Stem Wood and Bark Extracts of *Delonix regia* (Boj. Ex. Hook): Chemical Analysis and Antibacterial, Antifungal, and Antioxidant Properties

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In the present study, the fatty acid components of the wood, bark, and essential oil of wood from *Delonix regia* as well as its antibacterial, antifungal, and antioxidant properties were investigated for the potential ability to control plant and human pathogens. Myristic acid was found to be a major fatty acid in the wood and bark of *Delonix regia,* comprising 10.77% of wood and 9.63% of bark. According to the GC-MS results, naphthalene derivatives were detected in the essential oils from the wood samples. Heptadecane and acyclic hydrocarbons were found in a high percentage (14.05%). Methanol: chloroform (1:1 v/v) wood extract showed effective activity against *Bacillus subtilis*, *Sarcina lutea*, and *Staphylococcus aureus*, where the bark extract was most active against *Escherichia coli*. The essential oil showed good antibacterial activity against *Pectobacterium carotovorum*. The bark extract showed the maximum percentage inhibition of fungal mycelial growth against *Penicillium selerotigenum* (70.37%) and *Paecilomyces variotii* (77.78%), and the essential oil showed moderate inhibition against *Aspergillus nigra* (44.44%). The total antioxidant activity of essential oil, stem wood, and stem bark extract was 84.34%, 80.33%, and 70.21%, respectively.

Keywords: Delonix regia; Wood; Bark; Fatty acids; Essential oil; Antibacterial; Antifungal agents; Antioxidant activity

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INTRODUCTION

The monetary worth of annual global crop losses due to diseases has been estimated at 25 billion USD. A major part of this is due to fungal pathogens carried through seeds (Chandler 2005). Recently, the control of plant pathogens has required the use of alternative techniques because traditional handling with synthetic chemicals has been the cause of a variety of problems such as toxicity to users and impairment of beneficial organisms (Anderson *et al.* 2003; Whalen *et al.* 2003). Another important aspect is that pathogenic organisms have developed resistance to the active ingredient of some synthetic agrochemicals in response to selection pressure due to high dosage and continual application, causing great economic losses (Arteaga *et al.* 2005). An economical and efficient alternative for disease control is the use of natural products derived from plants (secondary metabolites), as it does not affect the environment and their residues are easily degraded (Wilson *et al.* 1999). There is significant potential to

expand biomass found by the large volumes of unused residues such as wood and bark from tree species. Currently, bark and wood found in large quantities as a result of pruning trees are emerging as a feedstock for energy production and extraction of valuable chemicals (Demirbas 2001).

Delonix regia or *Poinaana regia* (Boj. Ex. Hook) (Family: Fabaceae) is a medium-sized tree found in tropical countries (Shewale *et al.* 2012), and the different parts of the tree are used in traditional medicine (Ali *et al.* 1999). *D. regia* is an ornamental tree and is grown in parks, gardens, and along roadsides in residential and school compounds for shade and shelter (Webb *et al.* 1984). The yellow-brown wood is weak, brittle, and soft, although it is durable and resistant to water, and has been used for making fence posts and fuel (Sheikh 1993). The amount and chemical composition of the extracts are dependent on tree species, tree part age, season, and location of the tree (Bikovens *et al.* 2013). The extracts of *D. regia* consist of mixtures of various components, such as anthocyanin, carotenoids, flavonol, and phenolic acid from its flowers (Veigas *et al.* 2012; Adjé *et al.* 2010). Various types of non-polar compounds, including free fatty acids such as myristic, palmitic, stearic, oleic, and linoleic, have been found in seed oil of *D. regia* (Adewuyi *et al.* 2010; Hoasamani and Hosamani 1995).

The bark and flowers of *D. regia* have been reported to have broad spectrum antibacterial, antifungal, and anti-inflammatory properties (Salem 2013; Ahmad and Aquil 2003; Muruganandan *et al.* 2001). It has previously been reported that high concentrations of polyphenol compounds including anthocyanins, flavonols, and phenolic acids, found in the wood and bark of *D. regia* are the most bioactive natural compounds given their antioxidant and antibacterial properties (Einbond *et al.* 2003; Fatmawaty and Astuti 2013; Teow *et al.* 2007). Compounds such as lupeol, epilupeol, *β*-sitosterol, stigmasterol, and *p*-methoxybenzaldehyde were found in petroleum ether and dichloromethane fractions of a methanolic extract of *D. regia* stem bark (Jahan *et al.* 2010). Additionally, bark extracts showed a presence of gallic acid and other phenolic acids such as sorbic, sinapic, p-coumaric, m-coumaric, ferulic, caffeic, 3-hydroxybenzoic, 4-hydroxycinnamic, and 4-hydroxybenzoic acids (Shabir *et al.* 2011). Other chemical constituents such as kaempferol 3-rhamnoside (afzelin), quercetin 3-rhamnoside, kaempferol 3-glucoide 3(astragalin), kaempferol 3-rutinoside, kaempferol 3-neohesperidoside, quercetin 3-rutinoside, and quercetin 3-glucoside (isoquercetrin) were isolated from leaf extracts (Azab *et al.* 2013).

The antibacterial properties of *D. regia* bark extract might be due to the presence of flavonoids, alkaloids, and phenolic compounds (Salem 2013). The phytochemical investigation of extracts revealed the presence of auroxanthin, mutatochrome, and pyruvic acid (Jungalwala and Cama 1962) and bark extract contained *ß*-sitosterol, saponins, alkaloids, carotene, hydrocarbons phytotoxins, and flavonoids (Fatmawaty and Astuti 2013). The leaves are reported to have antibacterial and antimalarial properties (Ankrah *et al.* 2003; Parekh *et al.* 2005). However, no data were found regarding the composition of crude oil from wood, fatty acids from wood and bark as well as pharmacological evaluation of the wood of the plant.

