



**“PHARMACOGNOSTIC, PHYTOCHEMICAL AND
PHARMACOLOGICAL STUDY OF ETHANOL EXTRACT OF
Delonixregia BARRK”**

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ABSTRACT

The Fabaceae family includes the attractive tree *Delonixregia*. *DelonixregiaRafin* and *Delonixelata* are two species in the *Delonixgenus*. A blooming plant is called *Delonixregia*. It has five petals, four of which are the same colour but one of which is distinct and has white streaks. *Delonixregia* has been shown to have antibacterial, anti-inflammatory, antioxidant, and anti-diarrheal properties. It has been utilised in the traditional medical practises of several cultures, including those that cure rheumatism, arthritis, hemiplegia, leucorrhoea, and constipation. *Delonixregia* flowers have been utilised as tablet binders and as traditional herbal treatments for gynaecological diseases. According to research *Delonixregia* Contains Anti-cancer properties.

Key words: *Delonixregia*, Pharmacognosticevaluation, Phytochemicalanalysis, Anti-cancer property, Ethanol extract.

INTRODUCTION

Botanist WenselBojer found the flamboyant Gulmohar tree in its native Madagascar at the beginning of the 19th century. Some claim it to be the world's most colourful tree. It is covered in flamboyant clusters of 4-5-inch-wide flame-red flowers for several weeks in the spring and summer. They feature four spoon-shaped spreading crimson or orange-red petals that are about 3 in long, and one erect somewhat bigger petal (the standard) that is marked with yellow and white. Even up close, the individual flowers are remarkable. *Delonixregia* is a species of flowering plant in the bean family Fabaceae, subfamily Caesalpinioideae.

The stem bark of *Delonixregia* contains several important phytochemical constituents, such as alkaloids, flavonoids, tannins, coumarins, terpenoids, and phenolic compounds, which contribute to its medicinal properties. These bioactive compounds exhibit a wide array of biological activities, including antioxidant, antiinflammatory, antimicrobial, anticancer, antidiabetic, hepatoprotective and immunomodulatory among others.

Delonixregia has been extensively used by Ayurveda, the conventional Indian medical system, for a variety of medicinal purposes. *Delonixregia* is mentioned as having cooling, digestive, rejuvenating, and antibacterial effects in Ayurvedic scriptures. It is frequently used for conditions including urinary issues, lung issues, skin conditions, and other issues. To treat certain medical disorders, *Delonixregia* leaves, fruits, and stem bark are combined to create a variety of Ayurvedic remedies, including decoctions and powders.

MATERIAL AND METHODS

Plant material and Chemicals:

The stem bark of *Delonixregia*, a plant belonging to the Fabaceae family is used in this study. Bark of the *Delonixregia* were collected from Shirampur, Ahmednagar, Maharashtra, India. It has been authenticated at the herbarium of Department of Botany and Research Centre, PVP college of Arts, Science and Commerce, Pravaranagar, Loni, Ahmednagar, Maharashtra, India 413713 with the reference number /PVPC/Bot/2022-23/72.. All other chemical were used analytical grade.

Pharmacognostic study:

Study of macroscopic and microscopic of study of crude drug were carried out using various parameter

Macroscopic features of Stem bark:

Macroscopic features were studied by naked eyes and observation. size and shape, surfaces, fracture, texture, colour, odour and taste were done by observing visually.

Microscopic features:

Fresh bark pieces were boiled in water for 1 to 2 hrs. Thin sections were collected and washed with distilled water. Then transferred using No. 1 paint brush into Clearing agent Chloral hydrate 30% and kept for 10-15 minutes. Then transferred to staining agent Phloroglucinol – ConcHCl (1:1) which stains the tissues in pinkish red color. The sections were stained for 3-5 minutes and mounted on glass slide using glycerin. Then observed using Motic Electronic Microscope. (Khandelwal K. R. 2000).

Powder microscopy:

The shade dried powdered bark has been screened through sieve number 40 was used for the powdered analysis and to observe the microscopical powder characteristics. Powder consists of thin walled polygonal lignified cork cells, lignified phloem fibres, lignified xylem pitted vessels, lignified stone cells, stratified lignified Sclereids and calcium oxalate crystals. After staining of powder, the samples were observed under Motic Electronic Microscope with different magnifications.

