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### Phoenix roebelenii O'Brien DNA profiling, bioactive constituents, antioxidant and hepatoprotective activities

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#### ABSTRACT

**Objective:** To authenticate *Phoenix roebelenii* O'Brien, and investigate its leaves and fruits phytoconstituents, antioxidant, hepatotoxicity and hepatoprotective activities.

**Methods:** DNA profiling was carried out by random amplified polymorphic DNA-PCR). Total phenolic contents were estimated using Folin-Ciocalteu method. Chromatographic and spectral techniques were used for the phytochemical investigation. Petroleum ether extracts were investigated using GC-MS. Antioxidant activity was assayed by 1, 1-diphenyl-2-picrylhydrazyl radical scavenging method. Hepatotoxicity and hepatoprotective assessments were achieved by neutral red assay.

**Results:** Total phenolic contents of leaves and fruits were estimated as being 64.3 and 7.88 µg gallic acid equivalent/mg, respectively. Ten compounds were isolated for the first time from the aqueous methanol extract of leaves: apigenin 6, 8-di-C-β-glucopyranoside, quercetin 3-O-glucopyranoside, luteolin 3'-O-β-glucopyranoside, quercetin, apigenin, luteolin, baicalein and p-hydroxybenzoic, vanillic and caffeic acids. Major lipophilic constituents of petroleum ether leaves extracts are ricinoleic acid (51.12%) and phytol (10.64%), while those of fruits are linoleic acid (33.61%) and n-octadecanol (9.04%). Aqueous methanol and petroleum ether extracts of leaves and fruits exhibited antioxidant activity at IC<sub>50</sub> (60.25 and 51.70 µg/mL) and (6.3 and 21.0 µg/mL), respectively. The extracts hepatotoxicity is at IC<sub>50</sub> > 1000 µg/ml. Leaves and fruits petroleum ether extracts exhibited prominent hepatoprotective activity > 12.5 and 15 µg/mL, respectively.

**Conclusions:** The palm may be a new potential source as a natural hepatoprotective to be applied in pharmaceutical industries.

## 1. Introduction

The palm family (Arecaceae) has a long history of providing man with useful materials for his daily life[1]. Chemically, the family has been neglected despite of its economic importance, probably because of difficulty of collecting fresh material and getting it authenticated. The genus *Phoenix* is one of the most widely

cultivated groups of palms around the world[2]. *Phoenix roebelenii* O'Brien (*P. roebelenii*) is a species of date palm native to south eastern Asia. It is a popular ornamental plant in gardens and in tropical to warm temperate climate areas and well known as pygmy date palm or miniature date palm. Members of the genus *Phoenix* are characterized by different classes of phenolic compounds (tricin, luteolin and quercetin glycosides) constituting the major leaf components[3]. *Phoenix* species has been used for treatment of various infectious diseases, atherosclerosis, diabetes, hypertension and cancer[4-6]. Phenolic compounds have diverse beneficial biochemical effects on human health. They show antioxidant, hepatoprotective, anticancer and anti-inflammatory activities[7-10]. Reviewing the current literature, nothing has been reported about

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neither the phytochemical constituents nor biological activity of *P. roebelenii* O'Brien.

Accordingly, the aim of the present work was to authenticate *P. roebelenii* O'Brien growing in Egypt by carrying out PCR sequencing and to investigate its phenolic and lipoidal constituents. In addition, to *in vitro* investigate antioxidant, hepatotoxicity and hepatoprotective activities of the palm leaves and fruits extracts.