The aim of the present study was to investigate and evaluate unique antibacterial, antifungal, and antioxidant activities of the extracts from the wood and bark of *Delonix regia* grown in the city of Alexandria, Egypt.

EXPERIMENTAL

Plant Material and Preparation of Extracts

Delonix regia materials (wood and bark) were collected from pruning the tree species at the Faculty of Agriculture, Alexandria University, Alexandria, Egypt, during the month of August 2013. The plant was identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. The materials were air-dried under shade at room temperature and then milled into powder to obtain a 40- to 60-mesh product.

The pulverized stem wood and bark (50 g for each) was soaked in 300 mL of methanol:chloroform (1:1 v/v) (MECL) for 14 days and then filtered through a cotton plug, followed by Whatman filter paper number 1. The extracts were dried and concentrated using a rotary vacuum evaporator at 45 °C and stored in sealed vials at 4 °C until further use. The percentage yields of the MECL extract of *D. regia* were 10.13% and 12.55% (w/w) for wood and bark, respectively.

Essential Oil Extraction

About 200 g of milled *D. regia* wood was subjected to hydro-distillation using 1500 mL distilled water for 6 h in a Clevenger apparatus. The resulting oil was separated from the aqueous phase, dried over anhydrous Na_2SO_4, and measured (0.2 mL/100 g air-dried wood). The oil was kept dry in sealed Eppendorf tubes and stored at 4 °C before GC/MS analysis.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

A Trace GC Ultra/Mass spectrophotometer ISQ (Thermo Scientific) instrument equipped with a FID and a DB-5 narrow bore column (length 10 m × 0.1 mm ID, 0.17 μm film thickness; Agilent, Palo Alto, CA, USA) was used. Helium was used as the carrier gas (flow rate of 1 mL/min), and the oven temperature program was: 45 to 165 °C (4 °C/min) and 165 to 280 °C (15 °C/min) with post run (off) at 280 °C. Samples (1 μL) were injected at 250 °C, with split/split-less injector (50:1 split ratio) in the splitless mode flow with 10 mL/min.

The GC-MS was equipped with a ZB-5MS Zebron capillary column (length 30 m × 0.25 mm ID, 0.25 μm film thickness; Agilent). Helium (average velocity 39 cm/s) was used as the carrier gas and the oven temperature was held at 45 °C for 2 min then increased from 45 to 165 °C (4 °C/min), and 165 to 280 °C (15 °C/min). All mass spectra were recorded in the electron impact ionization (EI) at 70 electron volts. The mass spectrometer was scanned from m/z 50-500 at five scans per second. Peak area percent was used for obtaining quantitative data with the GC with HP-ChemStation software (Agilent Technologies) (Elansary and Ashmawy 2013) without using correction factors. Identification of the constituents was performed on the basis of MS library search (NIST and Wiley) (Adams 1995; Davies 1990).

Fatty Acids Determination and Methylation of Lipid

Samples of 10 g from each of air-dried wood and bark were weighed out into a conical flask containing 10 mL of concentrated HCl and boiled in a water bath until the sample completely dissolved. The conical flask was allowed to cool, the fats were extracted by shaking with 30 mL of diethyl ether, and the extract was collected into a weighed flask after allowing the layers to separate. The extraction process was repeated

three times more and the solvent distilled off. The fat was then dried at 100 °C, cooled, and weighed (Kirk and Sawyer 1991).

A lipid sample of 50 mg was weighed in a tube into which 50 mL of methanolic sulfuric acid (1 mL of concentrated sulfuric acid and 100 mL of methanol) and 2 mL of benzene were added. The tube was tightly closed and placed in a water bath at 90 °C for an hour and half. The tube then was cooled and 8 mL water and 5 mL petroleum ether were added. Subsequently, the tube was strongly shaken and the ethereal layer was separated out and evaporated. Table 1 shows the conditions used for characterization of fatty acids by GC. Standard fatty acids with C2-C25 were previously injected with the same conditions used by GC (Radwan 1978).

Table 1. GC Condition for Analysis of Fatty Acids

Device model	HP (Hewlett Packard) 6890 GC		
Column	HP-5 (5% diphenyl, 95% dimethyl polysiloxane), 30 m, 0.32 mm. ID, 0.25 µm film thickness		
Carrier gas/gas flow	Nitrogen/1 mL/min		
Detector/temperature	FID (flame ionization detector)/250 °C		
Injector temperature, Injection volume	220 °C, 2 µL in a splitless mode		
Oven program	Initial temp. 150 °C for 2 min		
Ramps	Rate °C/min	Final Temp. °C	Hold time
1	10	200	-
2	5	250	9 min

DPPH Radical-scavenging Assay

The percent of the total antioxidant activity (TAA %) was evaluated by the 2.2-diphenyl-1-picrylhydrazyl method (DPPH, Sigma-Aldrich) with some modifications made (Salem *et al.* 2013). Two milliliters of a stock solution of 0.1 mM DPPH reagent dissolved in pure methanol was added to a test tube containing 2 mL of the sample solution in methanol (200 µg/L), and the mixture was well mixed for 10 s and left to stand in the dark for 30 min at room temperature. Using a UV scanning spectrophotometer (Unico® 1200, Alexandria, Egypt), the absorbance was measured at 517 nm and the TAA was determined by the formula TAA (%) = (A-control–A-sample/A-control) × 100, where A-control is the absorbance of the control reaction (containing all reagents except the test compound) and A-sample is the absorbance of the test compound or the Tannic acid (positive control) solution. The control contained 2 mL of DPPH solution and 2 mL of methanol. The measurements of DPPH radical scavenging activity were carried out for three replicates.