Physico-chemical standard:

Loss on drying: Approximately 1.5 grams of powdered drug were dried in an oven and weighed to calculate the percentage of moisture loss using a gravimetric method

Determination of foreign content: Approximately 100 grams of dried bark powder were visually inspected, foreign matter was separated and weighed, and the percentage of foreign content was calculated (Khandelwal K. R. 2000).

Determination of total ash: About 2 grams of dried sample were incinerated, and the remaining ash was weighed to determine the percentage of total ash with respect to the air dried powder.

Determination of acid-insoluble ash:

The total ash was boiled with dilute hydrochloric acid, filtered, and the residue was incinerated to calculate the percentage of acid-insoluble ash.

Determination of water-soluble ash: The total ash was boiled with distilled water, filtered, and the residue was incinerated to calculate the percentage of water-soluble ash.

Extractive values: The alcohol-soluble and water-soluble extractives were determined by macerating the powdered drug in ethanol and chloroform water, respectively. The filtrates were evaporated and weighed to calculate the percentages of soluble extractives with reference to the air-dried powder (Khandelwal K.R. 2000).

Extraction:

The bark of *Delonixregia* was procured and authenticated from Department of Botany and Research Centre, PVP college of Arts, Science and Commerce, Pravaranagar, Loni, Ahmednagar, Maharashtra, India 41371. Authenticated bark was dried in shade and powdered coarsely using grinder. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of bark was macerated with Ethanol at 40 °C for 3-5 hours. The extract obtained were concentrated under reduced pressure to yield ethanolic extract. 2 gm of ethanolic extract were obtained from 200 gm of bark powder.

Preliminary phytochemical screening:

To determine the presence of different phytoconstituents, ethanolic and aqueous root bark extracts of *Delonixregia* were subjected to preliminary phytochemical screening. Carbohydrates, reducing sugars, alkaloids, glycosides, cardiac glycosides, flavonoids, triterpenoids, saponins and tannins were all subjected to tests. Qualitative chemical tests on the extracts were performed, and results were noted. *Delonixregia* bark extracts were quantitatively estimated to ascertain the total amount of different metabolites. Precipitation and filtration were used to quantify the total alkaloids content, and the Folin-Ciocalteu technique was used to calculate the total phenolic content. The total terpenoid content was estimated by weighing the material before and after extraction, and the total flavonoid content was ascertained using the aluminium chloride colorimetric technique.

Screening of extract for pharmacological actions:

Anti-cancer activity:

Onion root tip assay:

The following procedures were carried out in order to have the test organism ready for mitotic research. The outermost brownish scaly skin and dead roots of five healthy medium-sized onion bulbs weighing 25–28 g were gently scraped off around the disc. To ensure that the discs were immersed, the prepared bulbs were put in tubes filled with tap water and let to develop for three days. The growing process took conducted until the roots were about 1 cm long at room temperature (average temperature of 24–25°C), with an average humidity of 46.6% and partial exposure to sunshine. Then, for each bulb, the zero hour or stat Mitotic Index was established, which served as the foundation for the future mitotic research.

Squash Preparation:

The terminal 2-3 mm of the root meristems was cut and heated in a mixture of Acetocarmine:N/10 HCl in a 9:1 ratio. A watch glass containing the root tips was then heated until the tips were soft and darkly stained. A tip was then taken and squashed in a drop of fresh acetocarmine on a cleanslide after a cover slip was put. The slide was wrapped in 2 layers of filter paper and squashed by the application of direct vertical pressure of the thumb. The slides of mitosis thus prepared were scanned under the microscope at 40X in various fields. Cells showing various stages of mitosis and non-dividing cells were counted. 500-800 cells per onion bulb were counted.

Estimation of Mitotic Index (MI):

- 1) The bulbs were then put in 3 containers containing control, standard and extract at concentrations 1mg/ml and readings of MI were taken after 48 hours of exposure.
- 2) Each test was run in triplicate.
- 3) The data of MI was recorded.
- 4) The various stages of cell division were noted down separately.
- 5) The Mean and Standard Deviation (SD) were calculated.
- 6) The significance of the difference in MI at various times of exposure was calculated by Student ‘t’ test.

Mitotic index % = $\left(\frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}}\right) \times 100$

Potato disc assay:

Antitumor activity of twig ethanol of was assessed using the potato disc bioassay.

