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Pharmacognostical study, HPLC profiling of phenolic and flavonoid contents and biological evaluation of *Delonix regia* growing in Egypt

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Abstract
This study was aimed to carry out pharmacognostical features of different *D. regia* organs, quantification and HPLC profiling of phenolics and flavonoids of leaves extract, also to evaluate possible hepatoprotective activities of the leaves hydroalcoholic extract and its fractions. *Delonix regia* (Hook.) Raf. (Fabaceae) is an ornamental tree with flamboyant flowers. Total phenolic content (TPC) as gallic acid equivalent /100g dried extract (GAE/100g DE), and total flavonoid contents (TFC) as catechin equivalent /100g (CE/100g DE) of the leaves were carried and out using Folin–Ciocalteu's and aluminum chloride assays, respectively. TPC and TFC were 5.51g GAE/100g DE and 53.32g CE/100g DE respectively, with identification of 13 flavonoids, and 17 phenolics. Hesperidin showing the highest flavonoid content (48622.51 mg/100g DE), followed by quercetrin (711.5 mg/100g DE), while hydroxytyrosol was the major identified phenolic (1111.22 mg/100g DE) followed by catechin (1026.11 mg/100 g DE). Ethyl acetate fraction showed significant protection against the elevation in the levels of serum biochemical parameters, normal hepatocytes with minimum fatty changes, portal vain congestion and mild inflammatory cell infiltration around portal vain comparable to CCl4 group ($P< 0.001$). The potent and significant hepatoprotective activity of ethyl acetate fraction may be attributed to its high content of well-known antioxidant phenolic compounds. Thus, regarding our knowledge the present study may be the first one to test the hepatoprotective effect of *Delonix regia* fractions. This could suggest the use of *D. regia* as a natural chemopreventive agent against liver damage; liver toxicity by chlorinated agents.

Keywords: *Delonix regia*, Hepatoprotective; pharmacognostical, flavonoids and phenolics

Introduction
The Caesalpinioideae (Fabaceae) represent approximately 11% of all legume taxa with more than 2250 species mostly tropical and subtropical trees and shrubs [1]. Delonix is a genus of caesalpinioideae that is widely used in folk medicine. *Delonix regia* (Hook.) Raf. (*Poinciana regia* Boj. ex Hook, Gul mohar) is an ornamental tree (10-18 m) with fern-like bipinnately compound leaves and attractive red peacock flowers. It is native to Madagascar and widely grown in Egypt (especially in Cairo and Sinai) lining the streets and gardens with its beauty [2]. *Delonix regia* with an impressive range of medicinal and biological properties, has been used in folk medicine for the treatment of constipation, inflammation, arthritis, hemiplagia, gynecological disorders, and rheumatism [3]. Gulmohar leaves were reported to have antidiabetic [4], anti-inflammatory [5], antimicrobial and antioxidant activities [6]. The methanolic extract of aerial parts possesses hepatoprotective activity against CCl4-induced hepatotoxicity in rats [7]. Chemically, leaves are reported to contain lupeol, epilupeol, *β*-sitosterol [8] and phenolic acids (gallic, protocatechuic and salicylic acids) [9]. It is well established that phenolic antioxidants, namely flavonoids and phenolic acids, are commonly distributed mainly in plant leaves. Natural flavonoids and phenolic acids have increasing interest in food manufacturers and consumers due to its health effects [10].

Egypt is the second all-over the world in death caused by liver diseases reached 41,355 (8.92 %) of total death according to WHO data published in May 2014 [11]. Liver injuries induced by carbon tetrachloride were found to be the best model of xenobiotic-induced hepatotoxicity besides that changes were similar to those of the acute viral hepatitis. The principal causes of hepatotoxicity of CCl4 is trichloromethyl radical (active metabolite) which binds to the macromolecules inducing lipid peroxidative and degradation of biomembranes of the endoplasmic reticulum [12]. Literature survey indicated that no data are available on the anatomical features of *Delonix regia* and a few researches were reported conserning its phenolic content and hepatoprotective activity [13, 7]. Thus, the present study was done with three aims concerning *Delonix regia*; a pharmacognostical discription of the plant organs, total phenolic and flavonoid contents and HPLC profiling using RP-HPLC-UV, also to evaluate

possible hepatoprotective activity of hydroalcoholic leaves extract and its fractions to justify its use as a natural chemopreventive agents.

Materials and Methods

Materials

Gallic acid, (+)-catechin and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Sigma-Aldrich Co., Germany). Authentic phenolics and flavonoids for HLPC profiling were kindly supplied by Agricultural Research Center, Food Technology Research Institute, Giza, Egypt. Silymarin (CID Co., Giza, Egypt), Carbon tetrachloride (E. Merck Ltd., Bombay). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total antioxidant capacity (TAC) kits were purchased from Biodiagnostics Co. (Cairo, Egypt). All other chemicals and solvents used were purchased from local companies in Egypt and were of highest purity grade.

Plant Material and Botanical Study

Leaves of *Delonix regia* was collected from Egypt (6th October City) in May 2016. The taxonomical features were kindly confirmed by Agriculture Research Center, Cairo, Egypt. Voucher samples (52016/A) were kept in the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University. Specimens for morphological studies were dried according to standard herbarium techniques. Anatomical investigations were performed on cross-sections of the stem, petiole, leaf and fruit which were preserved in ethanol (70 %) containing glycerol (5%).