Antibacterial Activity Assay

Extracts, being wood and bark MECL and wood essential oil, with a concentration of 2000 µg/mL were evaluated as antibacterial agents against the growth of the Gram-positive bacteria *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, and *Staphylococcus aureus* ATCC 6538 as well as the Gram-negative bacteria *Escherichia coli* ATCC 8739 and *Pectobacterium carotovorum* subsp. *carotovorum* (strain No. ippbc038) using the Kirby-Bauer disc diffusion susceptibility test (Bauer *et al*. 1966). A sterile paper-filter disc, moistened with the extracts, was placed over the Petri dish containing the culture medium that was seeded uniformly with bacterial or fungal strains. The diameters of the inhibition zones (IZs) were measured in millimeters. Control discs

were impregnated with 20 µL of dimethyl sulfoxide (DMSO, Sigma-Aldrich) solution. Tetracycline (20 µg/disc) was used as a positive control with the tested bacteria. The experiment was done in triplicate, and the means ± standard deviations are reported. Minimum inhibitory concentrations (MICs) were determined by serial dilution (8, 16, 32, 64, 126, 250, 500, 1000, 2000, 4000, and 5000 µg/mL) of oil (wood) and MECL extract (wood, bark) using 96-well micro-plates (Eloff 1998).

Antifungal Activity Assay

The antifungal activity assay against the growth of *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus nigra* was performed following Satish *et al.* (2007), with some variation. About 15 mL of potato dextrose agar (PDA) medium containing the concentrated extract (2000 µg/mL) was poured into each Petri dish and allowed to solidify. A 1-cm disc of 7-day-old culture of the tested fungi was placed at the center of the Petri dish and incubated at 25 ± 3 ºC for seven days. After incubation, the colony diameter was measured in centimeters. A PDA medium with DMSO was used as a control. The percentage inhibition of mycelial growth, in terms of fungitoxicity of the extracts, was calculated using the following formula,

$$\% \text{ inhibition} = [(Mc–Mt)/Mc] \times 100 \quad (1)$$

where *Mc* is the average increase in mycelial growth in control and *Mt* is the average increase in mycelial growth in treatment (Singh and Tripathi 1999). The experiment was performed in triplicate.

RESULTS AND DISCUSSION

Figure 1 shows the GC/MS chromatogram analysis of the essential oil from *D. regia* wood. The chemical characterization is presented in Table 2.

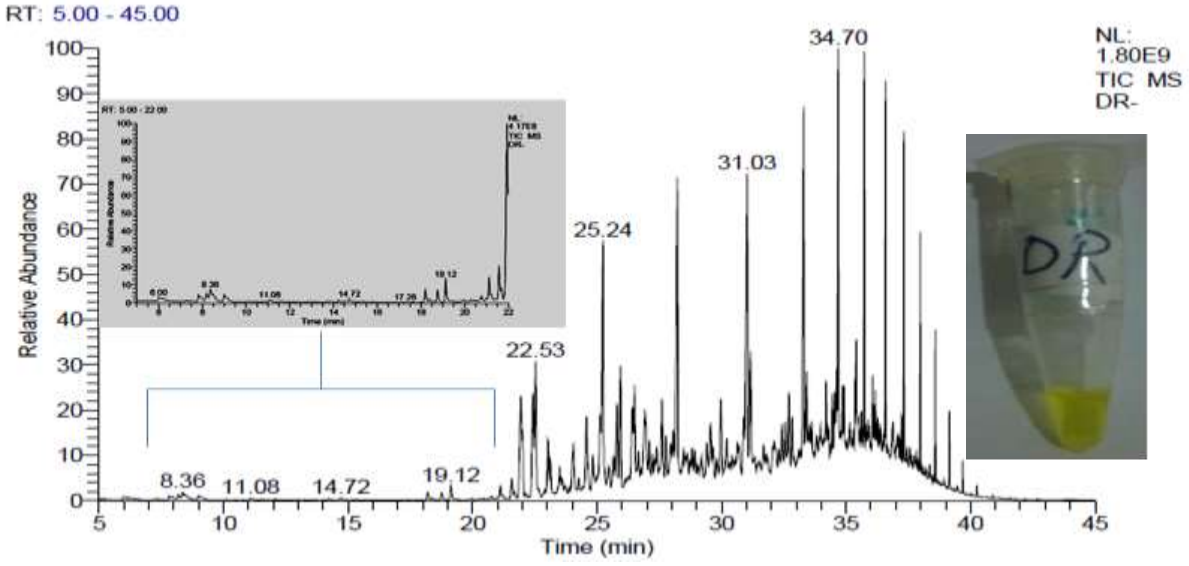

Fig. 1. GC/MS chromatogram of the essential oil from *D. regia* wood

The analysis in Fig. 1 shows that the chromatogram was split into two parts; the first from RT: 5:00 to 22:00 min and, according to the GC-MS results, naphthalene derivatives, a polycyclic aromatic hydrocarbons (1,5-dimethyl-naphthalene and 2-ethyl-naphthalene at 55.93% and 8.53%, respectively) were detected as the major compounds. Additionally, there were some monoterpenes found as volatile compounds but with trace amounts (3-carene, (R)-(+)-m-mentha-6,8-diene, and (1R)-(+)-α-pinene at concentrations of 4.37%, 2.25%, and 2.03%, respectively). Also, some derivatives of fatty acids were detected. The second part from RT: 22:43 to 45:00 min showed that the polycyclic aromatic hydrocarbons were detected as major compounds. The compounds 1,6,7-trimethyl-naphthalene (12.82%), and 1,7-dimethyl-naphthalene (11.72%) were observed as naphthalene derivatives. Acyclic hydrocarbons such as heptadecane (14.05%), heneicosane (8.29%), hexadecane (7.00%), octadecane (5.91%), pentadecane (5.75%) and eicosane (4.11%) were found. A previous report using GC-MS showed the presence of hydrocarbons in the seed oil of *D. regia* (Adewuyi *et al.* 2010). Working at elevated temperatures to obtain the essential oil could lead to chemical changes, *e.g.*, essential oils of chamomile (blue chamazulene originating from colorless matricin) (Bucar *et al.* 2013). Chamazulene was found in the present study, but in small amounts (1.75%).

