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Original Article

Pharmacognostical and Biological Studies of *Delonix regia* growing in Egypt: HPLC Profiles

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ABSTRACT

Background: *Delonix regia* (Hook.) Raf. (Fabaceae) is an ornamental tree with flamboyant flowers. **Objective:** to carry out pharmacognostical studies to evaluate the features of different *D. regia* organs, quantification and HPLC profiling of phenolics and flavonoids of leaf extract, also to evaluate possible hepatoprotective activities of the leaf hydroalcoholic extract and its fractions. **Materials and Methods:** Total phenolic content (TPC) as gallic acid equivalent /100 g dried extract (GAE/100 g DE), and total flavonoid content (TFC) as catechin equivalent /100 g (CE/100 g DE) of the leaves were carried out using Folin–Ciocalteu's and aluminum chloride assays, respectively. Hepatoprotective activity was determined against CCl_4 induced hepatotoxicity in rats. **Results:** TPC and TFC were 5.5 g GAE/100 g DE and 53.3 g CE/100 g DE respectively, with identification of 13 flavonoids, and 17 phenolics. Hesperidin was present as the highest flavonoid content (48622.5 mg/100 g DE), followed by quercetrin (711.5 mg/100 g DE), whilst hydroxytyrosol was the major identified phenolic compound (1111.2 mg/100g DE) followed by catechin (1026.1 mg/100 g DE). The 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 the portal vain comparable to CCl_4 group ($P< 0.001$). The potent and significant hepatoprotective activity of the ethyl acetate fraction may be attributed to its high content of antioxidant phenolic compounds. Thus, the present study may be the first to test the hepatoprotective effect of *Delonix regia* fractions. **Conclusion:** This study recommends the use of *D. regia* as a natural chemopreventive agent against liver damage and liver toxicity caused by chlorinated agents.

Key words: *Delonix regia*, Flavonoids, Hepatoprotective, Pharmacognostical, Phenolics.

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INTRODUCTION

The Caesalpinioideae (Fabaceae) represent approximately 11% of all legume taxa with more than 2250 species of 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 for 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] The leaves were reported to have antidiabetic,[4] anti-inflammatory,[5] antimicrobial and antioxidant activities.[6] A methanolic extract of aerial parts possesses hepatoprotective activity against CCl_4-induced hepatotoxicity in rats.[7] Chemically, the 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, including flavonoids and phenolic acids, are commonly distributed in plant leaves. Natural flavonoids and phenolic acids have increasing interest in food manufacturers and consumers due to their health effects.[10]

Egypt has the second highest rate of death globally caused by liver diseases which reached 41,355 (8.92 %) of total death according to WHO data published in May 2014.[11] Liver injuries induced by carbon tetrachloride (CCl_4) are the best model of xenobiotic-induced hepatotoxicity, with changes similar to those of the acute viral hepatitis. The principal causes of hepatotoxicity of CCl_4 is via the trichloromethyl radical (active metabolite), which binds to cellular macromolecules, inducing lipid peroxidative and degradation of biomembranes of the endoplasmic reticulum.[12] No data is 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 determination and HPLC profiling using RP-HPLC-UV, and to evaluate possible hepatoprotective activity of a 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 HPLC 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* were 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. A hydroalcoholic extract was prepared by powdering 1 kg of dried leaves, followed by percolating in ethanol (70%) until exhaustion. Ethanol was evaporated under reduced pressure (Rotavapor® R-300, BÜCHI, Switzerland). Subsequently, the dried hydroalcoholic extract (100 g) was sonicated with distilled water (500 ml) for 30 min. Using solvent-solvent extraction, the suspension was fractionated with *n*-hexane (6x500 ml), ethyl acetate (7x500 ml) and the remaining aqueous fraction. After evaporation, the extract and fractions were suspended in distilled water containing a few drops of Tween 80 to be used in biological activity assays.

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 extraction until exhaustion. Methanol was distilled and the extract was transferred to measuring flask (100 ml). The volume was adjusted with distilled water for the determination of total phenolic content. For determination of total flavonoid content, the same previous method was done but adjusts the measuring flask with ethanol. 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] Flavonoid quantification (calculated as catechin) was based on measuring the intensity of developed color when mixed with $ALCl_3$ using UV–visible spectrophotometer with reference to pre-established standard calibration curves.[14]

HPLC profiles of phenolics and flavonoids[15-16]

The hydroalcoholic extract (5 g) was extracted with aqueous acetone (70 %, 100 ml) using an Ultra-Turrax blender. After removing the 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 Packard HPLC system (series 1050) equipped with an autosampling injector, a solvent degasser, a quaternary HP pump (series 1050), a Lichrosorb RP-18 column (4.0 mm i.d., 250 mm; 5µm) (Merck, Darmstadt), and an 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 Packard software. The relative concentrations of the detected compounds were determined from the peak areas.

