



Constituents and Biological Activity of Sudanese *Delonix regia* (Leguminacea) Seed Oil

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Abstract *Delonix regia* contains many biologically interesting phytochemicals including flavonoids, alkaloids, steroids, saponins, tannins and carotenoids. Leaves are rich source of β -sitosterol and lupeol. *Delonix regia* is used traditionally against malaria and bacterial infections. Leave extract possesses a hypoglycemic effect. In this study, GC-MS analysis of *Delonix regia* oil revealed the presence of the following major constituents: (i) 9,12-octadecadienoic acid methyl ester (43.07%) (ii) hexadecanoic acid methyl ester (21.33%); (iii) 9-octadecenoic acid methyl ester (14.51%) and (iv) methyl stearate (14.12%). The studied oil showed weak activity against *Escherichia coli* and partial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. However, it did not exhibit any anticandidal activity. It also showed moderate antioxidant activity.

Keywords *Delonix regia*, Oil, GC-MS Analysis, Antimicrobial Activity, Antioxidant Activity

Introduction

The history of herbal medicines extends for centuries [1,2]. During the last decades there have been a renewed interest in herbal medicine, specially in developing countries [3,4]. In contrast to modern drugs, herbal medicine has fewer adverse effects beside being affordable [5]. This explains why the use of natural remedies considerably increased in recent decades [6].

Delonix regia (royal Poinciana) is a plant in the legume family (Leguminacea). This plant is well known for its seasonal red-orange bloom [7]. It has been reported that *Delonix regia* contains many biologically interesting phytochemicals including flavonoids, alkaloids, steroids, saponins, tannins and carotenoids [8-12]. Seeds contain galacomannon [13], while leaves are rich source of β -sitosterol and lupeol [13]. *Delonix regia* is used traditionally against malaria and bacterial infections [14-16]. It has been shown that the leave extract possesses a hypoglycemic effect [17].

Materials and Methods

Plant material

Delonix regia seeds were collected from a forest reserve around Damazin-Sudan. The plant was identified and authenticated by direct comparison with a reference herbarium sample.

Instruments

For GC-MS analysis a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μ m, thickness) was used.



Methods

Extraction of oil

Powdered seeds of *Delonix regia* (300g) were macerated with n-hexane for 72hr. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

Delonix regia seed oil was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument under the following chromatographic conditions:

Table 1: Chromatographic conditions

Column oven temperature	150 °C
Injection temperature	300 °C
Injection mode	Split
Flow control mode	Linear velocity
Total flow	50.0 ml/min
Column flow	1.54 ml/sec
Linear velocity	47.2 cm/sec
Purge flow	3.0 ml/min
Spilt ratio	-10

Antimicrobial assay

Microbial strains

The antimicrobial activity was screened using two G+ve strains: *Staphylococcus aureus*, *Bacillus subtilis*; two G-ve stains: *Escherichia coli*, *Pseudomonas aeruginosa* and the fungal species: *Candida albicans*.

Inoculum preparation

For bacteria, each of the bacterial strain was cultured in Mueller Hinton agar slants at 35 °C. Fungal strain was cultured in Sabouraud dextrose agar at 37 °C. The microbial growth was harvested using sterile saline solution (5 ml) and diluted to a viable cell count of 10^7 CFU/ml.

Antibacterial activity

The disc diffusion assay was used to screen the antibacterial activity. As basal layer, ten ml of Mueller Hinton agar was poured into sterile Petri dishes followed by 15 ml of seeded medium previously inoculated with bacterial suspension. Sterile filter paper discs (6mm) loaded with the test oil (100mg/ml) were placed onto the top of the Mueller Hinton agar plates. The plates were incubated at 35°C for 24h. After incubation inhibition zones were measured as indicator of antibacterial activity.

For antifungal activity, the same procedure was adopted but instead of Mueller Hinton agar Sabouraud dextrose agar was used and incubation continued here for 72h at 37°C.