Table 2. Chemical Composition of Essential Oil of *Delonix regia* Wood

RT: 5.00 - 22.00 min.				RT: 22.43 – 45:00 min			
RT (min)*	Constituent	Oil% †	Class	RT (min)*	Constituent	Oil%†	Class
6.00	(1R)-(+)-α-pinene	2.03	MT	22.43	1,7-dimethyl-Naphthalene	11.72	PAHs
7.28	α-pinene	0.36	MT	24.05	(2,3-Dimethyldecyl)benzene	1.55	ArH
7.82	α-Myrcene	2.36	MT	24.58	4-Methyl biphenyl	2.19	ArH
8.19	α-Phellandrene	1.54	MT	25.13	2-(1-methylethyl)-Naphthalene	1.51	PAHs
8.36	3-Carene	4.37	MT	25.24	Pentadecane	5.75	AcH
9.00	(R)-(+)-m-Mentha-6,8-diene	2.25	MT	25.80	1,6,7-trimethyl-Naphthalene	12.82	PAHs
11.08	Terpinolene	0.92	MT	28.05	4,4'-Dimethylbiphenyl	0.97	ArH
11.55	Linalool	0.22	MT	28.22	Hexadecane	7.00	AcH
11.72	Nonanal	0.15	MT	29.56	2,6,10-trimethyl-Pentadecane	1.51	AcH
13.10	cis-carveol	0.25	MT	29.97	Chamazulene	1.75	PAHs
13.70	2-Nonenal	0.09	MT	30.89	9-methyl-9H-Fluorene	1.16	PAHs
13.80	endo-Borneol	0.27	MT	31.03	Heptadecane	14.05	AcH
14.23	(-)-terpinen-4-ol	0.43	MT	31.16	2,6,10,14-tetramethyl-Pentadecane	2.82	AcH
14.44	p-cymen-8-ol	0.10	MT	32.71	Phenanthrene	1.55	PAHs
14.61	α-Terpineol	1.09	MT	32.84	undecyl-Benzene	0.90	ArH
15.34	verbenone	0.22	MT	33.29	Octadecane	5.91	AcH
17.28	1(4H)-Naphthalenone, 4a,5,8,8a-tetrahydro-4-hydroxy-, (4a,4ab,8ab)-	0.16	PAHs	33.42	2,6,10,14-tetramethyl-Hexadecane	1.91	AcH
17.60	2-Octenoic acid	0.11	FA	33.98	11-syn-bromo-	1.03	PAHs

					1,2,3,4,4a,9,10,10a-octahydro- 4a,10a-Methanophenanthren-9á-ol		
18.20	2-Methylnaphthalene	2.52	PAHs	34.20	2-methyl-Octadecane	1.99	AcH
18.38	(E,E)-2,4-decadien-1-al	5.29	FAld	34.55	2-methylanthracene	2.99	PAHs
18.62	Tridecane	0.09	AcH	35.41	n-Hexadecanoic acid	2.68	FA
18.76	1-Methylnaphthalene	2.34	PAHs	35.73	Eicosane	4.11	AcH
19.88	Falcarinol	0.11	AcH	36.07	2,3-dimethyl-Phenanthrene	0.85	PAHs
19.97	2,5-Octadecadiynoic acid, methyl ester	0.49	FA	36.59	Heneicosane	8.29	AcH
20.35	12,15-Octadecadiynoic acid, methyl ester	0.24	FA	36.89	(Z)-7-Hexadecenal	0.89	FAld
20.47	9,10-dehydro-Isolongifolene	0.23	MT	38.58	Tetracosane	1.16	AcH
20.69	1-Phenylheptane	0.26	ArH	Total		99.06%	
21.12	Biphenyl	5.22	ArH				
21.41	5,8,11-Heptadecatriynoic acid, methyl ester	0.15	FA				
21.57	2-ethyl-Naphthalene	8.53	PAHs				
21.94	1,5-dimethyl-Naphthalene	55.93	PAHs				
Total		98.32%					

* RT: Retention time (min). †Percentage of total FID area obtained on HP-5 capillary column. (MT): Monoterpene; (PAHs): Polycyclic aromatic hydrocarbons; (AcH); Acyclic Hydrocarbons; (ArH): Aromatic Hydrocarbon; (FA): Fatty acid; (FAld): Fatty aldehydes

Fatty Acids Detected in Wood and Bark

The total concentration of FA present in the wood and bark of *D. regia* were 0.177% and 0.386%, respectively. Additionally, the total amount found per sample was 0.514 and 0.200 g per 100 g, respectively. The nonpolar extractives (lipophilic components) from the *D. regia* wood and bark were mainly composed of fatty acids and fatty acid esters. GC analyses of fatty acids in the stem wood and bark of *D. regia* are presented in Table 3. The major fatty acids detected in stem wood (Fig. 2) were myristic acid (10.77%), erucic acid (8.532%), tridecanoic acid (6.48%), 14-pentadecenooic acid (5.55%), tetradecanoic acid (4.98%), pentadecanoic acid (4.26%), and palmitic acid (4.26%). The major fatty acid constituents found in bark (Figure 3) were myristic acid (9.63%), palmitic acid (7.75%), tridecanoic acid (6.08%), erucic acid (5.93%), tetradecanoic acid (4.91%), 14-pentadecenooic acid (3.66%), and pentadecanoic acid (3.58%).