Follwing design was followed:

- 600 µl test extract +150 µl Double Distilled Water (DDW) + 750 ul A. tumefaciens in PBS.
- Camptothecin was used as positive control replacing test extract.
- Potato discs (5 mmx8 mm in size) were collected from red-skinned potatoes (*Solanum tuberosum* L., Solanaceae) using sterilize cork borer.
- Each potato disc was overlaid with 50µl of appropriate inoculums with particular concentrations (10 ppm, 100 ppm, 1000 ppm) of test extract.
- Petri dishes were sealed by parafilm and incubated at room temp (27-30°C) for 3 weeks.
- After 3 weeks, potato discs were stained with Lugol's iodine (10% KI and 5% I₂) for 30 minutes and tumors were observed under stereo microscope, where the tumor cells lack starch (look like orange color).
- Each experiment was done in triplicate.

Percentage of tumor inhibition was calculated using standard formula. (Hossain al. (2007), (Russell D. Freed, Crop and Soil Sciences Department of Michigan State University, USA).

$$\text{Percentage Inhibition} = 100 - \frac{\text{Number of tumor with sample}}{\text{Number of tumor with control}} \times 100$$

RESULT AND DISCUSSION:

Pharmacognostic study:

Macroscopy:

Sr. No	Color	Dark Brown
1	Odor	Characteristics
2	Taste	Astringent
3	Size	30-40 cm long & 0.8-1 cm thick
4	Shape	Flat & Thick
5	Fracture & Fissure	Long Fissures, Furrowed, Scaly & Laminated

6	Inner surface	Corrugated
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Table No. 01: Macroscopical Characters

Microscopy:

Microscopic Characters	Observations
CorkCells	Thin walled 5-6 layers, flat, polygonal cells with reddish brown content
Phellodermal Cells	Non-separable
Sclerenchyma	Lignified, inner and radial walls more thick (U-shaped thickening)
Phloem	Single, isolated, pointed at end and lignified
Xylem	Bordered & pitted thickening
Cortex	Several layers of thin walled tangentially elongated cells containing reddish brown matter
MedullaryRays	Biseriate
CalciumOxalateCrystals	Square and prisms like crystals

Table No. 02: Microscopical Characters

T.S.of *Delonixregia* stem bark:

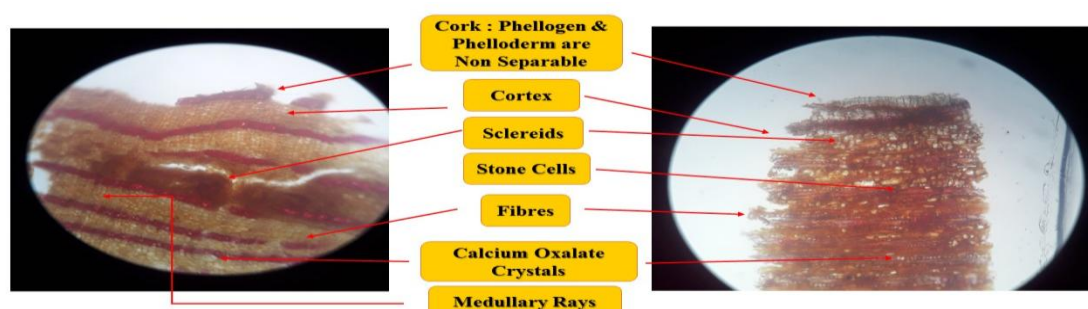


Figure No. 01: Photomicrograph of *Delonixregia* stem bark (Transverse Section)

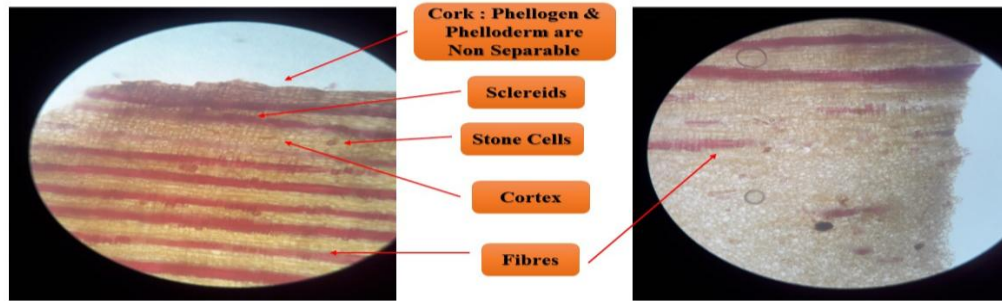


Figure No.02: Photomicrograph of *Delonixregia* stem bark (Longitudinal Section)