Extraction Process

Freshly collected *Delonix regia* leaves were dried in shade. Hydroalcoholic extract was prepared by powdering (1 Kg) followed by percolating in ethanol (70 %) till exhaustion. Ethanol was evaporated under reduced pressure (Rotavapor® R-300, BÜCHI, Switzerland). Subsequently, dried hydroalcoholic extract (100 g) was sonicated with distilled water (500 ml) for 30 min. Using solvent-solvent extraction, suspension was fractionated with *n*-hexane (6x500 ml), ethyl acetate (7x500 ml) and the remaining aqueous fraction. After evaporation, extract and fractions were suspended in distilled water containing few drops of Tween 80 to be used in biological activity.

Total Phenolics and Flavonoids Contents

Shade dried *Delonix regia* leaves powder (1 g) was defatted with *n*-hexane (10 ml, twice) followed by extraction with methanol (95%, 50 ml) using ultrasonic until exhaustion. Methanol was distilled and extract was transferred to measuring flasks (100 ml). The volume was adjusted with distilled water for the determination of total phenolic content. While for determination of total flavonoid content, adjusted measuring flasks with ethanol after the same previous method. By measuring the intensity of the color developed using UV–visible spectrophotometer (P/N 204-58000, Shimadzu Corporation, Kyoto, Japan), Phenolics (calculated as gallic acid equivalent) were complexed with Folin–Ciocalteu's phenol reagent with reference to a pre- established standard calibration curve [14]. While flavonoids quantification (calculated as catechin) was based on measuring the intensity of developed color when mixed with ALCl3 using UV–visible spectrophotometer with reference to pre-established standard calibration curves [14].

HPLC profiles of phenolics and flavonoids [15, 16].

Hydroalcoholic extract (5 g) was extracted with aqueous acetone (70 %, 100 ml) using an Ultra- Turrax blender. After removing acetone, the residue (3.2 g) was sonicated in 3 ml of methanol (5 min) then centrifuged at 1000 rpm (10 min). The supernatant was filtered through a 0.2 millipore membrane filter before HPLC analysis. Separation and determination of phenolics were performed using Hewlett packared (series 1050) equipped with autosampling injector, solvent degasser, quaternary HP pump (series 1050), a Lichrosorb RP-18 column (4.0 mm i.d., 250 mm; 5μm) (Merck, Darmstadt), and ultraviolet (UV) detector set at (280 and 330 nm for phenolics and flavonoids respectively). The column temperature was maintained at room temperature. Elution was carried out using methanol and acetonitrile (2:1) as a mobile phase at flow rate of 1 ml/min. Peak assignment was confirmed by injection of authentic phenolics and flavonoids. The retention time and peak area were used to calculate compound concentrations by the data analysis of Hewlett Packared software. The relative concentration of the detected compounds were determined from the peak areas.

Animals

Mature Wister strain male albino (56 rat, 160±10 g) and (24 mice, 25±5 g) were taken for this experiment. Animals were acclimatized for 7 days to our laboratory conditions prior to the experiment. Animals were housed in colony cage (6 rats per cage) at an ambient temperature of 25±2 ºC with normal light dark cycle and free access to standard food and water. The principles and instruction of laboratory animal care were followed throughout the experiment.

Hepatoprotective Assay

LD50 of hydroalcoholic extract of *Delonix regia* leaves were determined according to the Organization for Economic Co-operation and Development (OECD)-423 guidelines, for the acute toxicity class method [17]. Doses of 1, 3, and 5 g/kg were chosen as dose level that would be expected to allow the identification of dose producing evident toxicity in mice. Hepatoprotective protocol was carried out [18]. Rats were divided into 7 groups and treatment schedule for 7 days was as followed; Control group and CCL4 group; rats remain under normal conditions. Silymarin group; rats received 100 mg/kg p.o. once daily of silymarin. Hydroalcoholic extract group; rats received 200 mg/kg p.o. once daily [13]. *n*-hexane, ethyl acetate and remaining aqueous fractions groups; rats received 100 mg/kg p.o. once daily of each extract separately. On the 7th day all rats except control one subjected to hepatotoxicity by single intraperitoneal injection of 30 % CCL4 in corn oil (1ml/kg). After 24 h of hepatotoxicity (on the 8th day); blood was collected from rats of all groups by puncturing the retro orbital plexus in centrifuge tube and allowed to clot for 45 min. Serum was separated and various biochemical parameters i.e., aspartate amino transferase (AST), alanine amino transferase (ALT) [19], and total antioxidant capacity (TAC) [20] were estimated. The enzyme activity was expressed as units/liter (U/L) computed directly from the absorbance values.

Histopathological Investigation

After blood sampling for the biochemical analysis, animals were sacrificed; quickly dissected and a small portions of liver were washed in saline and quickly fixed in formalin (10 %). The specimens were processed by standard histopathological technique. Sections (6 μm) were prepared

and stained with Haematoxylin and Eosin (H&E), examined and photographed under microscope [21].