Table 3. Fatty Acid Concentration in Stem Wood and Bark of *Delonix regia*

Fatty acid (FA)	FA (g/100g lipid)		FA %		FA (g/100g sample)	
	Wood	Bark	Wood	Bark	Wood	Bark
Methyl ester of capric acid (C10:0)	0.001	-	0.394	-	0.004	-
Methyl ester of n-carpoic acid (C6:0)	-	0.001	-	0.08	-	0.003
Methyl ester of caprylic acid (C8:0)	-	0.003	-	0.45	-	0.002
Methyl ester of undecanoic acid (C11:0)	0.001	0.009	0.132	0.24	0.001	0.001
Methyl ester of lauric acid (C12:0)	0.005	0.012	1.39	1.58	0.013	0.006
Methyl ester of tridecanoic acid (C13:0)	0.021	0.046	6.48	6.08	0.061	0.023
Methyl ester of tetradecanoic acid (C14:1)	0.016	0.037	4.98	4.91	0.046	0.019
Methyl ester of myristic acid (C14:0)	0.034	0.073	10.77	9.63	0.100	0.038
Methyl ester of 14-pentadecenooic acid (C15:1)	0.017	0.027	5.55	3.66	0.052	0.014
Methyl ester of pentadecanoic acid (C15:0)	0.014	0.027	4.26	3.58	0.039	0.014
Methyl ester of 9-hexadecenoic acid (C16:1)	0.007	0.010	2.16	1.41	0.021	0.006
Methyl ester of palmitic acid (C16:0)	0.013	0.058	4.26	7.75	0.039	0.031
Methyl ester of heptadecenoic acid (C17:1)	0.009	0.002	0.56	0.29	0.005	0.001
Methyl ester of linolenic acid (C18:3, *cis*-9,12,15)	0.002	0.004	0.67	0.55	0.006	0.002
Methyl ester of linoleic acid (C18:2, *cis*-9,12)	-	0.008	-	1.02	-	0.004
Methyl ester of stearic acid (C18:0)	0.006	0.013	1.78	1.73	0.017	0.007
Methyl ester of 8,11,14-ecosatrienoic acid (C20:3)	0.001	-	0.39	-	0.004	-
Methyl ester of heneicosanoic acid (C21:0)	0.007	0.011	2.30	1.56	0.021	0.006
Methyl ester of docosadienoic acid (C22:2)	0.001	0.002	0.09	0.22	0.001	0.001
Methyl ester of erucic acid (C22:1, *cis*-13)	0.027	0.045	8.53	5.93	0.079	0.023
Methyl ester of behenic acid (C22:0)	0.001	-	0.34	-	0.003	-

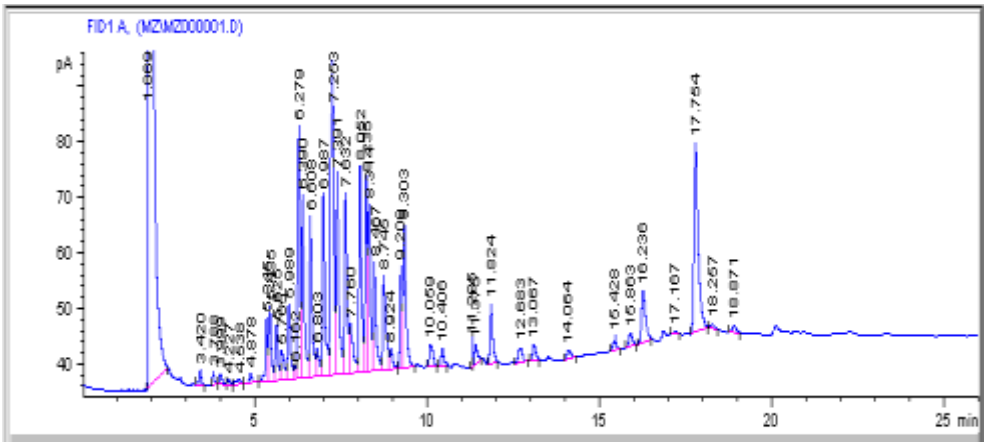

Fig. 2. GC chromatogram of the fatty acids from *D. regia* wood

Linoleic acid was the major fatty acid distributed across the lipid fractions of seed oil of *D. regia*, and the neutral lipids had the highest amount of stearic and linoleic acids (Adewuyi *et al.* 2010). Normal fatty acids such as myristic (1.1 %), palmitic (14.0%), stearic (11.0%), oleic (12.7%), and linoleic (50.7%) were found in *D. regia* seed oil (Hoasamani and Hosamani 1995). Palmitic acid was found to be 13.1% in *D. regia* seed oil (Adewuyi *et al.* 2010) while stearic acid was found at 12.1%.

