MORPHOLOGICAL, HISTOLOGICAL AND PHYTOCHEMICAL STUDY OF FLORAL LEAVES OF *DELONIX REGIA* (BOJER EX. HOOK.) RAF. AND THEIR ANTIOXIDANT ACTIVITIES

Nwe Oo[1]

Abstract

The morphological, histological and phytochemical studies were carried out at Botany Department, University of Yangon in 2019. According to morphological study, it is a tree with bipinnately compound leaves, pulvinus and leaf-like stipules present. Flowers are slightly zygomorphic and predominantly orange-red in color. Lower surface of sepals is green and upper is red. Four smaller petals are orange-red and one larger petal is white and yellow with reddish spots. In histological study (Trease and Evans, 2009), stomata present on the lower surface of sepals. Anthocyanin containing epidermal cells present only on the upper surface of sepals. In petals, anthocyanin containing cells present on both epidermis. Bundle sheath and starch sheath present in petals. In phytochemical study (Harborne, 1973, Sofowora, 1993 and Trease and Evans, 2009), reducing sugars, alkaloids and saponins were not detected in petals. Antioxidant activities were determined by DPPH assay (Blois, 1958 and Brand-Williams *et al*., 1995) at Botany Department, University of Mandalay in 2020. The IC_{50} value of sepals was 6.44 μg/ml and petals was 20.04 μg/ml.

Keywords: morphology, histology, phytochemistry, antioxidant activities

Introduction

Delonix regia (Bojer ex. Hook.) Raf. is a tree and belongs to the family Fabaceae. It is grown as shady and ornamental plant because of its beautiful and bright coloured flowers throughout Myanmar.

Its flowers are used as natural color and as an acid-base indicator. Chemical constituents of different classes such as; flavonoid, tannins, glycosides, phenolics, sterol (phytosterol) and terpenoids (triterpenoids) were reported from its flowers and leaves. Ethanolic extracts of flower and bark were investigated to anti- inflammatory activity in rats. Leaves and flowers are reported to possess antimalarial, anti-bacterial, anthelmintic, hepato-protective, diuretic and anti-oxidant (Hussain, *et al*., 2014).

It originated from Madagascar, where it is now almost extinct but widespread in most tropical and subtropical areas of the world. The bark yields thick mucilage of water – soluble gum. The seeds contain gum and the tree could provide timber and the large pods and wood are used for fuel (Igwe and Louis, 2014).

In traditional medicines, the flowers were used for curing chronic fever in Southwestern Bangladesh. In Nigeria, the flowers were used for antibacterial activity. Some tribes of India have used the seed for curing pyorrhea. The roasted and crushed leaves were wrapped in a cloth and inhaled just after scorpion bite; infusion of flowers was used in bronchitis, asthma and malarial fever. In several African countries, the water extracts of flowers were also used in traditional healthy beverages. It is a part of local medicine and traditional byproducts (Sharma and Saroj, 2015).

Its flowers especially sepals and petals have been used as vegetables and salad in some area. The aim of this study is to know the pharmacognostic properties of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.. The objectives are to study the morphological and histological characters of floral leaves and to determine their phytochemical constituents and antioxidant activity.

[1] Dr, Lecturer, Department of Botany, University of Mandalay

Materials and Methods

Collection of plants

Delonix regia (Bojer ex. Hook.) Raf. was collected from University of Yangon campus in 2019. The morphological characters of collected plant specimens were photographed and recorded their measurements in detail. The plant was identified with the help of literatures such as Hooker (1875) and Backer (1963).

Cleaning, drying and powdering

The collected samples were thoroughly cleaned to remove impurities and separated as sepals and petals. Then they were air dried to avoid direct loss of phytoconstituents from sunlight. The dried samples were coarsely powdered to study the preliminary phytochemical analysis and DPPH scavenging assays.