Statistical analysis

Analysis biological experiments were repeated at least three times. Data are presented as mean±SEM and statistically analyzed using Student's t-test using Graph Pad Prism15.0 (Graph Pad Prism Software Inc., San Diego, USA). The criterion for statistical significance was taken as $P < 0.05$.

Results and Discussion

Botanical macromorphology study

Fabaceae is the second largest family of flowering plants that exceed in the number of genera and species. It is widespread in distribution, divided into six subfamilies including Caesalpinioideae [22]. *Delonix regia* (Caesalpinioideae) is medium-sized, fast growing, deciduous tree. Horizontal branches forming a diameter that is wider than tree's height with crown umbrella shaped and spreading long branches. The stem is erect, cylindrical and showing a solid light green interior. It measures 0.2-0.3 cm in diameter. The surface is smooth and glabrous. It has fibrous fracture and characteristic odour and taste. The petiole is light green, cylindrical, pulvinate, and measures 8-10 cm length and 3-4 mm wide, and. Leaves are evergreen alternate bipinnately compound with 10-25 pairs of pinnae, each with 30-60 pairs of primary opposite leaflets or pinnae. Each pinnae is further divided into 10-20 pairs of secondary leaflets. The lamina is linear oblong in shape with rounded apex. The upper surface is dark green and lower surface is lighter. The surface is smooth, papery texture. It measures 1-1.5 cm in length and 0.5-0.7 cm in width. The leaf has a characteristic odour and taste. Flowers are arranged in loose terminal clusters, large (~10 cm across) and bright red in colour. The sepals are 5 fleshy and green on the outside but crimson on the inner side, pointed, finely hairy, about 2.5 cm long. Petals are separate and distinct. Out of five petals; one is larger and has a prominent white-to-creamy-yellow blotch. The other four are crimson. There are 10 stamens with red filaments. Pistil has a hairy one celled ovary about 1.3 cm long. Style is about 3 cm long. Fruiting occurs between August and October. Legumes are green and flaccid when young, turning to dark brown, hard, woody (30-75 cm long, 3.8 cm thick, 5-7.6 cm broad), ending in a short beak when mature. Indehiscent legumes have many horizontally partitioned seed chambers finally splitting into 2 parts. The conspicuous legumes hang down and remain attached most of the year even when the trees are leafless. Seeds are dark brown, slightly elongated to rod-shaped (2 cm long), glossy, smooth with hard seed coats and streaked.

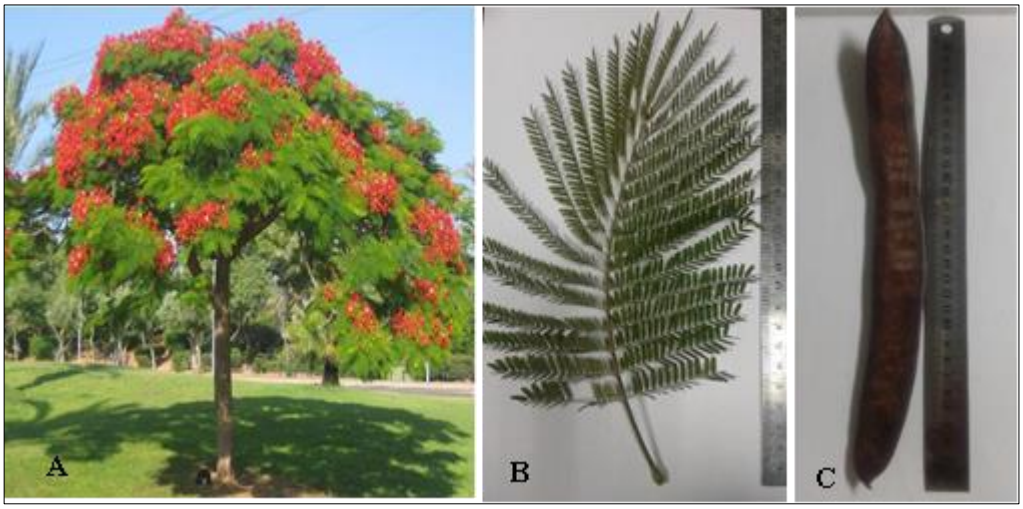

Fig 1: Photographs of *Delonix regia* (A) tree (X= 1/200), (B) leaf (X= 1/2), (C) legume (X= 1/3).

Microscopic features

The stem microscopic features

The stem is circular in outline. The epidermis is formed of polygonal cells with thin, straight anticlinal walls and covered with thin smooth cuticle, devoid of stomata. The cortex is formed of 8-11 rows of cellulosic, thick walled parenchyma with relatively narrow intercellular spaces. The endodermis is not distinct. The pericycle is formed of a continuous ring of 3-4 rows of lignified fibers. They are long, fusiform having thick straight walls, wide lumen and acute apices. The vascular tissue consists of a complete ring of phloem and xylem, separated by cambium and traversed by medullary rays. Thin walled phloem soft tissues formed of 6-8 rows. Thin walled tangentially elongated cells of cambium is formed of 2-4 rows. The xylem is formed of 20-30 rows of a continuous ring of radially arranged lignified xylem elements *viz.*, vessels, tracheids, wood fibers, wood parenchyma and traversed by medullary rays. Vessels are moderately wide having spiral thickening. Tracheids are elongated, having blunt apices, pitted walls and wide lumena. Wood fibers are long, fusiform with thick, straight walls, acute apices and wide lumena.