## 2. Materials and methods

### 2.1. General experiment

PCR amplification was performed in a Perkin-Elmer/Gene Amp<sup>®</sup> PCR system 9700 (PE Applied Biosystems). Ultraviolet spectra were recorded on Shimadzu UV 240 (P/N 204-58000). Nuclear magnetic resonance (NMR) measurements were carried out using Jeol EX-500 spectroscopy (Japan): 500 MHz (<sup>1</sup>H-NMR) and 125 MHz (<sup>13</sup>C-NMR). Electron ionization mass spectrometry was performed using thermo scientific spectrometer (70 eV). Gas chromatograph-mass spectrometry (GC-MS) analysis were carried on trace GC ultra/ISQ single quadrupole MS, TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). For preparation of petroleum ether extracts, a soxhlet apparatus was used. Column chromatography for isolation of phenolics was carried out on polyamide 6S (Riedel-De-Haen, AG, Sneeze Hanver, Germany) using methanol/H<sub>2</sub>O as eluent. Paper chromatography descending Whatman No.1 and 3 mm papers, using solvent systems: (1) water, (2) 15% HOAc (water:acetic acid 85:15), (3) 50% HOAc, (4) BAW (butanol:acetic acid:water, 4:1:5 upper layer) and (5) BBWP (benzene:butanol: water: pyridine 1:5:3:3, upper layer). BAW and BBWP were used for sugar identification. The purification was performed in Sephadex LH-20 (Pharmazia, Sweden). Solvents used for plant extraction were from SDFCL (industrial Estate, 248 Worli Road, Mumbai-30, India). Monolayer of hepatocytes examination was carried out with light microscope (Olympus, Saitama, Japan).

### 2.2. Chemicals

Methanol and sodium sulphate were from Readel-de Haën. The following compounds were also purchased: 4-(22-hydroxyethyl)-1-piperazine-ethanesulfonic acid (99%) (ACROS Co.), potassium chloride (S.R.L), D (+) glucose (Merck), calcium chloride (S.D), fetal bovine serum (Lonza), penicillin streptomycin (Gibco BRL), sodium thiopental, silymarin (Sigma), sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, potassium phosphate monobasic, ethylene glycol tetraacetic acid, collagenase type IV, culture medium RPMI-1640, trypan blue, insulin, neutral red and dimethyl sulfoxide (Sigma Co.).

### 2.3. Plant material

Fresh plant material (leaves and fruits) of *P. roebelenii* O'Brien (Areaceae) were collected from the Orman garden, Giza, Egypt and identified by Dr. M. El Gibali, former researcher of botany, National Research Centre, Egypt. A voucher specimen (M126) was deposited in the National Research Centre herbarium.

### 2.4. Random amplified polymorphic-DNA (RAPD)-PCR

Freeze-dried leaves of *P. roebelenii* were ground under liquid nitrogen to fine powder (50 mg) prior to DNA isolation. DNA was extracted using the cetyltrimethylammonium bromide [1% (w/v) N-cetyl-N, N, N trimethyl ammonium bromide] method[11]. Ten oligonucleotide primers were used for RAPD analysis in this study. PCR products were visualized on UV light and photographed using a Polaroid camera. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively. The banding patterns generated by RAPD-PCR marker analyses were compared to determine the genetic relatedness (Figure 1). The genetic similarity coefficient was estimated according to dice coefficient[12].

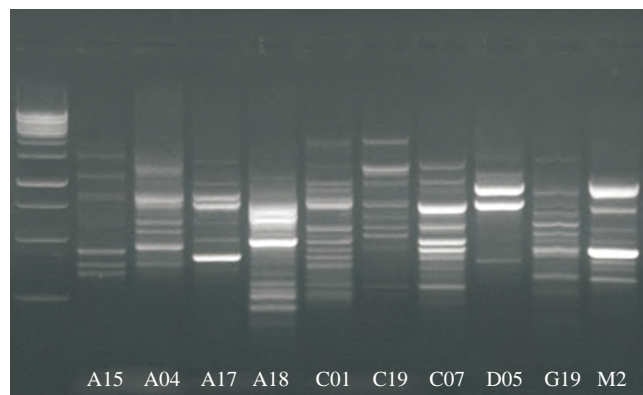


Figure 1. RAPD-PCR products for *P. roebelenii* O'Brien using ten decamer primers.

### 2.5. Phytochemical investigation

#### 2.5.1. Determination of total phenolic content of leaves and fruits

Air-dried plant leaves and fruits (1 g each) were extracted with 70% methanol by sonication at room temperature. The total phenolic content of the extracts were determined by the Folin-Ciocalteu method[13]. The total phenolic content was expressed in µg of gallic acid equivalents/mg of extract.