Histological study

The fresh samples were sectioned by free hand method according to Trease and Evans (2009). The following reagents were used for histological purposes;

1. Chloral hydrate solution as the clearing reagent
2. Saffranin solution as the stain for lignified and cutinized cell walls
3. Iodine solution used for starch.

Preliminary phytochemical study

Phytochemical screening was performed using standard procedures such as Harborne (1973), Trease and Evans (2009) and Sofowora (1993).

Test for glycosides

Two ml of aqueous extract was mixed with a few drops of aqueous NaOH. A yellow coloration will indicate the presence glycosides.

Test for reducing sugar

Two ml of the aqueous extract was treated with mixture of Fehling A and B solutions and heated gently. Orange red precipitates show reducing sugars.

Test for alkaloids (Wagner's reagent)

Two mL of aqueous extract was mixed with 0.2 mL dilute HCl, followed by 1 mL of Wagner's reagent. If the brown precipitation appears, alkaloid is presence.

Test for flavonoids

Two ml of the aqueous extract was mixed with a few drops of NaOH solution. If the mixture shows the yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, it indicates the presence of flavonoids.

Test for phenolic compound

Two mL of the aqueous extract was mixed with 1 to 2 drops of Iron III chloride ($FeCl_3$) was added. A blue, green, red or purple color is a positive test.

Test for tannins

Aqueous extract (2 ml) was mixed with a few drops of 5% $FeCl_3$ solution and concentrated sulphuric acid. The yellow brown precipitate is the presence of tannins.

Test for saponins

Aqueous extract (2 ml) was mixed with 2 ml of distilled water and shaken vigorously. The formation of stable foam indicates the presence of saponins.

Test for proteins

Aqueous extract (2 ml) was mixed with a few drops of Millon's reagent. The white precipitates turn red when heated. This appearance shows the protein presence.

Test for α-amino acids

A few drops of aqueous extract were spotted on a filter paper, allowed to dry and spray with ninhydrin reagent. It was dried at room temperature and then kept in an oven at 110˚C for a few minutes. The violet spot indicate α-amino acids presence.

Test for steroids/terpenoids

Ethanol extract (3 ml) was dissolved with 2 ml of chloroform. Then mixture was treated with concentrated sulphuric acid. The red color showed the steroids presence. Reddish brown color at interface showed the presence of terpenoids.

Antioxidant activity by using DPPH free radical scavenging assay

DPPH (2, 2-diphenyl- 1-picryl-hydrazyl) radical scavenging assay was carried out according to the methods of Blois (1958) and Brand-Williams *et al*. (1995). The experiment was performed at Department of Botany, University of Mandalay.

Preparation of 60 µM DPPH solution

DPPH powder (2.364 mg) was mixed with 95% ethanol (100 ml) and stirred thoroughly. This freshly prepared solution was kept in brown color flask and stored in the refrigerator at - 2˚C for no longer than 24 hours.

Preparation of test sample solution

Ethanolic extracts (0.1g) was dissolved with 95% ethanol (100 ml) to get 1000 µg/ml stock solution. Different concentrations, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml solutions were prepared from stock solution by the half dilution with 95% ethanol.

Preparation of standard ascorbic acid solution

Ascorbic acid (0.01 g) was mixed with 95% ethanol (100 ml) to get 100 µg/ml stock solutions. Different concentrations, 0.3906 µg/ml, 0.7813 µg/ml, 1.5625 µg/ml, 3.125 µg/ml, 6.25 µg/ml and 12.5 µg/ml solutions were prepared by the half dilution.

Procedure

Antioxidant activity of ethanolic floral leaves extracts was determined by DPPH scavenging assay. Blank solution was prepared 3 ml of 95% ethanol. 60 µM DPPH solution (1.5 ml) and 95% ethanol (1.5 ml) were used as control solution. The different concentrations of

test sample solution (1.5 ml) was mixed with 60 µM DPPH solution (1.5 ml) and allowed to stand in the dark for 30 minutes.