Animals

Mature male albino rats (56, 160±10 g) and male albino mice (24, 25±5 g) of Wister strain (7-9 weeks age) 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 or mice per cage) at an ambient temperature of 25±2 °C 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

The LD_{50} 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 and a hepatoprotective protocol was carried out.[18] Rats were divided into 7 groups and the treatment schedule for 7 days was as followed; Control group and CCL_4 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 once daily of hydroalcoholic extract.[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 the control group were subjected to hepatotoxicity by single intraperitoneal injection of 30 % CCL_4 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 by standard methods. 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. A small portion of liver were washed in saline and quickly fixed in formalin (10 %) for microscopic evaluation. The specimens were processed by standard histopathological technique. Sections (6 μm) were prepared and stained with haematoxylin and eosin (H and E), examined and photographed under microscope.[21]

Statistical analysis

Biological experiments were repeated at least three times. Data are presented as mean ±SD 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 in terms of 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 (Figure 1a). It has 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. Leaves are evergreen, alternate and bipinnately compound with 10-25 pairs of pinnae, each with 30-60 pairs of primary opposite leaflets or pinnae (Figure 1b). Each pinnae is further divided into 10-20 pairs of secondary leaflets. The lamina is linear to oblong in shape, with a rounded apex. The upper surface is dark green and lower surface is lighter in colour. The surface is smooth with a papery texture. Leaflets measure 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. The pistil has a hairy one celled ovary about 1.3 cm long. The 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

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

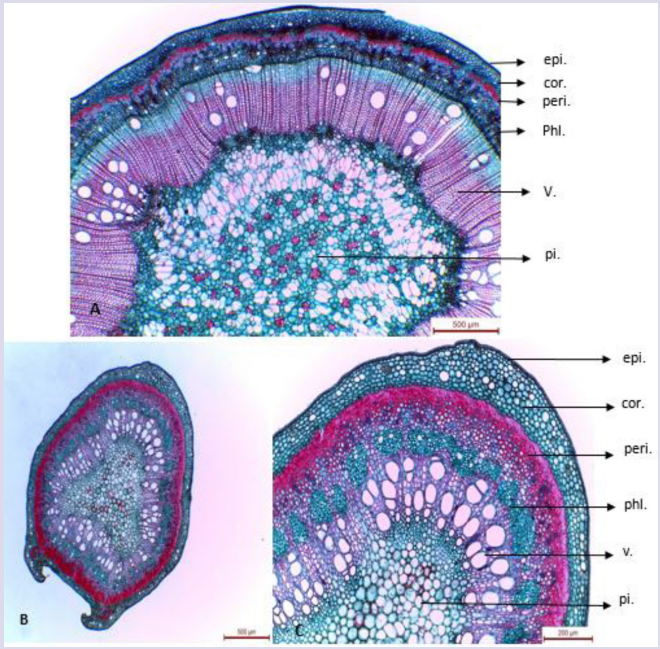

Figure 2: Micromorphology of *Delonix regia* views of the (A) T.S. in stem; (B) and (C) T.S. in petiole. cor. = cortex; epi = epidermis; peri = pericycle; phl = phloem; pi, =pith; v. = vessels.

when mature (Figure 1c). 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.

Microscopic features

The stem microscopic features

The stem is circular in outline (Figure 2a). The epidermis is formed of polygonal cells with thin, straight anticlinal walls 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. The thin walled phloem soft tissues are formed of 6-8 rows and the thin walled tangentially elongated cells of the cambium are 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. The vessels are moderately wide having spiral thickening. The tracheids are elongated, having blunt apices, pitted walls and wide lumena. The wood fibers are long, fusiform with thick, straight walls, acute apices and wide lumena. The wood parenchyma is 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.

The petiole microscopic features

The petiole is subcylindrical to planoconvex in outline, winged. 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 (Figure 2b). 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 2c).