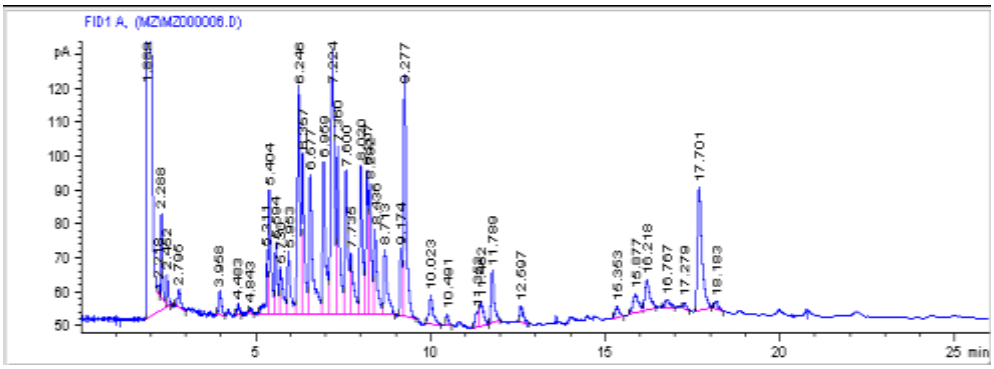

Fig. 3. GC chromatogram of the fatty acids from *D. regia* bark

Antibacterial and Antifungal Activities of Extracts

All tested essential oil and extracts exhibited antibacterial and antifungal activity to differing degrees (Table 4). Based on the investigations, MECL wood extract of *D. regia* was shown to be effective against *B. subtilis*, *S. lutea*, and *S. aureus* with respective IZ values of 16.66 ± 0.57 mm, 14.33 ± 1.15 mm, and 20.66 ± 0.57 mm, where the MECL extract of bark was most active against *E. coli* (21.33 ± 0.57 mm). The essential oil showed good antibacterial activity against *P. carotovorum* (17.66 ± 0.57 mm), while the MECL extract of bark showed no activity against *P. carotovorum*.

Table 4. Antibacterial and Antifungal Activities of Extract from Wood and Bark of *Delonix regia*

Extract	IZ of bacterial strains (mm)[a]					% Inhibition of fungal mycelial growth		
	B. subtilis	*S. lutea*	*S. aureus*	*E. coli*	*P. carotovorum*	*P. selerotigenum*	*P. variotii*	*A. nigra*
Essential oil	13.66 ± 1.15 (250)	15.00 ± 0.00 (64)	14.33 ± 1.15 (500)	20.66 ± 0.57 (16)	17.66 ± 0.57 (250)	44.44	74.07	44.44
MECL wood	16.66 ± 0.57 (32)	14.33 ± 1.15 (1000)	20.66 ± 0.57 (32)	18.33 ± 0.57 (32)	14.33 ± 1.15 (64)	25.93	66.67	14.81
MECL bark	11.66 ± 1.52 (500)	12.66 ± 0.57 (1000)	13.66 ± 1.15 (250)	21.33 ± 0.57 (126)	na (>5000)	70.37	77.78	3.70
Negative control	na	na	na	na	na	0	0	0
Positive control[b]	18	25	20	18	nt	nt	nt	nt

[a]Zone of inhibition in mm (include 5 mm disc). na - not active. nt – not tested
[b]Tetracycline (20 µg/disc). Values in parentheses are MICs values (µg/mL).

The MECL extract of bark showed the maximum percentage of inhibition of fungal mycelial growth against *P. selerotigenum* (70.37%) and *P. variotii* (77.78%). On the other hand, the essential oil showed moderate inhibition against *A. nigra* (44.44%).

The extracts from different parts of *D. regia* have been reported to possess anti-bacterial, anti-malarial, and anti-fungal properties (Ankrah *et al.* 2003; Aqil and Ahmad 2007; Dutta *et al.* 1998; Parekh *et al.* 2005). Some carotenoids such as 2-carotene, zeaxanthein, *etc*., present in the extract of *D. regia* (Jungalwala and Cama 1962) also exhibited antimicrobial activities. Jahan *et al.* (2010) reported that the zones of inhibition demonstrated by the petroleum ether, carbon tetrachloride, and dichloromethane fractions of extracts of *D. regia* stem bark ranged from 9 to 14 mm, 11 to 13 mm, and 9 to 20 mm, respectively, compared to kanamycin as a standard antibiotic with zones of inhibition ranged between 20 mm and 25 mm. Other studies reported that constituents such as 2,4-bis(1,1-dimethylethyl)-phenol, 12-methyl-tetradecanoic acid methyl ester, hexadecanoic acid methyl ester, phytol, and octadecanoic acid methyl ester have proven antimicrobial potential (Agoramoorthy *et al.* 2007; Bikovens *et al.* 2013; Namuli *et al.* 2011; Rani *et al.* 2011).

Total Antioxidant Activity

The total antioxidant activity (TAA %) of essential oil, stem wood, and stem bark MECL extracts were 84.34%, 80.33%, and 70.21%, respectively. The results in the present study are close to or higher than the value of tannic acid (TAA 83%). From the previous work, the TAA% from methanolic extract of stem bark was 78.35 ± 1.45 % (Salem 2013). The detected phenolic compounds in *D. regia* extracts, *i.e.*, caffeic acid, salicylic acid, ferulic acid, gentisic acid, chlorogenic acid, 3-hydroxybenzoic acid, 4-hydroxycinnamic acid, *p*-coumaric acid, gallic acid, and 4-hydroxybenzoic acid, are known to have antioxidant properties (Merkl *et al.* 2010; Proestos *et al.* 2005). The DPPH radical scavenging activity of *D. regia* extracts was found to be inconsistent with previous studies (Shabir *et al.* 2011).

CONCLUSIONS

The use of natural extracts in controlling plant diseases has little to no environmental impact, so they may become a viable option for the development of organic and sustainable agriculture. However, it is necessary to study the molecular and biochemical changes that these compounds may have on pathogens and plants. The present study has shown the fatty acid profile of wood and bark from *Delonix regia*, as well as the chemical characterization of the essential oil of its wood. The following points can be drawn from the present study:

1. Naphthalene derivatives such as 1,5-dimethyl-naphthalene, 2-ethyl-naphthalene, 1,6,7-trimethyl-naphthalene (12.82%), and 1,7-dimethyl-naphthalene were detected as polycyclic aromatic hydrocarbons in the essential oil of *D. regia* wood.
2. The major fatty acids detected in *D. regia* stem wood were myristic acid (10.77%), erucic acid (8.532%), and tridecanoic acid (6.48%). The major fatty acid constituents found in *D. regia* stem bark were myristic acid (9.63%), palmitic acid (7.75%), and tridecanoic acid (6.08%).