The different concentrations of standard ascorbic acid solution (1.5 ml) was mixed with 60 µM DPPH solution (1.5 ml) and allowed to stand in the dark for 30 minutes. The absorbance of these solutions was measured at 517 nm by using UV spectrophotometer (UV - Vis 2550). Lower absorbance showed the higher free radical scavenging activity. The measurements were carried out triplicates for each solution and calculated the percent inhibition by the following formula;

$$\% \text{ inhibition} = A_0 - A_1/A_0 \times 100$$

Where A_0 = the absorbance of control

A_1 = the absorbance of sample

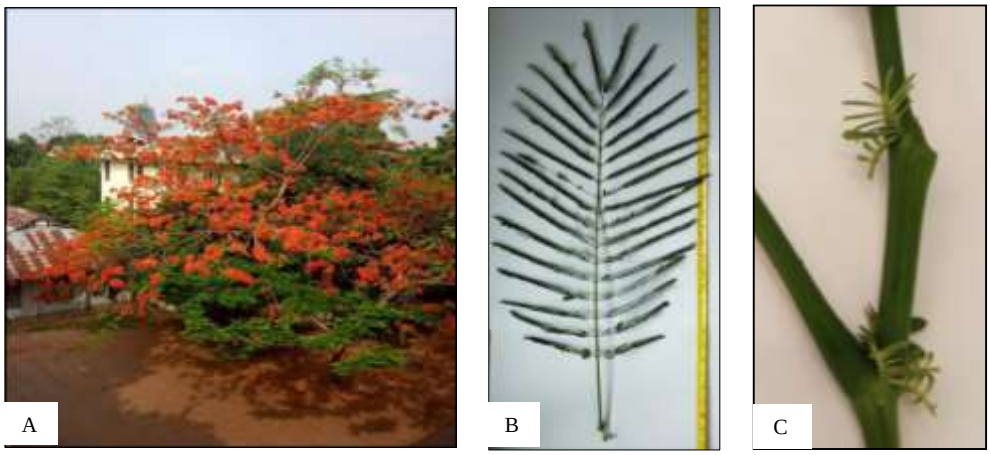
The 50% inhibitory concentration (IC_{50}) of test samples and standard ascorbic acid was calculated by nonlinear regression method of GraphPad Prism software.

Results

Morphological characters of *Delonix regia* (Bojer ex. Hook.) Raf.

Scientific Name	:	*Delonix regia* (Bojer ex. Hook.) Raf.
Family	:	Fabaceae
Common Name	:	Royal Poinciana, Flame tree, Flamboyant
Myanmar Name	:	Sein-pan

Tree, up to 15 meters height. **Leaves** bipinnately compound. **Leaflets** sessile, oblong, glabrous. **Stipules** leaf-like and caducous. Inflorescences racemose. **Flowers** bisexual, complete, irregular, slightly zygomorphic, pedicellate. **Calyx**; sepals 5, aposepalous, oblong-acuminate, reddish color inside, green color outside. **Corolla**; petals 5, apopetalous, smaller 4 petals orange-red, claw and lobe present, larger one petal white with reddish spot at upper portion, yellow with reddish spots at lower portion of lobe, claw reddish, margin of lobe reddish. **Androecium**; stamens 10, apostemonous, filament red at upper portion, white at lower portion. **Gynoeium;** carpels 1, monocarpellary, style filiform, stigma capitate, one ovule in the locule in T.S, marginal placentation. **Pods** blackish, style persistent. **Seeds** oblong-elliptic.