The leaflets and midrib microscopic features

A transverse section in the leaflets (Figure 3a) shows upper and lower epidermises, enclosing a dorsiventral mesophyll. The midrib (Figure 3b) is slightly prominent to the lower side showing one crescent shaped collateral vascular bundle. The epidermises of both surfaces are similar in shape. They are tangentially elongated cells with straight anticlinal walls. Both surfaces are covered with smooth cuticle. Each 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. The cortical tissue of the midrib region consists 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.

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 (Figure 3c). The epicarp is tangentially elongated, having straight anticlinal walls covered with smooth cuticle.

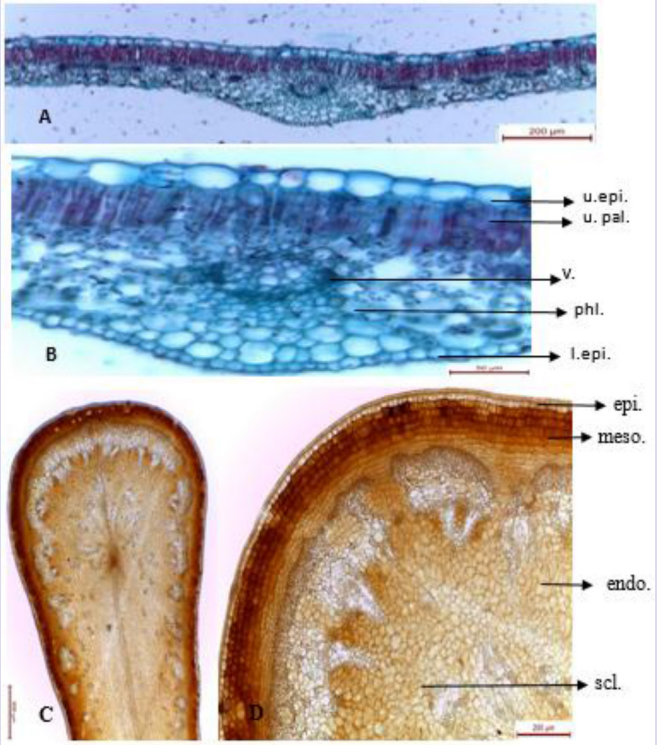

Figure 3: Micromorphology of *Delonix regia* views of the (A) T.S. in leaf, (B) T.S. in midrib, (C) and (D) T.S. in fruit. L. epi., lower epidermis; phl., phloem; u.epi., upper epidermis; u.pal., upper palisade; v., xylem vessels; endo, endocarp; epi., epicarp; meso., mesocarp; scl., sclereids.

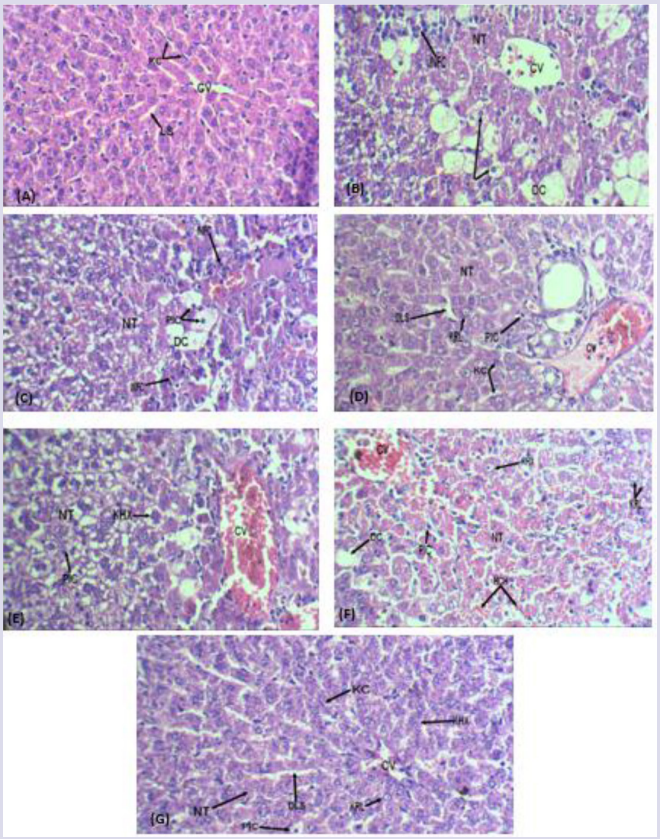

Figure 4: Liver photomicrograph of (A) control group showed normal hepatocytes and normal distribution of Kuppfer cells (KC) with normal liver sinusoids (LS), (B) CCL_4 group showed loss of hepatic histoarchitexture with marked infiltration of inflammatory cells (INFC), necrotic tissue (NT), degenerated 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.