GC-MS analysis

Delonix regia oil was extracted by hexane and analyzed by GC-MS. The analysis revealed the presence of 19 components. The constituents have been identified through retention times and mass spectra using (NIST) library. The total ions chromatograms is shown in Fig. 1 and the different constituents of the oil are presented in Table 1.



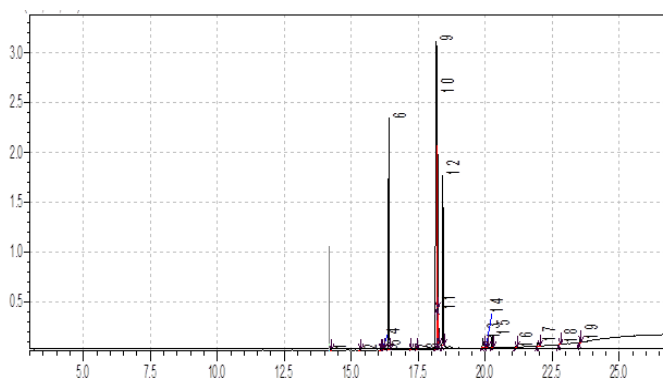


Figure 1: Total ions chromatograms of the oil

Table 2: Constituents of the oil

No.	Name	Ret. Time	Area	Area%
1.	Methyl tetradecanoate	14.181	272318	0.13
2.	Pentadecanoic acid, methyl ester	15.311	95228	0.04
3.	7,10-Hexadecadienoic acid, methyl ester	16.083	65050	0.03
4.	7-Hexadecenoic acid, methyl ester, (Z)-	16.148	75104	0.03
5.	9-Hexadecenoic acid, methyl ester, (Z)-	16.191	823955	0.38
6.	Hexadecanoic acid, methyl ester	16.399	46370759	21.33
7.	cis-10-Heptadecenoic acid, methyl ester	17.208	164886	0.08
8.	Heptadecanoic acid, methyl ester	17.420	345441	0.16
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.170	93620223	43.07
10.	9-Octadecenoic acid (Z)-, methyl ester	18.205	31552316	14.51
11.	9-Octadecenoic acid, methyl ester, (E)-	18.241	4428398	2.04
12.	Methyl stearate	18.414	30709618	14.12
13.	11-Octadecynoic acid, methyl ester	19.911	2878316	1.32
14.	cis-11-Eicosenoic acid, methyl ester	20.042	891597	0.41
15.	Eicosanoic acid, methyl ester	20.258	2567774	1.18
16.	Heneicosanoic acid, methyl ester	21.127	92842	0.04
17.	Docosanoic acid, methyl ester	21.964	1376728	0.63
18.	Tricosanoic acid, methyl ester	22.770	350701	0.16
19.	Tetracosanoic acid, methyl ester	23.543	738575	0.34

Major components are:

- (i) 9,12-octadecadienoic acid methyl ester (43.07%)
- (ii) Hexadecanoic acid methyl ester (21.33%)
- (iii) 9-Octadecenoic acid methyl ester (14.51%)
- (iv) Methyl stearate (14.12%).

The mass spectrum of 9,12-octadecadienoic acid methyl ester is shown in Fig.2. The molecular ion: $M^+[C_{19}H_{34}O_2]^+$ corresponds m/z 294 (R.T. 18.170). The peak at m/z 263 corresponds to loss of a methoxyl function. Figure 3 presents the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270 (at R.T. 16.399) corresponds $M^+[C_{17}H_{34}O_2]^+$, while the peak at m/z 239 is due to loss of a methoxyl. The EI mass spectrum of 9-octadecenoic acid, methyl ester is shown in (Fig. 4). The signal which appeared at m/z 296, with R.T. 18.205 is due to $M^+[C_{19}H_{36}O_2]^+$, while the peak at m/z 265 is due to loss of a methoxyl group. Fig. 5 shows the mass spectrum of methyl stearate. The signal at m/z 298 (R.T. 18.414) corresponds $M^+[C_{19}H_{38}O_2]^+$, while the peak at m/z 267 corresponds to loss of a methoxyl.