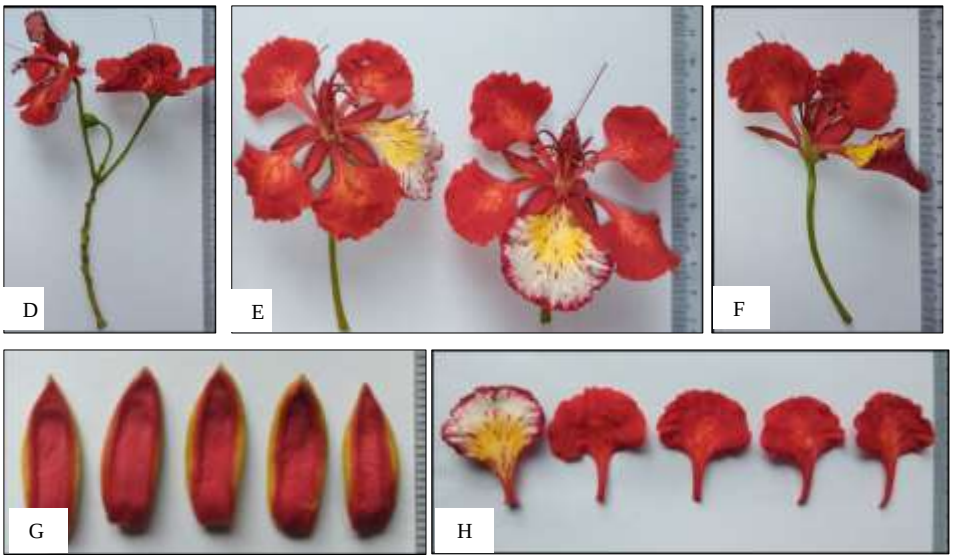


Figure 1 Morphological characters of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Habit
B. Leaf
C. Leaf-like stipules
D. Inflorescence
E. Flowers
F. L.S of flower
G. Sepals
H. Petals

Figure 2 Morphological characters of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Androecium
B. Pistils
C. T.S of ovary
D. L.S of ovary
E. Fruit
F. Seeds

Histological characters of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

In upper surface of sepals, polygonal-shaped parenchyma cells with red anthocyanin are present. Stomata are absent. In lower surface view, epidermal cells are polygonal in shaped and anomocytic stomata are present. No anthocyanin pigments found in the lower epidermal cells of sepals. In T.S of sepals, unicellular and uniseriate trichomes are found on the upper epidermis. In mesophyll, small vascular bundles arrange into ring. Numerous aerenchyma cell layers are found in the inner portion of the sepals. Mesophyll cells are not differentiated.

In both surface views of petals, striations and wavy epidermal cells with anthocyanin are abundantly present. In T.S of lobed petals, epidermal cells are larger than that of other mesophyll cells. Papillae are present on both epidermises. Mesophyll cells are aerenchymatous and vascular bundles are small with bundle sheath cells. There are 4 – 5 layers of mesophyll layers are found. The outline of clawed petal is crescent-shaped in T.S. Starch sheath is present in clawed petal.

