The composition of the lipoidal matter of the seeds of *Pleiogynium timorense* (DC.) Leenh

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Received 22 December 2014
Accepted 09 February 2015

Egyptian Pharmaceutical Journal
2015, 14:65–68

Background and objectives
Nothing dealing with the chemistry of the seeds of *Pleiogynium timorense* (DC.) Leenh. could be traced in the available literature. The objective of the present work was to investigate the lipoidal matter from *P. timorense* seeds and to isolate and identify the major sterols and triterpenes in the petroleum ether extract.

Materials and methods
The petroleum ether extract of *P. timorense* seeds was prepared. The extract was subjected to column chromatography using silica gel (type 60-230 mesh) as an adsorbent. The unsaponifiable matter and fatty acid methyl esters were analyzed by GC/MS.

Results and conclusion
GC/MS analysis of the unsaponifiable matter from the petroleum ether extract of *P. timorense* seeds revealed the identification of 70.04% of unoxygenated compounds and 23.34% oxygenated compounds. Hydroxylated compounds, ketones, and steroidal compounds represent 23.24, 0.20, and 3.19%, respectively. 1-Heptene (66.47%) was the major compound in the USM, followed by butylated hydroxy toluene (21.07%). GC/MS analysis of fatty acid methyl esters showed that nine fatty acid methyl ester derivatives could be identified, representing 95.84% of the total composition. The major fatty acid was 9,12-octadecadienoic acid (linoleic acid) (33.8%). Saturated fatty acids represent 36.93% of the total fatty acid content, whereas monounsaturated and diunsaturated fatty acids represent 25.11 and 33.8% of the total fatty acid content, respectively. Column chromatography of the petroleum ether extract led to the isolation of two compounds [α–amyrin and 5,24(28)-cholestadien-24-methylen-3β-ol].

Keywords:
α-amyrin, 5,24(28)-cholestadien-24-methylen-3β-ol, lipids, *Pleiogynium timorense*, sterols

Egypt Pharm J 14:65–68

1687-4315

Introduction

Pleiogynium timorense (DC.) Leenh. (Anacardiaceae) is an evergreen tree indigenous to tropical and subtropical regions. It is known in Arabic as Gambozia and cultivated in Egypt as an ornamental plant [1,2]. *P. timorense* has edible fruits used in the preparation of jellies, jams, and preserves [3,4]. Previous phytochemical studies resulted in the isolation of quercetin, myricetin, rutin, quercitrin, hyperin, lupeol, and β-sitosterol from the leaves. Aqueous and alcoholic extracts of the leaves showed significant antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* [5]. The ethanolic extract of the leaves showed significant hypoglycemic, antioxidant, and anti-inflammatory properties. In addition, 12 phenolic compounds were isolated from the leaves of the plant, which include kaempferol, gallic acid, kaempferol-3-*O*-β-d-galactopyranoside, kaempferol-3-*O*-β-d-glucopyranoside, quercetin-3-*O*-β-d-galactopyranoside, quercetin-3-*O*-β-d-glucopyranoside, kaempferol-3-*O*-β-d-6˝-methylglucuronopyranoside, kaempferol-3-*O*-β-d-glucuronopyranoside, myricetin-3-*O*-α-l-rhamnopyranoside, 3,5-di-*O*-galloylquinic acid, 1,4,6-tri-*O*-galloyl-β-d-glucopyranose, and 1,3,4,6-tetra-*O*-galloyl-β-d-glucopyranose [6]. Cyanidin-3-glucoside was isolated from the fruits [7], which is reported to have antioxidant activity. The present study was undertaken to investigate the lipoidal matter composition of *P. timorense* seeds.

Materials and methods

Plant material

Fruits of *P. timorense* were collected from Zoo Garden, Giza, Egypt, in April 2010, and were kindly authenticated by Dr. Tereez Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman Botanical Garden, Giza, Egypt, and confirmed by the taxonomist, Dr. M. El-Gebaly, NRC. The fruits were air-dried, and the seeds and the pericarp were separated, powdered, and stored in dark well-closed containers.

Preparation of the lipoidal matter

The powder of the air-dried seeds of *P. timorense* (800 g) was exhaustively extracted with light petroleum (60°–80°C) in a continuous extraction apparatus. The

extract was evaporated under vacuum to yield 28 g of dry residue, representing 3.5% of the air-dried seeds.

Investigation of the lipoidal matter

Saponification of the petroleum ether extract

The petroleum ether extract (1 g) was subjected to saponification according to the method reported by Tsuda *et al.* [8]. Percentages of the unsaponifiable matter and the total fatty acid were found to be 38 and 60%, respectively.

Preparation of fatty acid methyl esters

Free fatty acids obtained by saponification were methylated according to the method reported by Finar [9].