Wood parenchyma, circular with thick and pitted walls. Medullary rays are uniseriate radially extended and formed of elongated thick pitted walled cells. The pith is formed of wide ring of polygonal large parenchyma cells with thin anticlinal walls. Numerous prisms of calcium oxalate are scattered (figure 2).

The petiole microscopic features

The petiole is subcylindrical to planoconvex in outline, winged and it is formed of epidermis which is composed of tangentially elongated cells with straight anticlinal walls covered with thin smooth cuticle and showing non- glandular stellate hair. The cortex is formed of 6-8 rows of thick walled parenchyma. The endodermis is not distinct. The pericycle is formed of 5-7 rows of lignified pericyclic fibers. The vascular tissue is formed of wide collateral vascular bundles composed of phloem and xylem and crossed by medullary rays. The phloem is formed of 5-7 rows of thin walled phloem soft

tissues. The cambium is formed of 2 rows of thin-walled tangentially elongated cells. The xylem is formed of lignified xylem vessels with spiral and annular thickening, cellulosic thin-walled wood parenchyma and traversed by bi or tri to multiseriate thin walled-medullary rays. The pith is formed of large rounded cellulosic thick walled parenchyma cells with narrow intercellular spaces, prismatic crystals of ca-oxalate are scattered (figure 2).

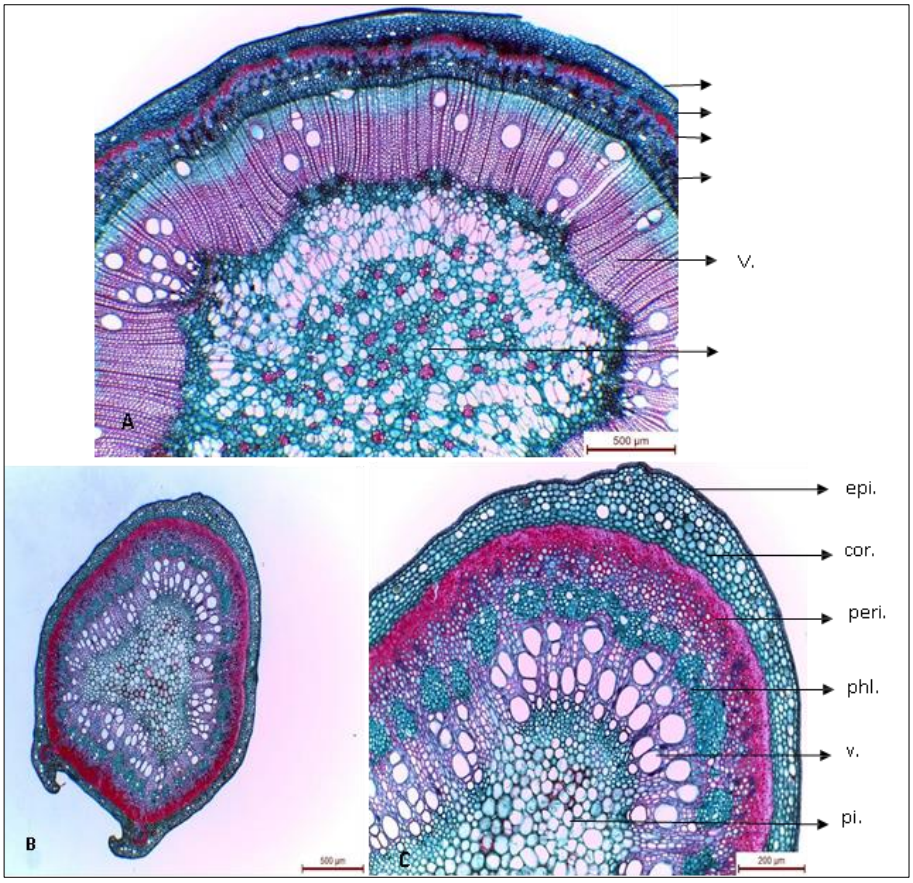

Fig 2: Micromorphology of *Delonix regia* views of the T.S. in stem (A); petiole (B), (C); cor., cortex; epi, epidermis; peri, pericycle; phl, phloem; pi, pith; v., vessles

The leaflets and midrib microscopic features

A transverse section in the leaflets shows upper and lower epidermises, enclosing a dorsiventral mesophyll. The midrib is slightly prominent to the lower side showing one crescent shaped collateral vascular bundle. The epidermises of both surfaces are nearly similar in shape. They are tangentially elongated cells with straight anticlinal walls. Both surfaces are covered with smooth cuticle. Leaflet has paracytic stomata and non-glandular unicellular hair arising from cicatrix. The cells of neural epidermis are polygonal isodiametric, having straight thin walls, covered with thin smooth cuticle and showing paracytic stomata. The mesophyll is heterogenous, differentiated into palisade and sponge tissue. The palisade is formed of one row of columnar, closely packed cells having straight anticlinal walls and containing green plastids. The spongy tissue is composed of 2-3 rows of thin walled parenchymatous cells (figure 3). The cortical tissue of the midrib region consist of axially elongated thin walled cells having straight anticlinal walls, showing narrow intercellular spaces. The endodermis is indistinct. The pericycle is formed of non-lignified fibers. The vascular tissue is crescent shaped collateral type. It composed of the xylem which is composed of lignified vessels, wood parenchyma and wood fibers and traversed by uniseriate medullary rays. The xylem vessels show spiral and annular thickening. Wood fibers are long with wide lumena and blunt apices. Wood parenchyma consists of thin walled parenchyma present between xylem vessels. The cambium is formed of 2 rows of tangentially elongated thin walled cellulosic cambiform cells. The phloem tissue consists of soft tissue of 2-3 rows of thin walled phloem elements (figure 3).