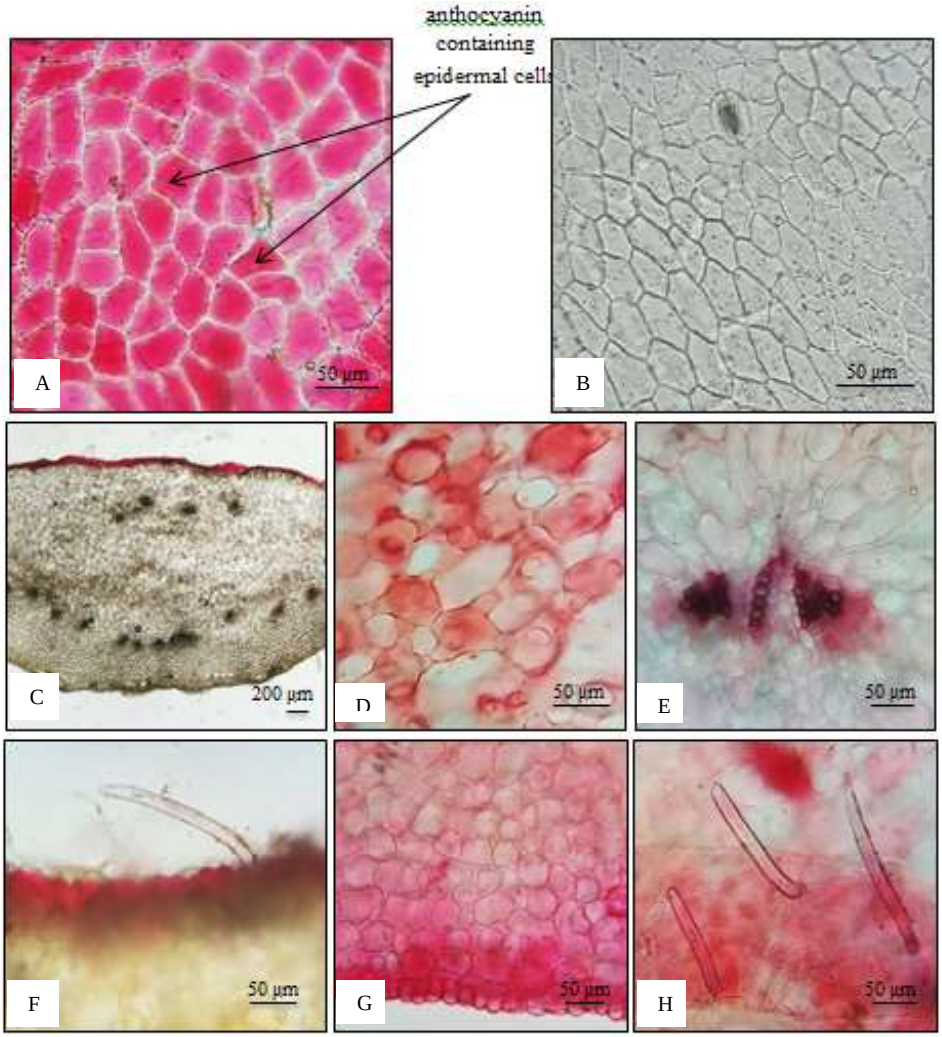


Figure 3 Histological characters of sepals of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Upper surface B. Lower surface
C. Transverse section D. Spongy cells
E. Vascular bundle F. Upper portion
G. Lower portion H. Unicellular and uniseriate **trichomes**