#### 2.5.2. Extraction and isolation

The air-dried powdered palm leaves (1 kg) were extracted with MeOH/H<sub>2</sub>O (7:3) by percolation on hot (3 × 4L). The solvent was evaporated under reduced pressure at 60 °C to yield 228 g dried aqueous methanol extract (PLAME). PLAME was defatted with CHCl<sub>3</sub> (3 × 1 L) afforded chloroform extract (12 g). The defatted PLAME residue (180 g) was

extracted with methanol and followed by water (three times for each solvent). The methanol fraction residue (80 g) was chromatographed on polyamide column chromatography (5 cm × 100 cm) using H<sub>2</sub>O, followed by H<sub>2</sub>O/MeOH mixtures of decreasing polarity to yield ten main fractions (A–J). Fraction B was eluted on Sephadex L20 column using H<sub>2</sub>O:MeOH (1:1) then subjected to preparative paper chromatography using BAW and followed by elution on Sephadex L20 column using methanol to yield compound 1 (12 mg). Column chromatography of fraction B on polyamide column (gradient elution with CHCl<sub>3</sub>/MeOH) gave one major sub-fraction B1. Column chromatography of B1 on Sephadex L20 column using MeOH afforded compound 2 (11 mg). Fraction I was chromatographed on Sephadex column using H<sub>2</sub>O:MeOH (1:1) followed by MeOH for elution, afforded compounds 3 (45 mg). Column chromatography of fraction J on polyamide column (2.5 cm × 45 cm), gradient elution with CHCl<sub>3</sub>/MeOH, gave four major sub-fractions: J1, J2, J3 and J4, each was subjected to column chromatography on Sephadex L20 using MeOH afforded compounds: 4 (12.5 mg), 5 (5 mg), 6 (4 mg) and 7 (40 mg).

The water fraction residue (30 g) was chromatographed on Sephadex L20 column using H<sub>2</sub>O:MeOH (1:1) yielding three major sub-fractions, each was subjected to column chromatography on Sephadex using MeOH to yield compounds 8 (10 mg), 9 (62 mg) and 10 (42 mg). The isolated compounds (1–10) were subjected to chemical and spectroscopic methods of analysis for their structure elucidation[14–16].

### 2.5.3. GC-MS analysis of the lipophilic fractions

#### 2.5.3.1. Extraction of lipophilic fractions

A total of 50 g of powdered plant leaves and fruits of *P. roebelenii* were extracted with (2500 mL) petroleum ether (60–80 °C) each, by Soxhlet method. The solvent was distilled off under vacuum at reduced temperature 45 °C to yield 3 g leaves petroleum ether extract (PLPEE) and 5 g fruits petroleum ether extract (PFPEE) residues.

#### 2.5.3.2. Saponification of the lipophilic fraction and formation of fatty acid methyl esters

One gram of petroleum ether extract (PLPEE, PFPEE) was saponified according to the method described by Abdeddaim *et al.*[17] by refluxing with alcoholic potassium hydroxide (10%) for 45 min to yield the unsaponifiable matter of leaves (LUSM) and of fruits (FUSM) as well as fatty acids fraction. The fatty acids fraction was subjected to methylation according to the method adopted by Mendonça *et al.*[18] by refluxing with absolute methanol (50 mL) and sulphuric acid (3 mL) for 2 h to yield fatty acid methyl esters of leaves (LFAME) and of fruits (FFAME). The produced LUSM, FUSM, LFAME and FFAME are kept in a desiccator for GC-MS analysis.

#### 2.5.3.3. GC-MS analysis for unsaponifiable matter

A 1 µL unsaponifiable matter sample (LUSM, FUSM) was injected

into gas chromatographic apparatus and separation was carried out on DB-5 fused silica (5% phenyl methyl polysiloxane). The carrier gas (helium) pressure was maintained at 13 psi and the column flow rate was 1 mL/min. The oven temperature was maintained at 50 °C isothermal for 3 min, and then heated to 300 °C at a rate of 5 °C/min. The temperature of the injector during the injection was 220 °C.

## 2.6. Biological assay

### 2.6.1. Determination of antioxidant activity

Radical scavenging activity of 70% aqueous methanol extract of *P. roebelenii* leaves and fruits were estimated by 1, 1-diphenyl-2-picrylhydrazyl radical assay[19]. The half maximal scavenging capacity (SC<sub>50</sub>) values for each extract and ascorbic acid was estimated via dose curve.