The pod microscopic features

The pod wall appears elongated funnel shaped like in outline showing three distinct tissue layers; epicarp and mesocarp followed by a wide fleshy parenchymatous endocarp. The epicarp is tangentially elongated, having straight anticlinal walls covered with smooth cuticle. The mesocarp is formed of a narrow band of 5-7 rows of collenchyma cells. The endocarp is formed of thin walled cellulosic parenchymatous cells. Collateral vascular bundle is scattered in the inner region; with xylem to the inside and phloem to the outside. The phloem is consisting of soft tissue formed mainly of thin-walled parenchyma cells, sieve tubes and companion cells. The xylem is formed of lignified spiral and annular vessels. The pericycle is present and forming crystal sheath. The endocarp is formed of columnar thin walled cellulosic parenchymatous cells with occasional scattered non lignified sclereids. The inner region is formed of 2-3 rows of thin-walled elongated parenchyma cells (figure 3).

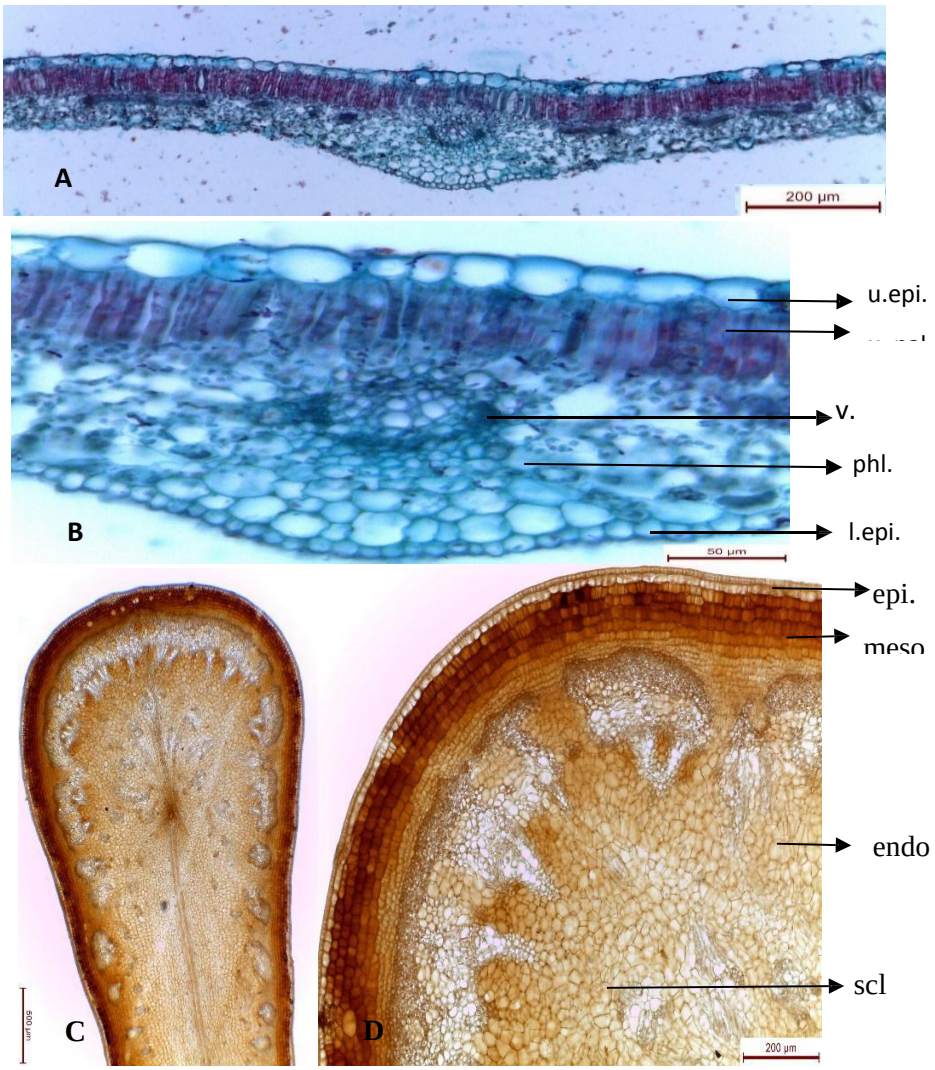

Fig 3: Micromorphology of *Delonix regia* views of the T.S. in leaf (A), midrib (B), fruit (C), (D); L. epi., lower epidermis; phl., phloem; u.epi., upper epidermis; u.pal., upper palisade; v., xylem vessels; endo, endocarp; epi., epicarp; meso., mesocarp; scl., sclereids

