified strands of stone cells interspread in this region. Stone cells were rectangular to somewhat oval, highly thickened and lignified. Stone cells bearing numerous simple pits were also noticed. Secondary phloem of sieve tubes, companion cells, phloem parenchyma and stone cells were observed. Medullary rays 2 - 4 serriate were present shown in **Fig. 2.**

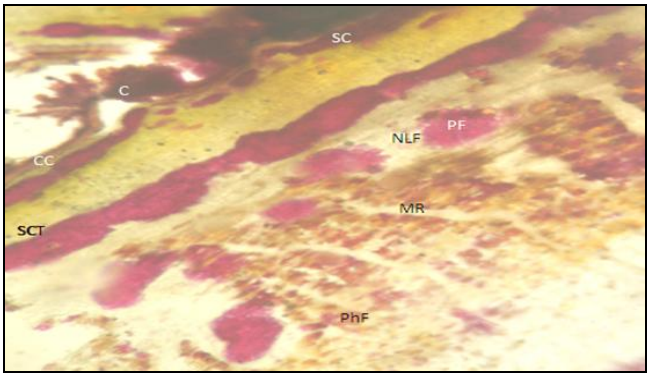
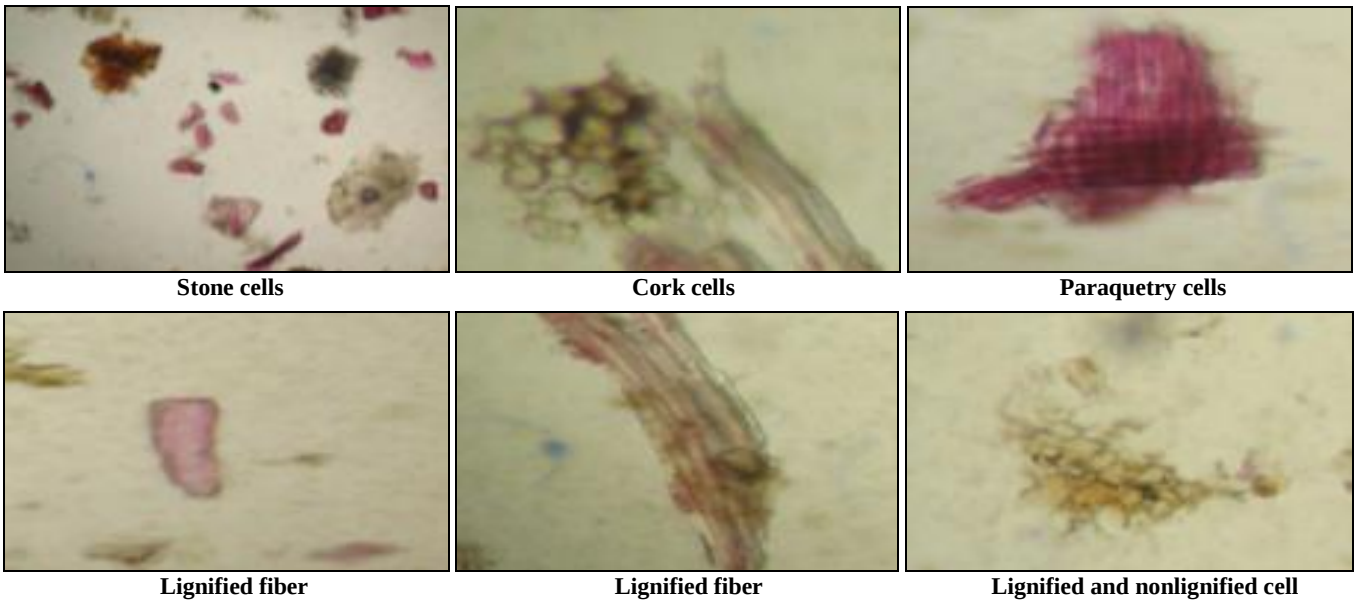


FIG. 2: TRANSVERSE SECTION OF STEM BARK OF *DELONIX REGIA* RAFIN

C: Cork; CC: Cork cambium; SC: Stone cells; SCT: Secondary cortex; PF: Pericyclic fiber; NLF: Non lignified fibers; MR: Medullary rays; PhF: Phloem fiber

Powder study of stem bark under microscope showed stone cells, cork cells and parquetry cells. It also showed lignified and non-lignified cells shown in **Fig. 3**.



Stone cells | **Cork cells** | **Paraquetry cells**

Lignified fiber | **Lignified fiber** | **Lignified and nonlignified cell**

FIG. 3: POWDER STUDY OF STEM BARK OF *DELONIX REGIA* RAFIN.

Histochemical Colour Reaction Tests: Transverse sections of *Delonix regia* stem bark when treated with various chemicals reagents for the tests of cell components showed change in colour as shown in **Table 1**.