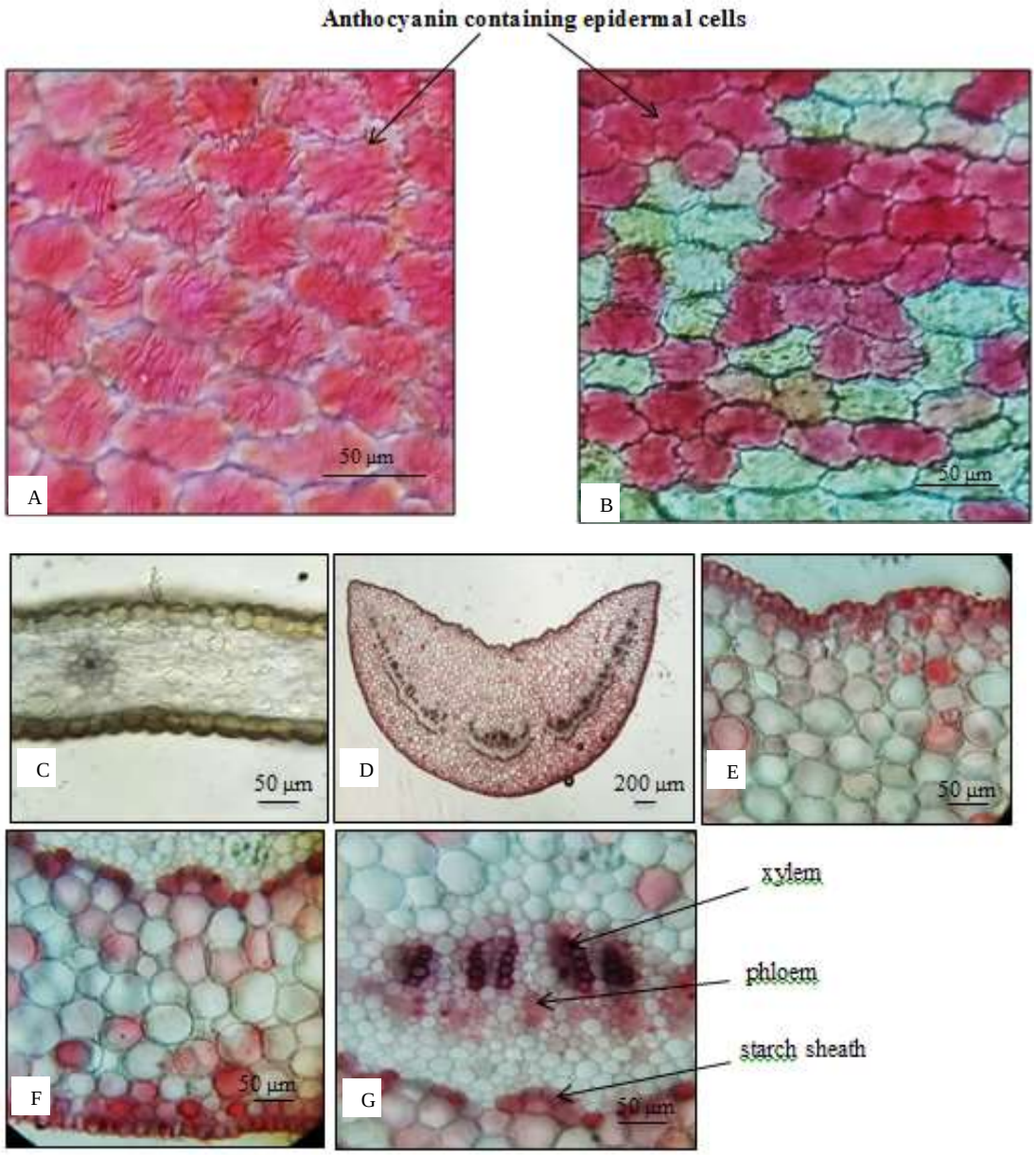


Figure 4 Histological characters of petals of *Delonix regia* (Bojer ex. Hook.) Raf.

A. Upper surface

B. Lower surface

C. Transverse section of lobed petals

D. Transverse section of clawed petals

E. Upper portion of clawed petals

F. Lower portion of clawed petals

G. Vascular bundles of clawed petals

Samples of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

Figure 5 Fresh sepals samples

Figure 6 Dried and powdered sepals

Figure 7 Fresh petals samples

Figure 8 Dried and powdered petals

Preliminary phytochemical study of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