Total phenolic and flavonoid content

Hydroalcoholic extract of *Delonix regia* leaves afforded 120 g (12 %). Fractionation (100 g DE) afforded 10 g, 13 g, and 77 g residues for *n*-hexane, ethyl acetate, and remaining aqueous fractions respectively. The total phenolic contents (TPC) and total flavonoid contents (TFC) were determined in *Delonix regia* leaves extract. Extraction with methanol show greater efficacy in the extraction of polar phytochemicals such as phenolics and flavonoids [22]. The extracted amounts of total phenolics and total flavonoids were 5.51g GAE/100g DE and 53.32g CE/100g DE respectively. The result is aligning with those reported previously [9]. The presence of phenolics and flavonoids affects the antioxidant activity and other biological properties of the plant extracts produced.

HPLC flavonoids profile

Hydroalcoholic extract (70 %) is a highly polar solvent, known to be an efficient and widely used to extract phenolics and flavonoids from plant materials. The presence of phenolics and flavonoids affects the antioxidant activity and other biological properties [23]. Relative concentrations of the detected phenolics and flavonoids were determined by peak areas; where thirteen flavonoid compounds were identified by matching their retention times against those of the standards. Hesperidin was the predominant flavonoid showing the highest abundance (48622.51 mg/100g), followed by quercetrin (711.5 mg/100g) and luteolin-7-glucose (634.81 mg/100g). Apigenin (18.08 mg/100g) and kaempferol (31.97 mg/100g) were existed in relatively smaller concentrations (table 1). However, our results cannot be compared with literatures, because this is the first work of its kind on the preliminary identification and quantification of flavonoids of this plant.

Table 1: HPLC flavonoids profile of hydroalcoholic extract of *Delonix regia* leaves

No.	Results Flavonoids (mg/100 g DE)	
1	Apigenin-6-arabinose-8-galactose	543.48±8.18
2	Apigenin-6-rhamnose-8-glucose	84.15±0.89
3	Luteolin-7-glucose	634.81±8.71
4	Hesperidin	4862 2.51±190.23
5	Rosmarinic acid	134.96±5.31
6	Apigenin-7-glucose	54.43±0.35
7	Apigenin-7-*O*-neohespiroside	74.60±0.48
8	Kampferol 3,7-dirhamnoside	505.60±1.13
9	Quercetrin	711.50±2.8
10	Kampferol-3-(2-*P*-comaroyl) glucose	264.03±1.87
11	Acacetin-7-neohesperside	60.09±0.46
12	kaempferol	31.97±0.33
13	Apigenin	18.08±0.3
Total identified flavonoids		5174 0.21
Percentage of total identified flavonoids (%)		51.74 %

Values are presented as mean ± SE of triplicate observations.

HPLC phenolics profile

Hydroxytyrosol (3-Hydroxytyrosol) was the major identified phenolic (1111.22 mg/100 g). Catechin and pyrogallol were present in relatively high amounts (1026.11, 453.84 mg/100 g respectively). Ellagic and vanillic acid were the most abundant phenolic acids 441.54 and 402.91 mg/100g respectively (table 2). All of the detected phenolic compounds are known to have antioxidant properties *viz*; hydroxytyrosol can protect cells against injury due to oxidation process [24]. Ellagic acid; the well-known antioxidant; has chemoprotective effect by reducing oxidative stress [25]. According to Shabir *et al*, gallic acid, salicylic acid and protocatechuic acid were the most abundant phenolic acids in the leaves [9], but they did not identify other phenolics in their study which were detected in the present work, *i.e.*, Hydroxytyrosol, catechin, pyrogallol, ellagic acid and vanillic acid.

Table 2: HPLC phenolics profile of hydroalcoholic extract of *Delonix regia* leaves

No.	Results Phenolic compounds (mg/100g)	
1	Gallic acid 7.88	±0.42
2	Pyrogallol 453.	84±3.22
3	3-Hydroxytyrosol 1111.22±9.31	
4	Protocatechuic acid 122.	79±1.91
5	Catechin 1026.11±8.72	
6	Catechol 304.	94±0.31
7	*P*-hydroxybenzoic acid 183.	66±0.11
8	Caffeic acid 67.2	6±0.03
9	Vanillic acid 402.	91±2.71
10	*P*-coumaric acid 161.	23±0.98
11	Ferulic acid 41.4	2±0.08
12	Iso-ferulic acid 81.5	3±0.17
13	α-coumaric acid 76.6	9±0.21
14	Ellagic acid 441.	54±3.3
15	Benzoic acid 302.	30±2.19
16	3,4,5-methoxy-cinnamic acid 84.8	0±0.34
17	Cinnamic acid 4.80	±0.01
Total identified phenolics 487		
Percentage of total identified phenolics (%) 4.87		4.92%

Values are presented as mean ± SE of triplicate observations.