Powder characteristics:

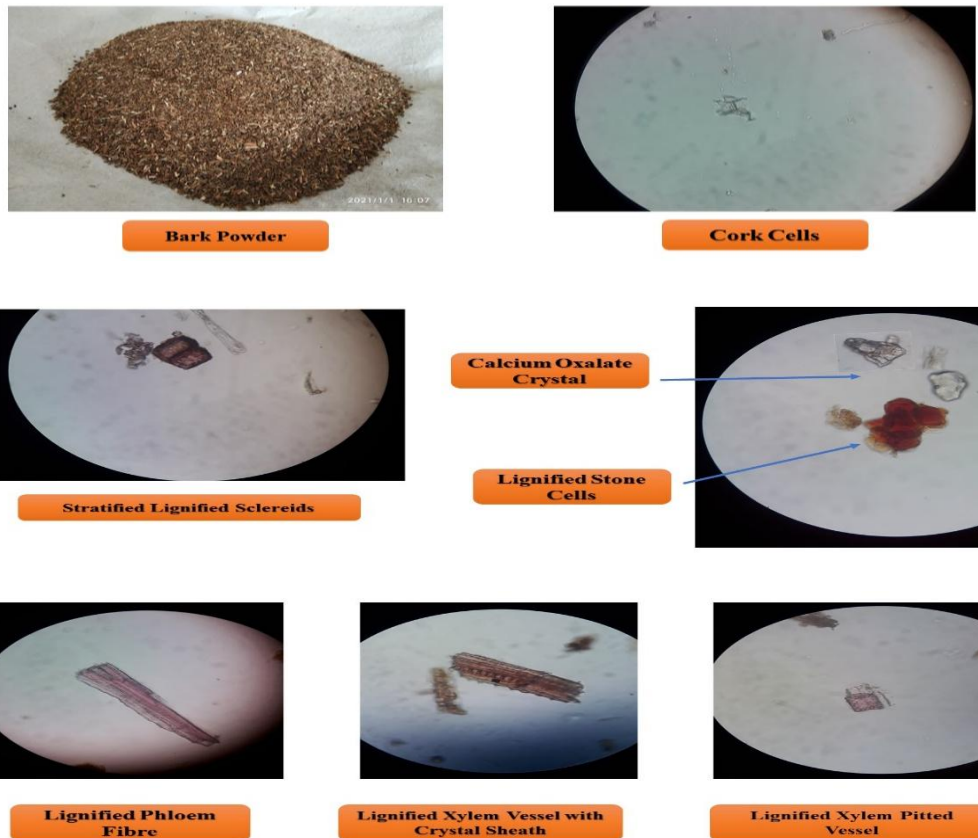


Figure No. 03: Photomicrograph of *Delonixregia* stem bark Powder Characters

Microchemical test:

Sr. No.	Reagent	Observation	Characteristics
1	Phloroglucinol + Conc. HCl (1:1)	Pinkish	Lignified cells: fibre's, stone cells, Sclereids, cork cells
2	Iodine	Blue	Starch
3	Ruthenium red	No Pink Colour	No Mucilage cells
4	Acetic acid	Insoluble	Calcium oxalate crystals

	Dil. Hydrochloric acid	Soluble	
5	Alcoholic Picric acid	No Yellow Colour	No Aleurone grains found
6	Sudan red III	No Red colour	Oil globules absent

Table No. 03: Microchemical test

Physicochemical standard:

The provided physico-chemical standards include parameters such as Loss on Drying,

Total Ash, Water Soluble Ash, Acid Insoluble Ash, Water Soluble Extractive, and Alcohol Soluble Extractive. These parameters measure the moisture content, inorganic residue, solubility in water and alcohol, and other characteristics of the sample. These results are important for assessing the quality and purity of the sample.

Parameter	Result
Loss on Drying	2.3 % w/w
Total Ash	Not more than 9.3 % w/w
Water soluble ash	Not more than 4.2 % w/w
Acid Insoluble ash	Not more than 3.36 % w/w
Water soluble extractive	Not less than 13.33 % w/w
Alcohol soluble extractive	Not less than 16 % w/w

Table No. 04: Physico-chemical standards

Phytochemical screening table:

The phytochemical screening of the sample using ethanolic and aqueous extracts revealed the presence of various phytoconstituents. Both extracts showed high levels of carbohydrates, reducing sugars, alkaloids, glycosides, cardiac glycosides, flavonoids, triterpenoids and tannins. These phytoconstituents are known for their potential medicinal properties and may contribute to the therapeutic value of the sample. However, saponins were only detected in the ethanolic extract, while they were absent in the aqueous extract. These findings provide valuable information about the chemical composition of the sample and suggest its potential use in pharmaceutical or natural product research.