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. Some collateral vascular bundles are scattered in the inner region; with xylem to the inside and phloem to the outside. The phloem consists 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.

Total phenolic and flavonoid contents

The hydroalcoholic extract of *Delonix regia* leaves afforded 120 g (12 % extracted material). 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 content (TPC) and total flavonoid content (TFC) were determined in *Delonix regia* leaves extract. Extraction with methanol show greater efficacy in the extraction of the polar phenolics and flavonoids 22. The extracted amounts of total phenolics and total flavonoids were 5.5 g GAE/100 g DE and 53.3 g CE/100g DE respectively. This is similar to the levels reported previously.[9] The presence of phenolics and flavonoids affects the antioxidant activity and other biological properties of the plant extracts produced.[10]

HPLC flavonoids profile

The hydroalcoholic extract (70%) is a highly polar solvent, which is 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. Thirteen flavonoid compounds were identified by matching their retention times against those of the flavonoid standards. Hesperidin was the predominant flavonoid, showing the highest abundance (48622.5 mg/100 g DE), followed by quercetrin (711.5 mg/100 g DE) and luteolin-7-glucose (634.8 mg/100 g DE). Apigenin (18.1 mg/100 g DE) and kaempferol (32.0 mg/100 g DE) were present in smaller concentrations (Table 1). However, our results cannot be compared with the literature as this is the first work of its kind reporting the preliminary identification and quantification of flavonoids of this plant species.

HPLC phenolics profile

Hydroxytyrosol (3-Hydroxytyrosol) was the major identified phenolic (1111.2 mg/100 g DE). Catechin and pyrogallol were present in relatively high amounts (1026.1, 453.8 mg/100 g DE respectively). Ellagic and vanillic acid were the most abundant phenolic acids (441.5 and 402.9 mg/100 g DE) 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 effects 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] although they did not identify other phenolics in their study. In our study, hydroxytyrosol, catechin, pyrogallol, ellagic acid and vanillic acid were also identified.

Hepatoprotective activity

Liver injuries induced by carbon tetrachloride are a good model to study induced hepatotoxicity in animal models by initiating lipid peroxidation, 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 (2014), death caused by liver diseases in Egypt reached 41,355 (8.9%) of total deaths, and this ranks Egypt as the second globally for mortality due to hepatotoxicity.[11] Oxidative stress is a redox disequilibrium in which the pro-oxidant/antioxidant balance is shifted in the pro-oxidants. Exposure to toxic chemicals, pollutants and drugs can cause cellular injuries through activation of reactive oxygen species (ROS).[26] The presence of significant proportions of phenolics (hesperidin, quercetrin and hydroxytyrosol) with their reported free radical scavenging, and hepatoprotective activities,[10,24] in addition to, the reported antioxidant activity of *Delonix regia*[6] added a clue for the authors to evaluate the hepatoprotective activity of the leaf hydroalcoholic extract and its fractions. The LD_{50} 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 for this study. Rats receiving these treatments showed normal values for the serum biochemical parameters determined at the selected dose regimen (Table 3). After CCL_4 injection (8th day), there was a significant (P<0.01) elevation in serum biochemical parameters AST and ALT of CCL_4 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 CCl_4 group (P< 0.05). Biochemical parameters approached the control level in rats pretreated with hydroalcoholic extract and fractions comparable to CCl_4 group (P<0.01). Moreover, the ethyl acetate fraction group was significant different (P<0.01) when compared to CCl_4 group, although not significantly different when compared with silymarin group (P>0.05) (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.[27-28] Data represented for TAC showed that CCl_4 administration caused a significant decrease (P<0.01) in TAC compared to the control group. Pretreatment of rats with the extract and its fractions significantly increased (P<0.01) the level of TAC compared to the CCl_4 treated group. The ethyl acetate fraction showed a significant

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

No.	Flavonoids	Results (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	48622.51±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	51740.21
	Percentage of total identified flavonoids (%)	51.74 %

Values are presented as mean ± SE of triplicate observations.

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

No.	Phenolic compounds	Results (mg/100 g DE)
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	67.26±0.03
9	Vanillic acid	402.91±2.71
10	*P*-Coumaric acid	161.23±0.98
11	Ferulic acid	41.42±0.08
12	Isoferulic acid	81.53±0.17
13	α-Coumaric acid	76.69±0.21
14	Ellagic acid	441.54±3.3
15	Benzoic acid	302.30±2.19
16	3,4,5-methoxy-cinnamic acid	84.80±0.34
17	Cinnamic acid	4.80±0.01
	Total identified phenolics	4874.92
	Percentage of total identified phenolics (%)	4.87 %

Values are presented as mean ± SE of triplicate observations.