GC/MS analysis

Both the unsaponifiable and the saponifiable fractions were studied to identify their contents using GC/MS analysis. The constituents were identified by comparison of their mass spectral fragmentation patterns with those of the available database libraries, Wiley (Wiley International, Colorado, USA) and NIST (Nat. Inst. St Technol., Colorado, USA), and/or published data [10,11]. Quantitative determination was carried out on the basis of the peak area integration. GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters of *P. timorense* seeds was carried out using conditions described in Table 1.

The Experiment

An NMR Jeol ECA spectrometer (JEOL Corporation, Tokyo, Japan), 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, using $CDCl_3$ as a solvent, was used. All chemical shifts (δ) are given in ppm units with reference to TMS as the internal standard, and the coupling constants (*J*) are given in Hz. The gas chromatograph was coupled with a mass spectrometer [an Agilent 6890 gas chromatograph coupled with an Agilent mass spectrometric detector, 70 eV (Agilent Technologies, Santa Clara, California, USA), ESR, Brucker, Elexsys, E 500 (Frankfurt, Germany)].

Extraction and isolation of major sterols and triterpenes

Air-dried powdered seeds of *P. timorense* (800 g) were exhaustively extracted with light petroleum (60°–80°C) in a continuous extraction apparatus. The extract was evaporated under vacuum to yield 28 g of extract, representing 3.5% of the air-dried powder. Part of the extract (20 g) was subjected to column chromatography using silica gel (type 60-230 mesh, 200 g; E. Merck, Darmstadt, Germany) as an adsorbent, and elution was carried out with *n*-hexane, followed by *n*-hexane-ethyl acetate mixtures of increasing polarity. Fractions (50 ml each) were collected and concentrated separately to a small volume. All fractions were screened by thin-layer chromatography on silica gel 60 F_{254} plates using hexane: ethyl acetate (9 : 1) as the solvent system and *P*-anisaldehyde-sulphuric acid spray reagent, followed by heating at 110°C for 5 min. Similar fractions were pooled to form two major groups and the solvents were evaporated separately under reduced pressure.

Group 1

It contained one major spot (R_f = 0.38) of violet color with the *P*-anisaldehyde-sulphuric acid spray reagent. The fraction was freed of the solvent under reduced pressure; the residue was dissolved in the least amount of chloroform and subjected to purification by rechromatography on preparative thin-layer chromatography using hexane: ethyl acetate (8.5 : 1.5). The bands were eluted with chloroform. The chloroformic eluate was filtered and concentrated to a small volume to yield compound L_1 (5 mg).

Group 2

It contained one major spot (R_f = 0.3) of violet color, with the *P*-anisaldehyde-sulphuric acid spray reagent. The fraction was freed of the solvent under reduced pressure. The residue obtained was purified as in group I to yield compound L_2 (10 mg).

Results and discussion

A total of 28 compounds were identified (Table 2) by GC/MS of the unsaponifiable matter, representing 96.68% of the total unsaponifiable content. The analysis revealed that the identified components consisted of 70.04% unoxygenated compounds and

Table 1 Conditions of GC/MS analysis of the unsaponifiable matter and fatty acid methyl esters

Condition	Unsaponifiable matter	Fatty acid methyl esters
Column	Capillary column of fused silica, 30 m length, 0.32 mm ID and 0.25 μm thickness	
Stationary phase	TR-5MS (5% phenyl polysil phenylene siloxane)	
Carrier gas	Helium at 1 ml/min, 13 psi	
Temperature programming	At 70°C isothermal for 5 min 70°–290°C at a rate of 4°C/min at 290°C isothermal for 10 min	At 140°C isothermal for 5 min 140°–200°C at a rate of 5°C/min at 200°C isothermal for 3 min
Detector temperature	280°C	220°C
Ion source temperature	270°C	200°C
Ionization voltage	70 eV	

23.34% oxygenated compounds: 23.24% hydroxylated compounds, 0.10% ketones, and 3.19% steroids. 1-Heptene (66.47%) was the major compound in the USM, followed by butylated hydroxy toluene (21.07%). About nine fatty acid methyl ester derivatives could be identified (Table 3), representing 95.84% of the total composition. The major fatty acids were 9,12-octadecadienoic acid (linoleic acid) (33.8%), followed by 9-octadecenoic acid (oleic acid) (24.14%) and 14-methyl-pentadecanoic acid 12.08%. Saturated fatty acids represent 36.93% of the total fatty acid content, whereas monounsaturated and diunsaturated fatty acids represent 25.11 and 33.8% of the total fatty acid content, respectively.