3. The methanol-chloroform wood extract of *D. regia* showed effective activity against *B. subtilis*, *S. lutea*, and *S. aureus*, with IZ values of 16.66 ± 0.57 mm, 14.33 ± 1.15 mm, and 20.66 ± 0.57 mm, respectively, where the methanol-chloroform extract of *D. regia* bark was the most active against *E. coli* (21.33 ± 0.57 mm). The essential oil showed good antibacterial activity against *P. carotovorum* (17.66 ± 0.57 mm).
4. The methanol-chloroform extract of *D. regia* bark showed the maximum percentage of inhibition of fungal mycelial growth against *P. selerotigenum* (70.37%) and *P. variotii* (77.78%). On the other hand, the essential oil showed moderate inhibition against *A. nigra* (44.44%).
5. The total antioxidant activity (TAA %) of essential oil, stem wood, and stem bark MECL extract was 84.34%, 80.33%, and 70.21%, respectively, and the results in the present study were close to or higher than that for tannic acid (TAA 83%).

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REFERENCES CITED

Adewuyi, A., Oderinde, R. A., Rao, B. V. S. K., Prasad, R. B. N., and Anjaneyulu, B. (2010). "Chemical component and fatty acid distribution of *Delonix regia* and *Peltophorum pterocarpum* seed oils," *Food Sci. Technol. Res.* 16(6), 565-570.

Adams, R. P. (1995). "Identification of essential oil components by gas chromatograph /quadrupole mass spectroscopy," Carol Stream, IL: Allured Publishing.

Adjé, F., Lozano, Y. F., Lozano, P., Adima, A., Chemat, F., and Gaydou, E. M. (2010). "Optimization of anthocyanin, flavonol, and phenolic acid extractions from *Delonix regia* tree flowers using ultrasound-assisted water extraction," *Ind. Crops Prod.* 32(3), 439-444.

Agoramoorthy, G, Chandrasekaran, M., Venkatesalu, V., and Hsu, M. J. (2007). "Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India," *Braz. J. Microbiol.* 38(4), 739-742.

Ahmad, I., and Aquil, F. (2003). "Broad spectrum antibacterial and antifungal activities and potency of crude, alcoholic extract and fraction of *Delonix regia* flowers," 2nd World Congress on "Biotechnological Development of Herbal Medicine", NBRI, Lucknow, UP, India, page 74.

Ali, M. S., Azhar, I., Amtul, Z., Ahmad, V. U., and Usmanghani, K. (1999). "Antimicrobial screening of some Caesalpiniaceae," *Fitoterapia* 70(3), 299-304.

Anderson, B. S., Hunt, J. W., Phillips, B. M., Nicely, P. A., de Vlaming, V., Connor, V., Richard, N., and Tjeerdema, R. S. (2003). "Integrated assessment of the impacts of agricultural drain water in the Salinas River," *Environ. Pollut.* 124(3), 523-532.

Ankrah, N. A., Nyarko, A. K., and Addo, P. G. A. (2003). "Evaluation of efficacy and safety of a herbal medicine used for the treatment of malaria," *Phytother. Res.* 17(6), 697-701.

Aqil, F. M., and Ahmad, I., (2007). "Antibacterial properties of traditionally used Indian medicinal plants," *Meth. Findings Experim. Clini. Pharm.* 29(2), 79-92.

Arteaga, S., Andrade, C. A. and Cárdenas, R. (2005). "*Larrea tridentata* (creosote bush), an abundant plant of Mexican and US-American deserts and its metabolite nordihydroguaiaretic acid," *J. Ethno pharmacol.* 98(3), 231-239.

Azab, S. S., Abdel-Daim, M., and Eldahshan, O. A. (2013). "Phytochemical, cytotoxic, hepatoprotective and antioxidant properties of *Delonix regia* leaves extract," *Med. Chem. Res*. 22(9), 4269-4277.

Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. (1966). "Antibiotic susceptibility testing by a standardized single disk method," *Am. J. Clin. Pathol.* 45(4), 493-496.

Bikovens, O., Roze, L., Pranovich, A., Reunanen, M., and Telysheva, G. (2013). "Chemical composition of lipophilic extractives from grey alder (*Alnus incana*)," *BioResources* 8(1), 350-357.

Bucar, F., Wube, A., and Schmid, M. (2013). "Natural product isolation – How to get from biological material to pure compounds," *Nat. Prod. Rep.* 30(4), 525-545.

Chandler, J. (2005). "Cost reduction in SIT programmes using exosect auto-dissemination as part of area wide integrated pest management," *Int. J. Pest Cont.* 47(5), 257-260.

Davies, N. W. (1990). "Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and Carbowax 20M phases," *Journal of Chromatography A* 503, 1-24. British Pharmacopeia (1998). (Vol. II). HMSO: London.

Demirbas, A. (2001). "Biomass resource facilities and biomass conversion processing for fuel and chemicals," *Energ. Convers. Manage.* 42(11), 1357-1378.

Dutta, B. K., Rahman, I., and Das, T. K. (1998). "Antifungal activity of Indian plant extracts," *Mycoses.* 41(11-12), 535-536.

Einbond, L. S., Reynertson, K. A., Luo, X. D., Basile, M. J., and Kennelly, E. J. (2003). "Anthocyanin antioxidants from edible fruits," *Food Chem.* 84(1), 23-28.

Elansary, H. O., and Ashmawy, N. A. (2013). "Essential oils of mint between benefits and hazards," *Journal of Essential Oil Bearing Plants* 16(4), 429-438.