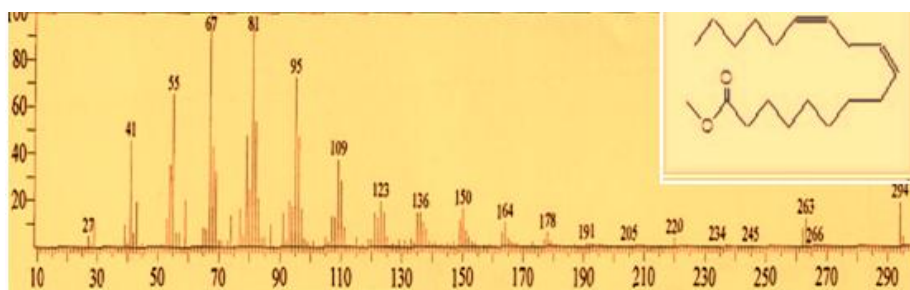


Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

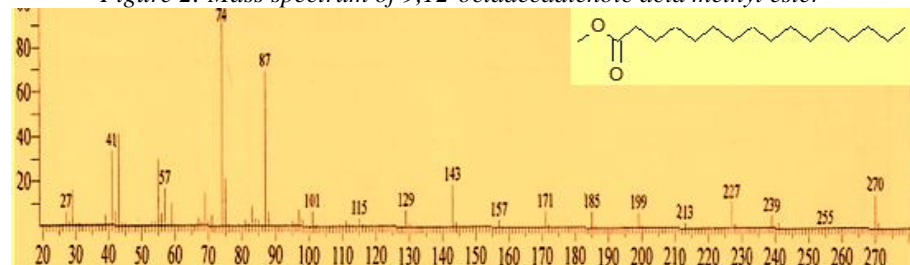


Figure 3: Mass spectrum of hexadecanoic acid methyl ester

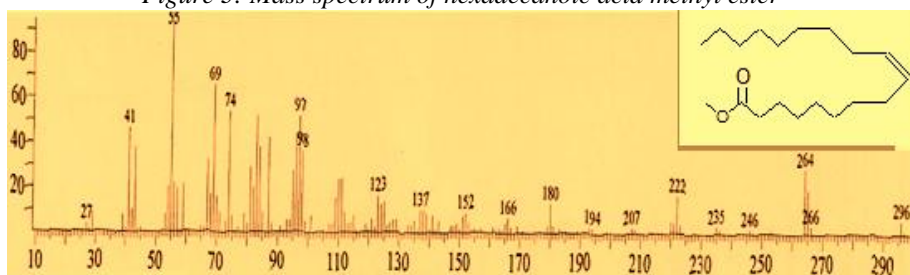


Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

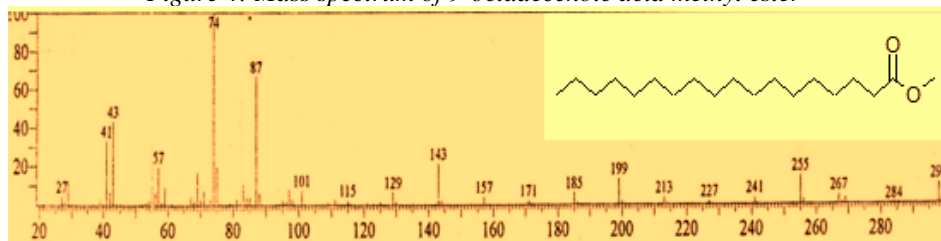


Figure 5: Mass spectrum of methyl stearate

Antimicrobial assay

The oil was evaluated for antimicrobial activity against standard microorganisms using disc diffusion method. The average of the diameters of the growth inhibition zones are presented in Table 3. Results were interpreted in conventional terms: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicillin, gentamicin and clotrimazole were used as positive controls. The studied oil showed weak activity against *Escherichia coli* and partial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. However, it did not exhibit any anticandidal activity.