### 2.6.2. Hepatoprotective activity

#### 2.6.2.1. Isolation and culture of rat hepatocytes monolayer

Primary culture of rat hepatocytes was prepared according to Seglen's method[20], modified by Kiso *et al.*[21], using Wistar male rats (250–300 g) obtained from the animal house of the National Research Center, Cairo. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

#### 2.6.2.2. Evaluation of hepatotoxicity

After pre- incubation of primary culture of rat hepatocytes for 22–24 h, the monolayer was checked under inverted microscope (Olympus) for attachment. Then, the monolayer was washed twice with phosphate buffer saline (PBS). In order to determine IC<sub>50</sub>, different concentrations of PLAME, PFAME, PLPEE and PFPEE were prepared. The range of concentrations used started from 125 to 1000 µg/mL that induced death of half the number of cells. *P. roebelenii* extracts were dissolved in dimethyl sulfoxide (1% maximum concentration). For each concentration, three replicates were carried out. The plate was incubated for 2 h in CO<sub>2</sub> incubator. After cell incubation with the extract, cell viability was determined using neutral red assay[22]. Absorbance produced by viable cells was read at 540 and 630 nm dual wavelength using automatic kinetic microplate reader (Labsystems Multiskan RC reader). Each experiment was carried out in triplicate to confirm validity of results. A graph was plotted with x-axis showing different concentrations of extracts used, y-axis showing absorbance percentage of viable cells.

#### 2.6.2.3. Evaluation of hepatoprotective activity

Monolayer from primary cultures of rat hepatocytes was performed

in the 96-well plate, incubated for 22-24 h, washed twice with PBS. Different concentrations were prepared from each of the extracts of *P. roebelenii*, starting from 12.5 µg/mL and increasing concentration in ascending order by dissolving in dimethyl sulfoxide (1% maximum concentration). For each concentration, three replicates were carried out, in addition to controls which were: cell control (cells only), 50% of cell control, paracetamol (25 mmol/L) negative control (cells + paracetamol) and sylimarin (50 µg/mL) positive control (reference) (cells + sylimarin + paracetamol). The plate was incubated for 2 h, washed twice with PBS. Paracetamol (25 mmol/L) was added to each well except on that of the cell control (100% and 50%), and incubated for 1 h. Following incubation, the monolayer was washed again with PBS. The half maximal inhibitory concentration (IC<sub>50</sub>) was determined using neutral red assay, by comparing the results to that of the control wells that contain: cell control (2 × 10<sup>6</sup> cells/mL), a total of 50% of cell control (1 × 10<sup>6</sup> cells/mL) and positive control (cells + sylimarin + paracetamol) which was able to protect the cells from the hepatotoxic effect of paracetamol. The concentration of the extract that was able to protect the cells from the hepatotoxic effect of paracetamol by 100% was considered hepatoprotective.

#### 2.6.2.4. Statistical analysis

All data presented are based on means of triplicate absorbance determinations. Experiments were repeated twice for all samples.

### 3. Results

#### 3.1. DNA profiling analysis

Total genomic DNA profiling of *P. roebelenii* O'Brien grown in Egypt was performed using 10 random primers. The number of banding patterns generated by each primer was recorded to obtain the plant DNA profile, in order to compare it with previously reported phenotypic characters as well as for chemical investigations. The ten primers (A-15, A-04, A-17, A-18, C-01, C-19, C-07, D-05, G-19 and M-02) of arbitrary sequences generated 91 fragments in *P. roebelenii* O'Brien (Table 1). The obtained RAPD-PCR products are represented in Figure 1.

**Table 1**

Total numbers of RAPD-PCR fragments in *P. roebelenii* O'Brien.