Hepatoprotective activity

Liver injuries induced by carbon tetrachloride were found to be the best model with changes similar to xenobiotic and viral hepatotoxicity. Silymarin a well-known hepatoprotective compound; was reported to have a protective effect on hepatocytes plasma membrane [18]. According to WHO data published in May 2014, death caused by liver diseases in Egypt reached 41,355 (8.92 %) of total death, and this ranks Egypt as the second all over the world [11]. The presence of significant proportions of hesperidin, quercetrin and hydroxytyrosol with their reported free radical scavenging, anti-lipid peroxidation and hepatoprotective activities [10, 24]. In addition to, the reported antioxidant activity of all organs of *Delonix regia* [6] added a support for the authors to evaluate the hepatoprotective activity of the leaves hydroalcoholic extract and its fractions against CCl4 induced liver damage. The LD50 of hydroalcoholic extract of *Delonix regia* leaves was found more than 5 g/kg, p.o. in mice. Therefore, 200 mg/kg of hydroalcoholic extract and 100 mg/kg of fractions are considered to be convenient and safe doses. Rats receiving treatments showed normal values for the serum biochemical parameters determined at the selected dose regimen. After CCL4 injection (8th day), there was a significant ($P< 0.01$) elevation in serum biochemical parameters AST and ALT of CCL4 group compared to the control group. Rats pretreated with silymarin were protected considerably against the elevation in the levels of the biochemical parameters when compared with the CCl4 group ($P< 0.05$). Biochemical parameters came towards the control level in rats pretreated with hydroalcoholic extract and fractions comparable to CCl4 group ($P< 0.01$), moreover; ethyl acetate fraction group was significant different ($P< 0.01$) when compared to CCl4 group and non-significantly when compared with silymarin group ($P> 0.05$). The results were summarized in (table 3). The potent and significant hepatoprotective activity of ethyl acetate fraction of *Delonix regia* leaves may be attributed to its high content of phenolic compounds [26, 27]. Data represented for TAC showed that CCl4 administration caused a significant decrease ($P< 0.01$) in TAC as compared to control group. Pretreatment of rats with extract and fractions significantly increased ($P< 0.01$) the level of TAC as compared to CCl4 treated group. Ethyl acetate fraction showed a significant decrease in the enzyme levels regarding their respective normal values which indicates stabilization of the hepatocyte cell membrane as well as repairing of hepatic tissue damage caused by CCl4 [2]. Since, the preventive action of the liver damage induced by CCL4 has widely been used as a marker of hepatoprotective activity of drugs in general and as mentioned earlier that the liver toxicity is one of the death leading diseases in Egypt

Table 3: Effect of *Delonix regia* leaves extract and fractions on serum biochemical parameters and total antioxidant capacities in CCl4-Induced hepatotoxicity rats

Groups	**Biochemical parameters** (U/L)		
	AST	**ALT**	**TAC**
Control	26.1 ±1.4	31.11±0.97	108.3±1.29
CCL4	170.1±1.28a	198.42±3.36a	73.61±2.17a
Silymarin	64.28±1.4b	80.87±0.77b	117.3±1.64b
Hydroalcoholic extract	98.7±7.06b	153.17±6.48b	87.08±2.73b
n-hexane fraction	93.08±6.73b	108.73±8.14b	117.5±2.74b
Ethyl acetate fraction	65.69±4.83bc	74.61±3.37bc	82±3.58bc
Remaining aqueous fraction	107.6±4.61b	127.34±8.58b	126.7±9.13b

Values are given as mean ± SD for groups of six animals each. ues are statistically significant at $p< 0.01$

a statistically significant from control group at $p< 0.01$

b statistically significant from CCL4 group at $p< 0.01$

c statistically non-significant from silymarin group at $p> 0.05$

Histological and Histochemical investigation

The microscopic observations provided good information about organ morphology to confirm the biochemical studies. There was no histopathological or histochemical alteration observed and normal histological structure of liver cells was recorded in control group, which shows normal hepatocytes

with well-preserved cytoplasm, nucleus, and central vein (figure 4). In CCL4 group the liver sections showing total loss of cellular architecture with enlarged nuclease, marked degenerative changes and fatty changes with dilated liver sinusoids, as well as areas of inflammatory cell infiltration with clear pyknotic reaction other nuclear changes can be seen (karyolysis and karyorrhexis). Pretreatment of rats with silymarin resulted in moderate cellular degeneration with more or less normal hepatocytes with mild fatty changes with necrotic tissue area (NT) and dilated liver sinusoids. Rats pretreated of with leaves extract and fractions of *Delonix regia* before CCL4 toxicity showed mild degenerative changes with mild apoptotic reactions, pyknosis (PIC) and karyolysis (KRL) with mild necrotic tissue appearance (NT), mild frequency of Kuppfer cells (KC) and mild dilatation of liver sinusoids (DLS) and more or less normal tissue histoarchitexture normal hepatocytes. Rats pretreated of with ethyl acetate fraction showed mild nuclear changes and apoptosis ranging from pyknosis, karyolysis and karyorrhexis, mild necrotic changes and normal appearance of Kuppfer cells, mild dilatation of liver sinusoids with normal tissue histoarchitexture (figure 4).