Sr. No	Type of Phytoconstituent	ethanolic extract	Aqueous extract
1	Carbohydrates	+++	+++
2	Reducing sugars	+++	+++
3	Alkaloids	+++	+++
4	Glycosides	+++	+++
5	Cardiac glycosides	+++	+++
6	Flavonoids	+++	+++
7	Triterpenoids	+++	+++
8	Saponins	+++	---
9	Tannins	+++	+++
10	Steroids	---	---

Table No. 05: Preliminary Phytochemical Screening

Quantitative Estimation:

Sr. No	Phytochemical Content	Content Amount	Method	Calculated using
1	Total Alkaloid Content	23 mg/gm	Standard Method using Ammonium Hydroxide	Formula
2	Total Phenolic Content	132.7 mg/gm	Folin-Ciocalteu method	Calibration Curve
3	Total Flavonoid Content	79.29 mg/gm	Aluminium chloride colorimetric method	Calibration Curve
4	Total Terpenoid Content	20.21 mg/gm	Standard Method Using PET ether	Formula

Table No 06: Quantitative Estimation of Extract

Thin layer chromatography:

Ethanol crude extracts with the solvent system in below table showed a spots which were visualized after the exposure of iodine crystals. The Rf value is calculated by using the formula as follows:

Rf Value = Distance travelled by the compound /Distance travelled by the solvent

Sr.No	Extracts	Solvent system	Rf Value
1.	Ethanol extract of <i>Delonixregia</i>	N -butanol:Glacial acetic acid:H ₂ O (4:1: 5)	0.23
2.	Ethanol extract of <i>Delonixregia</i>	Toluene: Ethyl acetate:Ethanol (8: 2: 2)	0.22
3.	Ethanol extract of <i>Delonixregia</i>	Toluene:Ethyl acetate: Ethanol (8:2:1)	0.25

Table No. 07: TLC of ethanol extracts of *Delonixregia*.

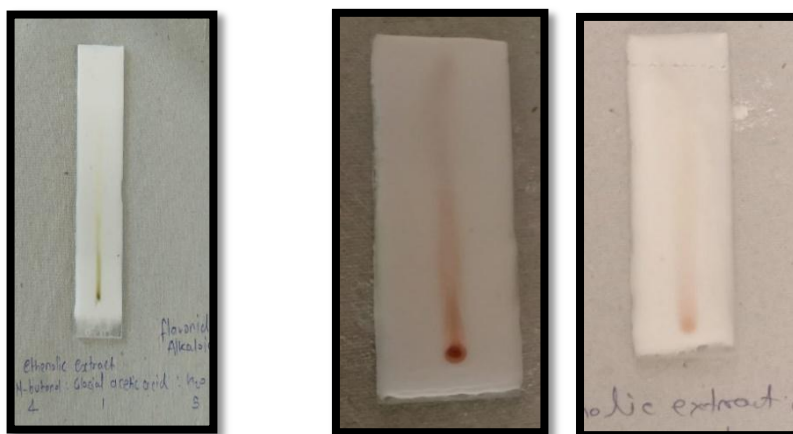


Figure No.04: Thin Layer Chromatography Plate

Column chromatography:

Fractionation of *Delonixregia*. Stem 1gm of *Delonixregia* extract was subjected for Column chromatography. The movement of the various parts of the mixture move at various speeds when the mobile phase and the mixture that needs to be separated are introduced from the top of the column. Compared to components with greater adsorption and affinity to the stationary phase, those with lower adsorption and affinity move more quickly. The elements that move quickly are eliminated first, while the elements that move slowly are eliminated last. Initial fractionation of 1g of the extract was done with toluene, then ethanol. The insoluble

substance was the number of the chemicals in each fraction of the leaves was determined using thin-layer chromatography (TLC) and mobile phases such N - butanol, Ethyl acetate (4:6).The product was obtained 0.34 gm. product.