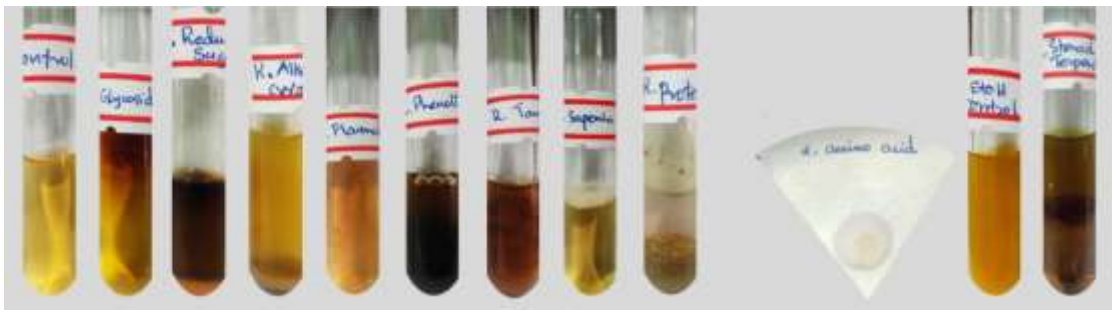


Figure 9 Phytochemical constituents of sepals

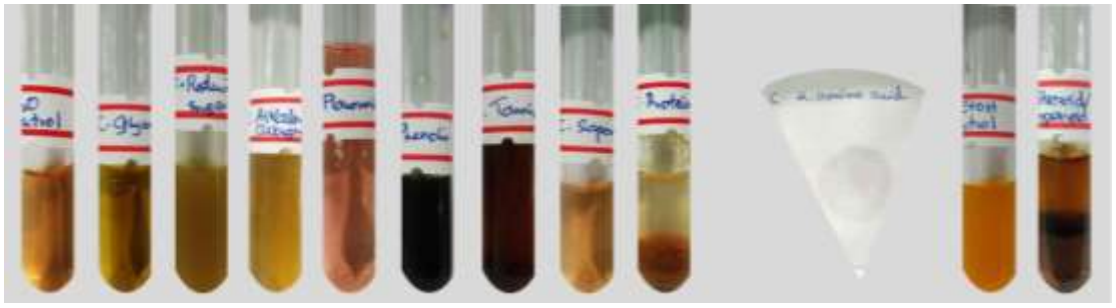


Figure 10 Phytochemical constituents of petals

Table 1 Preliminary phytochemical constituents of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf.

No.	Tests	Observations		Results	
		Sepals	Petals	Sepals	Petals
1.	Glycosides	Yellow color	Yellow color	+	+
2.	Reducing sugars	Orange ppt.	No ppt.	+	–
3.	Alkaloids	Brown ppt.	No ppt.	+	–
4.	Flavonoids	Colorless	Colorless	+	+
5.	Phenolic compounds	Blackish ppt.	Blackish ppt.	+	+
6.	Tannins	Yellow brown ppt.	Yellow brown ppt.	+	+
7.	Saponins	Foaming	No foaming	+	–
8.	Proteins	White ppt. turned red when heated.	White ppt. turned red when heated.	+	+
9.	α-amino acids	Pink spot	Pink spot	+	+
10.	Steroids/ Terpenoids	Red color/ Reddish brown interface	Red color/ Reddish brown interface	+	+

\+ = present
– = absent

Antioxidant activity of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf. by DPPH free radical scavenging assay

Table 2 Percent inhibition of ethanolic floral leaves extracts of *Delonix regia* (Bojer ex. Hook.) Raf.

Concentrations (µg/ml)	Absorbance			Inhibition (%)		IC_{50} value (µg/ml)	
	Sepals	Petals	Control	Sepals	Petals	Sepals	Petals
6.25	0.130	0.176	0.243	46.50	27.57	6.44	20.04
12.5	0.089	0.172	0.243	63.37	29.22		
25	0.046	0.092	0.243	81.06	62.14		
50	0.039	0.067	0.243	83.95	72.43		
100	0.032	0.047	0.243	86.83	80.66		
200	0.026	0.020	0.243	89.30	91.77		

Table 3 Percent inhibition of standard ascorbic acid

Concentrations (µg/ml)	Absorbance		Inhibition (%)	IC_{50} value (µg/ml)
	Ascorbic acid	Control		
0.3906	0.198	0.243	18.52	2.79
0.7813	0.196	0.243	19.34	
1.5625	0.175	0.243	27.98	
3.125	0.116	0.243	52.26	
6.25	0.076	0.243	68.72	
12.5	0.010	0.243	95.88	