TABLE 1: HISTOCHEMICAL COLOUR REACTIONS ON TRANSVERSE SECTION (STEM)

Reagents	Colour	Cell component
5% Ferric chloride	Dark green	Tannins present
Iodine	brown	Carbohydrate present
Picric acid	Green	Alkaloid present
Phloroglucinol+Hydrochloric acid	Xylem vessesls become Pink	Lignin present
Millon's regaent	Violet	Proteins present

Physico-chemical parameters: The various parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying were established and shown in **Table 2**. The extractive values with colour by successive extraction method are summarized in **Table 3**.

TABLE 2: ASH VALUES AND LOSS ON DRYING

Parameter	%(w/w)
Total ash	8
Acid insoluble ash	2.005
Water soluble ash	3.0
Sulphated ash	1.4
Loss on drying	45

TABLE 3: SUCCESSIVE EXTRACTIVE VALUE AND COLOUR OF EXTRACT UNDER VISIBLE LIGHT

Solvent	Extraction Time(h)	Colour of extract	Extractive value(%w/w)
Petroleum ether (60 - 80 ^{0}C)	18	Green	0.370
Chloroform	18	Buff	0.430
Acetone	18	Brown	0.291
Methanol	18	Dark brown	0.850
Aqueous	18	Light brown	0.262

Fluorescence Analysis: The plant material may be identified from their adulterants on the basis of fluorescence nature. The powder of stem bark was treated with different chemical reagents and results are reported in **Table 4**.

TABLE 4: FLUORESCENCE ANALYSIS OF POWDERED STEM OF *DELONIX REGIA* RAFIN.

Powdered drug+reagent	Visible/Day light	UV 254 nm (short)	UV 365 nm (long)
Powder as such	Yellowish white	Yellow	Pale yellow
Powder + 5% NaOH	orange brown	Light green	Yellow
Powder +1% picric acid	Brown	Yellowish green	Black
Powder + acetic acid	Yellow	Yellow	Yellow
Powder + distilled water	Yellow	Yellow	Yellow
Powder + 5% Iodine	Black	Black	Black
Powder + 5% $FeCl_3$	Yellow	Yellow	Black
Powder+conc. Nitric acid	Brown	Fluorescent green	Black
Powder+conc. H_2SO_4	Brown	Black	Dark green
Powder +dilute H_2SO_4	Reddish brown	Cream	Fluorescent Yellow
Powder + dilute HCl	Yellow	Yellow	Fluorescent Yellow
Powder+ conc. HCl	Brown	Fluorescent green	Black
Powder + 5% KOH	Orange brown	Reddish brown	Fluorescent Yellow
Powder + ammonia	Reddish brown	Reddish brown	Yellow
Powder +ethyl acetate	Yellow	Yellow	Fluorescent Yellow
Powder +chloroform	Yellow	Yellow	Off white
Powder + ethanol	Yellow	Yellow	Yellow
Powder + potassium permagnate	Black	Black	Black

Quantitative studies: The other quantitative studies for foaming index, swelling index, crude fiber content, hemolytic activity, foreign organic matter and bitterness value were performed. The results are tabulated in **Table 5**.

TABLE 5: QUANTITATIVE STUDIES OF *DELONIX REGIA* RAFIN.

Estimation	Observations
Foaming index	> 100
Swelling index	-
Crude fiber content	42%
Hemolytic activity	Non hemolytic
Foreign organic matter	0.60%w/w
Bitterness value	Non bitter

Preliminary Phytochemical Screening: The various extracts of powdered stem bark like petroleum ether, chloroform, ethylacetate, acetone, methanol and aqueous extracts were subjected for investigation of various phytoconstituents. It revealed the presence of different phytoconstituents, like carbohydrate, protein, glycosides, flavonoid, sterol and triterpenoid, phenolic and tannins compounds in different extracts as in **Table 6**.

TABLE 6: PHYTOCHEMICAL SCREENING OF EXTRACTS OF *DELONIX REGIA* RAFIN.

Test	P	C	A	E	M	W
Carbohydrate	-	+	-	-	+	+
Alkaloid	-	-	-	-	-	-
Glycoside	-	-	+	-	+	+
Phenolic compound and Tannin	-	-	+	-	+	-
Protein and amino acid	-	-	-	-	+	+
Flavonoid	-	-	-	-	+	+
Sterol and triterpenoid	+	-	-	-	+	+

Here, P-petroleum ether extract; C-chloroform extract; E-ethylacetate extract; A-acetone extract; M-methanolic extract; W- water extract. Here, (+) is present and (-) is absent

CONCLUSION: The scientists from past few decades are keen and sincere to evaluate many ethno medicinally used plants, due to their specific healing properties, desirable action, easy availability and less toxicity. The stem bark of *Delonix regia* Rafin. is still used in treatment of various disorders by many populations. The pharmacognostical standardization on this plant gives the idea about identification, standardization

and monograph of the plant. It is also important to evaluate the medicinal and therapeutic action of this plant.

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CONFLICT OF INTEREST: The authors have no conflict of interest.

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