Table 3: Inhibitory effect of *Delonix regia* oil

Sample	Sa	Bs	Ec	Ps	Ca
Oil(100mg/ml)	10	10	13	9	--
Ampicilin(40mg/ml)	30	15	--	--	--
Gentamicin(40mg/ml)	19	25	22	21	--
Clotrimazole(30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus*; Ec.: *Escherichia coli*; Pa.: *Pseudomonas aeruginosa*; Bs.: *Bacillus subtilis*;
Ca.: *Candida albicans*



Antioxidant activity

The antioxidant capacity of *Delonix regia* oil was measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test sample against stable DPPH radical. The change in color is measured spectrophotometrically at 517 nm. As depicted in Table 4, the oil exhibited moderate antioxidant activity. Propyl gallate was used as a positive control.

Table 4: Antioxidant activity of the oil

Sample	Antioxidant activity
Propyl gallate	89 ±0.01
<i>Delonix regia</i> oil	50.1 ±0.25

References

- [1]. Cragg GM, Newman DJ., Biodiversity: A continuing source of novel drug leads, *Pure Appl Chem.*, 2005; 77:7–24.
- [2]. Silva NCC, Fernandes A., Júnior Biological properties of medicinal plants: A review of their antimicrobial activity, *Venom Anim Toxins incl Trop Dis.* 2010; 16:402–13.
- [3]. Tao Z, Shi A, Zhao J., Epidemiological perspectives of diabetes. *Cell Biochem Biophys.*, 2015; 73:181–5.
- [4]. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety, *Front Pharmacol.*, 2014; 4:177.
- [5]. Kumar S, Saini M, Kumar V, Prakash O, Arya R, Rana M, et al. Traditional medicinal plants curing diabetes: A promise for today and tomorrow, *Asian J Tradit Med.*, 2012; 7: 178–88.
- [6]. Firenzuoli F, Gori L. Herbal medicine today: Clinical and research issues., *Evid Based Complement Alternat Med.*, 2007; 4(Suppl 1):37–40.
- [7]. *Delonix Regia*; Department of Plant Sciences; Aridus, Vol. 16, no. 1, 2004.
- [8]. Edward F, Dennis G, Watson. *Delonix Regia*- royal Poinciana. Face sheet ST-228, a series of Environmental Institute of food and Agricultural Sciences, University of Florida. 1993.
- [9]. Jungalwala FB, Chama HR. Carotenoids in *Delonix regia* (Gulmohor) Flower, *Biochem. J.*, 1962; 85-93.
- [10]. Parekh J, Chanda SV. In vitro Activity and Phytochemical Analysis of some Indian Medicinal Plants, *Turk J Biol.*, 2007; 31, 53-58.
- [11]. <http://www.sc.chula.ac>. Chemical constituents of *Delonix regia* flowers.
- [12]. http://www.sc.chula.ac.th/department/chemistry/npr_u/6Senior/Abs-senior.html chemical constituents of *Delonix regia* plant.
- [13]. Jungalwala FB, Cama, H.R., Carotenoids in *Delonix regia* flower, *Biochem J.*, 1962; 85, 1.
- [14]. Aqil, F. and Ahmad, I., Antibacterial properties of traditionally used Indian medicinal plants, *Methods and Findings in Experimental and Clinical Pathology*, 2007; 29(2): 79-92.
- [15]. Ankrah, N.A., Nyarko, A.K., Addo, P.G., Ofosuhene, M., Dzokoto, C., Marley, E., Addae, M.M., Ekuban F.A., Evaluation of efficacy and safety of a herbal medicine used for the treatment of malaria, *Phytother. Res.*, 2003; 17(6):697-701
- [16]. Dutta, B.K., Rahman, I. and Das, T.K., Antifungal activity of Indian plant extracts. *Mycoses*, 1998; 41 (11-12): 535-536.
- [17]. Hassan, N., Das, A.K., Hossain, T., Jahan, R., Khatun, A., Rhamatullah, M., *Afr. J. Trad. Complement Altern. Med.*, 2011; 8(1): 34-36.