The column chromatography separation technique was performed for Ethanolic extract the result shown in

Type of Elution: Gradient column

Size: Length 40cm, diameter 1cm

Drop/min: 2-3



Figure No. 5: Column Chromatography

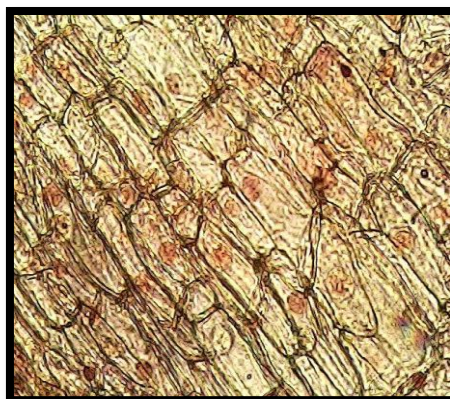
**Screening of extract for pharmacological actions:Anti-cancer activity:
InvitroantimitoticactivityOnion roottip method:**



Control



Extract



Standard

Figure No.06: Mitosis onion root tip assay

Extract	Concentration mg/mL	Total No. of Cells	Cell in Division	Mean	Mitotic Index %
Control	0.1 mg/ml	200	57	57.33	28.66
	0.1 mg/ml	200	61		
	0.1 mg/ml	200	54		
Standard	0.1 mg/ml	200	162	168.66	84.33
	0.1 mg/ml	200	169		
Sample-SU	0.1 μ l	200	98	107.00	53.5
	0.1 μ l	200	114		
	0.1 μ l	200	110		

Table No. 08: Onion root tip assay calculation

Treatment not only brought down the frequency of dividing cells but also produced a good number of anomalies in the mitotic cells when you compared with standard reports. The Sample also caused chromosomal and mitotic aberrations including accumulation of prophase, sticky chromosomes at metaphase, spindle disturbance at prophase and anaphase bridges.

Potato disc assay:

Sr no	Treatment	No of Tumors	Percentage inhibition
1	Control	12	
2	Camptothecin(0.5mg/ml)	4	28.7%
3	<i>Delonixregia</i> (0.5mg/ml)	10	69.2%
4	<i>Delonixregia</i> (0.05mg/ml)	7	50.5%
5	<i>Delonixregia</i> (0.01mg/ml)	6	39.2%

Table No.09: Potato disc assay calculation



Figure No.07: Antitumor potato disc assay A- Broth medium with *Agrobacterium tumefacein*

B – Surface sterilization of Potatowith 20% bleach solution, C – Potato cubes in petridish and form tumor

Treatment shows the effect of samples tested on crown gall tumor inhibition on potato discs. The average and the maximum inhibition for each sample were calculated. The maximum inhibition was observed with conc of 0.5 mg/ml. Others appear to cause a satisfactory inhibition. Tumors appear in the plates as brown spots which are in fact nodes and these spots give the number of tumors which appear on the disc. Most of the extracts that cause inhibition make the outer layer of the potato discs mucous.

Conclusion:

In conclusion, pharmacognostic assessment, phytochemical analysis, and pharmacological activity assessment were all part of the thorough investigation carried out on the ethanol extract from the stem bark of *Delonixregia*. The pharmacognostic analysis showed the stem bark's macroscopic and microscopic properties, revealing important details about its external features and internal organisation. Important characteristics including moisture content, inorganic residue, and extractive values which are essential for determining the quality and purity of the sample were measured using the physico-chemical standards. The ethanolic and aqueous extracts' phytochemical screening revealed the presence of a number of phytoconstituents, including reducing sugars, alkaloids, cardiac glycosides, flavonoids, triterpenoids and tannins. These phytoconstituents show that the stem bark of *Delonixregia* is suitable for further pharmaceutical and natural product research and contribute to its potential therapeutic capabilities. By using onion tip root test and the potato disc assay, the pharmacological activity evaluation of the extract concentrated on its anticancer properties. The findings showed a considerable reduction in tumour development, indicating the extract's potential as an anticancer treatment. These findings call for more research to clarify the underlying processes and assess the therapeutic potential of *Delonixregia* stem bark since they offer preliminary proof of its pharmacological action. This extensive study highlights the pharmacognostic characteristics, phytochemical composition, and pharmacological activity of *Delonixregia* stem bark, which is a useful medicinal plant. Utilising this plant's

medicinal potential for drug development and natural medicine requires more investigation into its bioactive ingredients and their modes of action.

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