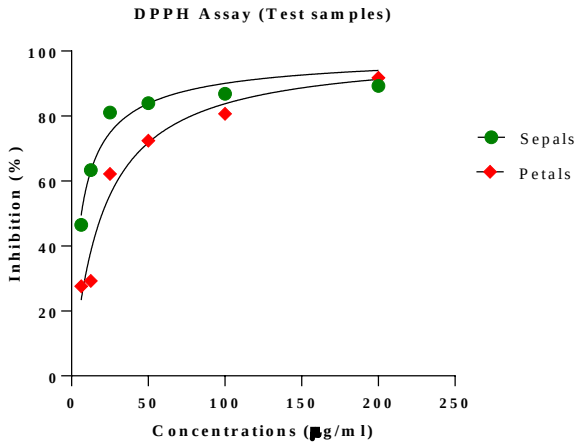


Figure 11 Line graph showing percent inhibition of ethanolic floral leaves extracts of *Delonix regia* (Bojer ex. Hook.) Raf.

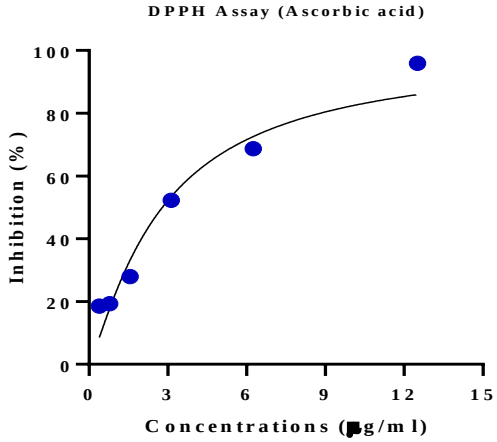


Figure 12 Line graph showing percent inhibition of standard ascorbic acid

Discussion and Conclusion

In this paper, morphological and histological character of floral leaves of *Delonix regia* (Bojer ex. Hook.) Raf. were studied and the determination of their phytochemical constituents and antioxidant activities were performed. Some distinct morphological characters were the presence of bipinnately compound leaves, caducous leaf-like stipules, showy and bright colored flowers, sepals color, different petals size and color etc. These characters were in agreement with those mentioned by Hooker (1875) and Backer (1963).

The histological characters of sepals and petals were similar with leaves. The lower surface of sepals was green and possesses stomata for photosynthesis. Wallis (1967) reported that the mesophyll of sepals is usually undifferentiated and resembles the spongy tissue of a foliage leaf. In this study, the mesophylls of sepals were undifferentiated into palisade and spongy layers. The histology of lobed petals was similar with lamina. The presence of papillae is agreed with Wallis (1967) who reported that this is the useful anatomical characters of petals. Clawed petals were very similar with petiole structure. Starch sheath was found around the vascular bundles. According to Borghi & Fernie (2017), these stored carbohydrates may be for the bright red color appearance of this flower.

In phytochemical study, alkaloids, saponins and reducing sugar were not found in petals. This study pointed out that the sepals possess more phytochemical constituents than the petals. Shabir, *et al*., (2011) have reported that IC_{50} value of this flower was 14.80 μg/ml in DPPH method. Vivek *et al*., (2013) also stated that IC_{50} value was 24.88 μg/ml. However, their reports were for all floral parts of this plant. In this study, the antioxidant activities of sepals and petals were separately reported. IC_{50} value for sepals is 6.44 μg/ml and 20.04 μg/ml for petals.

Gan *et al*., (2017) mentioned that polyphenols and alkaloids are major antioxidants. Phenolic compounds and flavonoids (including anthocyanin) are polyphenols (Miguel, 2011). In this study, these polyphenols were detected qualitatively in both parts but alkaloids were not present in petals. Therefore, it can be concluded that the more effective free radical scavenging activity of sepals may be due to the presence of more antioxidant compounds than petals. The quantitative determinations of antioxidant compounds from this flower will be studied in future. Further researches may also be carried out to isolate the pure antioxidant compounds from the floral parts of this plant, especially from sepals.

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