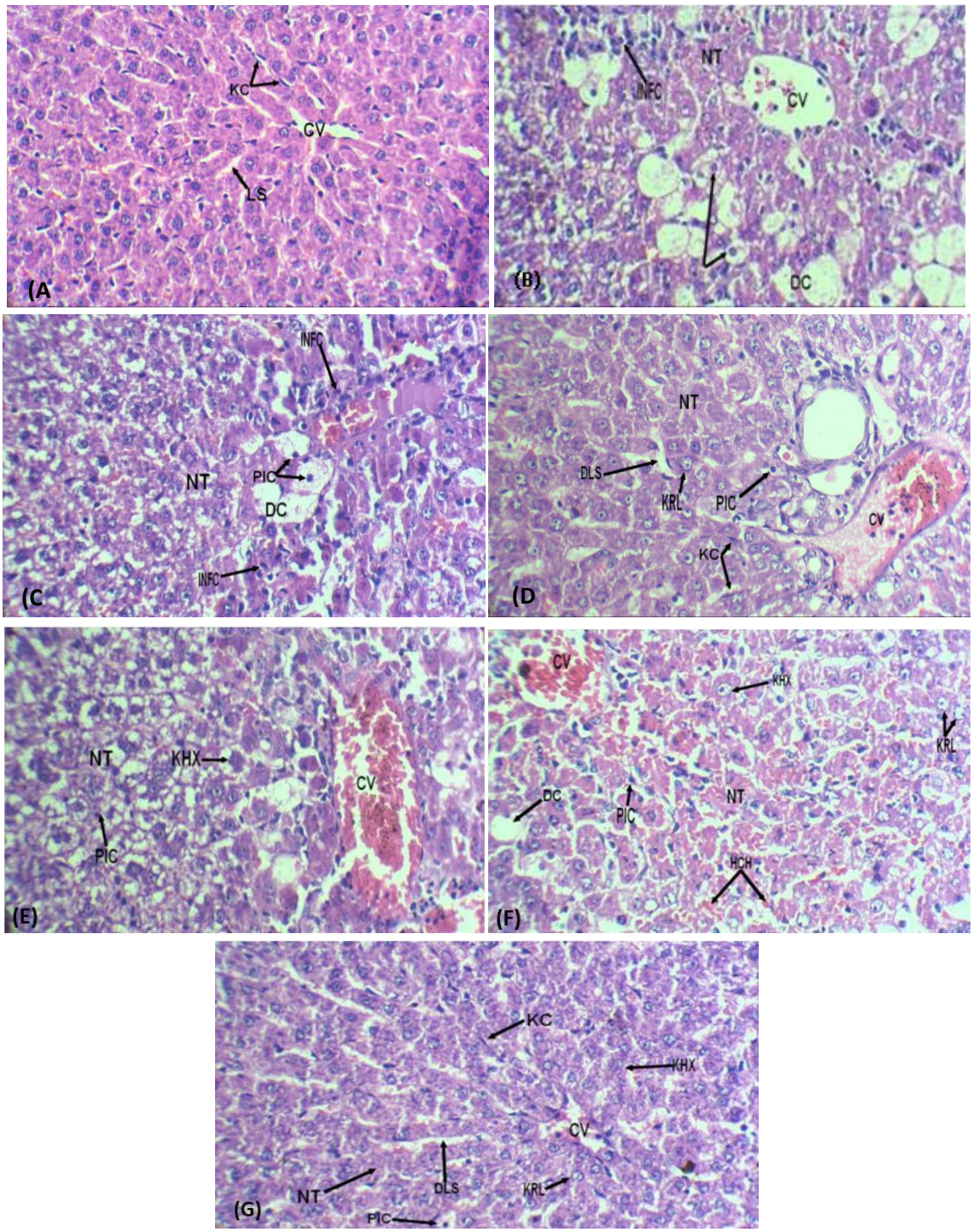

Figure 4: liver photomicrograph of (A) control group showed normal hepatocytes and normal distribution of Kuppfer cells (KC) with normal liver sinusoids (LS), (B) CCL4 group showed loss of hepatic histoarchitexture with marked infiltration of inflammatory cells (INFC), necrotic tissue (NT), degenerated cells (DC) with nuclear changes, (C) silymarin group showing marked infiltration of inflammatory cells with pyknotic changes (PIC), moderate degenerative changes, (D) hydroalcoholic extract group, (E) *n*-hexane fraction group, and (F) remaining aqueous fraction showed mild degenerative changes with mild apoptotic reactions, pyknosis (PIC) and karyolysis (KRL) and more or less normal tissue histoarchtexture normal hepatocytes, (G) ethyl acetate fraction group showed mild nuclear changes, mild necrotic changes and normal appearance of Kuppfer cells, mild dilatation of liver sinusoids with normal tissue histoarchitexture.

Conclusion

In conclusion, this work figures out the macromorphological characters of different organs of *D. regia* which were described and illustrated in order to identify the plant organs in entire form. In addition this study declared that *D. regia* leaves possess hepatoprotective activity which may be attributed to its antioxidant activity by its high phenolic and flavonoids contents. The study has established that *D. regia* is holding a great expectation for food and pharmaceutical applications as a chemopreventive agent. However, further detailed studies are required to clarify the exact role of the potential active constituents. Such aim will enable us to proceed further for exploration of the extract in subsequent *in-vivo* studies and later in controlled clinical trials.

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