Table 2 GC/MS analysis of the unsaponifiable matter from *Pleiogynium timorense* seeds

Compound	*RRt	Relative area (%)	Molecular weight
1-Heptene	**1**	**66.47**	**98**
1-Nonene	3.7	0.23	126
1-Tetradecene	4.9	0.35	196
Butylated hydroxy toluene	5.7	21.07	220
Tridecanol	6.1	0.78	200
Spathulenol	6.7	0.08	220
Pygmaein	6.8	0.11	194
Thujopsanol	6.9	0.10	222
1-Hexadecene	7.2	0.85	224
Hexadecane	7.3	0.13	226
β-Atlantol	7.4	0.16	220
Khusimone	7.5	0.10	204
Tetradecanol	8.2	0.66	214
Pentadecanol	8.6	0.11	228
Phytol	8.8	0.28	296
Docosene	9.0	0.42	308
Docosane	9.1	0.09	310
Tetracosene	9.8	0.20	336
Tetracosane	9.9	0.20	338
Cholesterol	10.3	0.17	386
Pentacosane	10.6	0.12	352
Heptacosane	11.1	0.21	380
Octacosene	11.3	0.08	392
Squalene	11.4	0.24	410
Nonacosane	11.6	0.25	408
Hentriacontane	12.3	0.20	422
β-Sitosterol	13.15	1.01	414
α-Amyrin	13.9	2.01	426

RRT, relative retention time.

Compound L_1: white powder (5 mg), m.p. (181°–183°C), a single spot in hexane : ethyl acetate (8.5 : 1.5) (R_f = 0.38), violet color with the *P*-anisaldehyde-sulphuric acid spray reagent.

Mass spectral data: 426(M^+), 411 (M^+-CH_3), 409 (M^{+1}-H_2O), 218 (100%) 203 (28%) and 189 (29%). The mass spectrum showed a molecular ion peak at *m/z* 426 for the molecular formula $C_{30}H_{50}O$, in addition to the following peaks at *m/z* 411(M^+-CH_3), 409 (M^{+1}-H_2O), 218 (M-208 ($C_{14}H_{24}O$), and 203 (218-CH_3)+. On the basis of the spectral data, melting point, and comparison with an authentic sample, compound L_1 was identified as α-amyrin. This compound was previously isolated from *P. timorense* leaves [5]. This is the first report in the seeds of the plant.

Compound L_2: white powder (10 mg), m.p. (142°–143°C), gave a single spot in hexane : ethyl acetate (8.5 : 1.5) (R_f = 0.3), gave violet color with the *P*-anisaldehyde-sulphuric acid spray reagent.

^{1}H-NMR spectral data of compound L_2 ^{1}H-NMR, $CDCl_3$: δ 5.35(H-6, broad s), δ 3.9(H-3, m), δ 4.6, δ 4.5 (H-28, s), δ 0.82(3H-18, s), δ 0.84(3H-19, s), δ 1.4 (3H-26,d, *J*=7 Hz), δ 1.4(3H-27,d, *J* = 7 Hz), δ 0.9 (3H-21,d, *J*=6.5 Hz), δ 2.2(H-25, m)

^{13}C-NMR in $CDCl_3$ spectral data of compound L_2: The spectrum showed peaks at δ 140.813 (C-5), δ 147.5 (C-24), 129.7 (C-6), 111.3 (C-28), 67 (C-3), 19.3 (C-21), 11.8 (C-19,C18), 22.6 (C-26, C27), 30.9 (C-23), 33.8 (C-25), 34.5 (C-22), 35.6 (C-20). Thus, compound L_2 is identified as 5,24 (28)-cholestadien-24-methylen-3β-ol, which was isolated, for the first time, from the plant.

Conclusion

This study showed the identification of 28 compounds of unsaponifiable matter from the seeds of *P. timorense*,

Table 3 GC/MS analysis of fatty acid methyl esters from *Pleiogynium timorense* seeds

Compound	*RRt	Relative area (%)	Molecular weight
14-Methyl-pentadecanoic acid methyl ester	0.78	12.08	270
9-Hexadecenoic (palmitoleic) acid, methyl ester	0.80	0.25	268
Hexadecanoic acid methyl ester (methyl palmitate)	0.81	7.75	270
11-Hexadecenoic (palmitoleic) acid methyl ester	0.84	0.37	268
Octadecanoic acid methyl ester (methyl stearate)	0.94	7.91	298
9-Octadecenoic acid methyl ester (methyl Oleate)	0.95	24.14	296
9,12-Octadecadienoic acid methyl ester (methyl linoleate)	**1**	**33.80**	**294**
Eicosanoic acid methyl ester (methyl arachidate)	1.08	9.19	326
13-Eicosenoic acid methyl ester	1.09	0.35	324

RRT, relative retention time.

which were identified by GC/MS. 1-Heptene (66.47%) was the major compound in the USM. About nine fatty acid methyl ester derivatives could be identified. The major fatty acid was 9,12-octadecadienoic acid (linoleic acid) (33.8%).

Acknowledgements

The authors thank the National Research Centre, Egypt, for funding this work.

Conflicts of interest

There are no conflicts of interest.

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