Table 3: Effect of *Delonix regia* leaves extract and fractions on serum biochemical parameters and total antioxidant capacities in CCl_4-Induced hepatotoxicity rats.

Groups	Biochemical parameters (U/L)		
	AST	ALT	TAC
Control	26.1 ±1.4	31.11±0.97	108.3±1.29
CCL_4	170.1±1.28[a]	198.42±3.36[a]	73.61±2.17[a]
Silymarin	64.28±1.4[b]	80.87±0.77[b]	117.3±1.64[b]
Hydroalcoholic extract	98.7±7.06[b]	153.17±6.48[b]	87.08±2.73[b]
***n*-hexane fraction**	93.08±6.73[b]	108.73±8.14[b]	117.5±2.74[b]
Ethyl acetate fraction	65.69±4.83[bc]	74.61±3.37[bc]	82±3.58[bc]
Remaining aqueous fraction	107.6±4.61[b]	127.34±8.58[b]	126.7±9.13[b]

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

[a] statistically significant from control group at p< 0.01

[b] statistically significant from CCL_4 group at p< 0.01

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

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 CCl_4.[2] Since, the liver toxicity is one of the death leading diseases in Egypt, *Delonix regia* may be used as a valuable hepatoprotective plant.

Histological and Histochemical investigation

The microscopic observations provided good information about organ morphology to confirm the biochemical studies (Figure 4). 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 4a). In CCL_4 group (Figure 4b), the liver sections showed total loss of cellular architecture, with an 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 (Figure 4c). Rats pretreated of with leaves extract and fractions of *Delonix regia* before CCL_4 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 relatively normal tissue histoarchitexture and normal hepatocytes (Figures 4d-g). 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 4g).

CONCLUSION

This work reports the macro and micromorphological characters of different organs of *D. regia* which were described and illustrated in order to identify the plant organs in the entire form. In addition, this study demonstrates that *D. regia* leaves possess hepatoprotective activity which may be attributed to its antioxidant activity by high phenolic and flavonoid contents. The study has established that *D. regia* has potential 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.

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CONFLICT OF INTEREST

The author decalre no conflict of interest.

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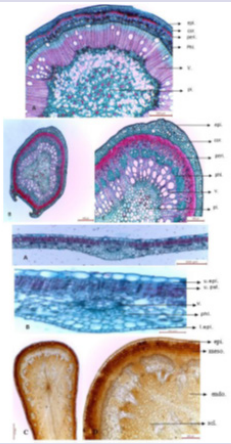
PICTORIAL ABSTRACT

Delonix regia



Groups	Biochemical parameters (U/L)		
	AST	ALT	TAC
Control	26.1 ±1.4	31.11±0.97	108.3±1.29
CCl_4	170.1±1.28^a	198.42±3.36^a	73.61±2.17^a
Silymarin	64.28±1.4^b	80.87±0.77^b	117.3±1.64^b
Hydroalcoholic extract	98.7±7.06^b	153.17±6.48^b	87.08±2.73^b
n-hexane fraction	93.08±6.73^b	108.73±8.14^b	117.5±2.74^b
Ethyl acetate fraction	65.69±4.83^{bc}	74.61±3.37^{bc}	82±3.58^{bc}
Remaining aqueous fraction	107.6±4.61^a	127.34±8.58^b	126.7±9.13^b

No.	Flavonoids	Results (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	48622.51±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	51740.21
	Percentage of total identified flavonoids (%)	51.74 %

SUMMARY

- Total phenolic content and total flavonoid content of *Delonix regia* leaves were 5.5 g GAE/100 g DE and 53.3 g CE/100 g DE respectively with identification of 13 flavonoids, and 17 phenolics. Hesperidin was the highest flavonoid, whilst hydroxytyrosol was the major identified phenolic. The ethyl acetate fraction showed significant protection against the elevation in serum biochemical parameters. The study recommends the use of *D. regia* as a natural chemopreventive agent.

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Research Scopes:

I. Active natural products.

II. Chromatographic and Structure elucidation techniques.

Bibliometrics (June 2018):

Google Scholar: h-index = 3, 36 total citations.

Research Gate: 13 publications; RG Score 10.65, 1312 reads.