Eloff, J. N. (1998). "A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria," *Planta Medica* 64, 711-713.

Fatmawaty, F., and Astuti, H. (2013). "Antimalarial activity of *Delonix regia* on mice with *Plasmodium berghei*," *J. Nat. Prod.* 6, 61-66.

Hoasamani, K. M., and Hosamani, S. K., (1995). "Component fatty acids of *Delonix regis* seed oil-A source of 7-(2-octacyclopropen-1-yI) heptanoic acid and 8-(2-octacyclopropen-1-yl) octanoic acid," *Eur. J. Lipid. Sci. Tech.* 97(11), 420-422.

Jahan, I., Rahman, M. S., Rahman, M. Z., Kaisar, M. A., Islam, M. S., Wahab, A., and Rashid, M. A. (2010). "Chemical and biological investigations of *Delonix regia* (Bojer ex Hook.) Raf," *Acta Pharmaceut.* 60(2), 207-215.

Jungalwala, F. B., and Cama, H. R. (1962). "Carotenoids in *Delonix regia* (Gul Mohr) flower," *Biochem. J.* 85(1), 1-8.

Kirk, R. S., and Sawyer, R. (1991). *Pearson's Chemical Analysis of Foods, 9th Ed.*, Longman Scientific and Technical, Harlow, Essex, UK.

Merkl, R., Hrádková, I., Filip, V., and Šmidrkal, J. (2010). "Antimicrobial and antioxidant properties of phenolic acids alkyl esters," *Czech J. Food Sci.* 28(4), 275-279.

Muruganandan, V., Srinivas, K., Tandan, S. K., Jawahar, L., Chandra, S., and Raviprakash, V. (2001). "Anti-inflammatory and analgesic activity of some medicinal plants," *J. Med. Arom. Plants Res.* 22(4a), 56-85.

Namuli, A., Abdullah, N., Sieo, C. C., Zuhainis, S. W., and Oskoueian, E. (2011). "Phytochemical compounds and antibacterial activity of *Jatropha curcas* Linn. Extracts," *J. Med. Plants Res.* 5(16), 3982-3990.

Parekh, J., Jadeja, D., and Chanda, S. (2005). "Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity," *Turk. J. Biol.* 29(4), 203-210.

Proestos, C., Chorianopoulos, N., Nychas, G. J. E., and Komaitis, M. (2005). "RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity," *J. Agric. Food Chem.* 53(4), 1190-1195.

Radwan, S. S. (1978). "Coupling of two-dimensional thin-layer chromatography with gas chromatography for the quantitative analysis of lipid classes and their constituent fatty acids," *J. Chrom. Sci.* 16(11), 538-542.

Rani, M. J. P., Kannan, P. S. M., and Kumaravel, S. (2011). "Screening of antioxidant activity, total phenolics and gas chromatograph and mass spectrometer (GC-MS) study of *Delonix regia*," *Afr. J. Biochem. Res.* 5(12), 341-347.

Salem, M. Z. M. (2013). "Evaluation of the antibacterial and antioxidant activities of stem bark extracts of *Delonix regia* and *Erythrina humeana* grown in Egypt," *J. Forest Prod. Ind.* 2(2), 48-52.

Salem, M. Z. M., Ali, H. M., El-Shanhorey, N. A., and Abdel-Megeed, A. (2013). "Evaluation of extracts and essential oil from *Callistemon viminalis* leaves: Antibacterial and antioxidant activities, total phenolic and flavonoid contents," *Asian Pac. J. Trop. Med.* 6(10), 785-791.

Satish, S., Mohana, D. C., Ranhavendra, M. P., and Raveesha, K. A. (2007). "Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp.," *J. Agric. Tech.* 3(1), 109-119.

Shabir, G., Anwar, F., Sultana, B., Khalid, Z. M., Afzal, M., Khan, Q. M., and Ashrafuzzaman, M. (2011). "Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf]," *Molecules* 16(9), 7302-7319.

Sheikh, M. I. (1993). *Trees of Pakistan*, GOP-USAID Forestry Planning and Development Project, Islamabad, Pakistan.

Shewale, V. D., Tushar, A., Deshmukh, L., Patil, S., and Patil, V. R. (2012). "Anti-inflammatory activity of *Delonix regia* (Boj. Ex. Hook)," *Adv. Phar. Sci.* 789713, 4.

Singh, J., and Tripathi, N. N. (1999). "Inhibition of storage fungi of blackgram (*Vigna mungo*) by some essential oils," *Flav. Frag. J.* 14(1), 1-4.

Teow, C. C., Truong, V. D., McFeeters, R. F., Thompson, R. L., Pecota, K. V., and Yencho, G. C. (2007). "Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours," *Food Chem.* 103(3), 829-838.

Veigas, J. M., Divya, P., and Neelwarne, B. (2012). "Identification of previously unreported pigments among carotenoids and anthocyanins in floral petals of *Delonix regia* (Hook.) Raf," *Food Res. Int.* 47(1), 116-123.

Webb, D. B., Wood, P. J., Smith, J. P., and Henman, G. S. (1984). "A guide to species selection for tropical and sub-tropical plantations," Tropical Forestry Papers, No. 15. Oxford, UK: Commonwealth Forestry Institute, University of Oxford, p. 256.

Whalen, M. M., Wilson, S., Gleghorn, C., and Loganathan, B. G. (2003). "Brief exposure to triphenyltin produces irreversible inhibition of the cytotoxic function of human natural killer cells," *Environ. Res*. 92(3), 213-220.

Wilson, C. L., Ghaouth, A. E., and Wisniewski, M. E. (1999). "Prospecting in nature´s storehouse for biopesticides," *Mex. J. Phytopath.* 17(1), 49-53.

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