Primer code	Sequences (5'- 3')	RAPD fragments
A15	5'-TTCCGAACCC-3'	8
A04	5'-AATCGGGCTG-3'	7
A17	5'-GACCGCTTGT-3'	7
A18	5'-AGGTGACCGT-3'	12
C01	5'-TTTCGAGCCAG-3'	15
C19	5'-GTTGCCAGCC-3'	11
C07	5'-GTCCCCGACGA-3'	9
D05	5'-TGAGCGGACA-3'	4
G19	5'-GTCAGGGCAA-3'	10
M02	5'-ACAACGCCTC-3'	8
Total		91

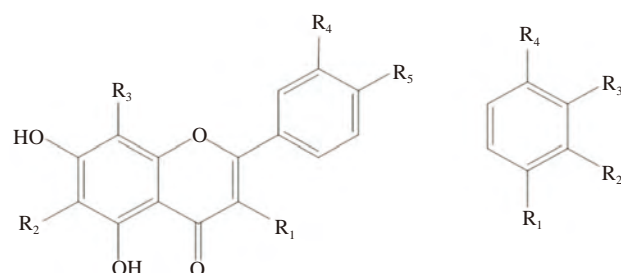
#### 3.2. Chemical investigation

##### 3.2.1. Total phenolic content

The total phenolic contents of the aqueous methanol extract of the leaves and fruits of *P. roebelenii* O'Brien were 64.3 and 7.88 µg of gallic acid equivalents/mg, respectively.

##### 3.2.2. Phenolics from *P. roebelenii* leaves

Phytochemical investigation of defatted aqueous methanol extracts of *P. roebelenii* leaves led to the isolation of 10 phenolic compounds identified as: apigenin 6,8-di-C-β-glucopyranoside (1), quercetin 3-O-glucoside (2), luteolin 3'-O-β-glucopyranoside (3), quercetin (4), apigenin (5), luteolin (6), baicalein (7), p-hydroxybenzoic acid (8), vanillic acid (9) and caffeic acid (10) (Figure 2).



- 1: R<sub>1</sub> = H, R<sub>2</sub> = R<sub>3</sub> = C-glucose, R<sub>4</sub> = H, R<sub>5</sub> = OH  
 2: R<sub>1</sub> = O-glucose, R<sub>2</sub> = R<sub>3</sub> = H, R<sub>4</sub> = R<sub>5</sub> = OH  
 3: R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = H, R<sub>4</sub> = O-glucose, R<sub>5</sub> = OH  
 4: R<sub>1</sub> = OH, R<sub>2</sub> = R<sub>3</sub> = H, R<sub>4</sub> = R<sub>5</sub> = OH  
 5: R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = R<sub>4</sub> = H, R<sub>5</sub> = OH  
 6: R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = H, R<sub>4</sub> = R<sub>5</sub> = OH  
 7: R<sub>1</sub> = R<sub>2</sub> = R<sub>3</sub> = R<sub>4</sub> = H, R<sub>5</sub> = OH  
 8: R<sub>1</sub> = OH, R<sub>2</sub> = R<sub>3</sub> = H, R<sub>4</sub> = COOH  
 9: R<sub>1</sub> = COOH, R<sub>2</sub> = H, R<sub>3</sub> = OCH<sub>3</sub>, R<sub>4</sub> = OH  
 10: R<sub>1</sub> = R<sub>2</sub> = OH, R<sub>3</sub> = H, R<sub>4</sub> = CH = CHCOOH

**Figure 2.** Compounds isolated from leaves of *P. roebelenii*.

##### 3.2.3. Lipophilic constituents of petroleum ether extracts of *P. roebelenii* leaves and fruits

The GC-MS analysis of *P. roebelenii* leaves and fruits [unsaponified (LUSM, FUSM) and saponified (LFAME, FFAME)] petroleum ether fractions revealed the presence of 59 and 46 phytochemical compounds, respectively, that could contribute to the medicinal quality of the plant. The identification of the compounds was confirmed on the basis of peak area, retention time and molecular formula. The active principles with their relative retention time, molecular weight and peak area in percentage are presented in Tables 2 and 3. The first compounds identified with less retention time for LUSM and FUSM were hexadecane and tetradecane (0.58, 0.66 relative retention time) respectively, whereas for LSM and FSM, palmitic acid and methyl 14-methyl-pentadecanoate were the last compounds appearing at relative retention time (0.66, 0.8) respectively.

#### 3.3. Biological assay

##### 3.3.1. Antioxidant assay

*P. roebelenii* leaves and fruits aqueous methanol extracts

**Table 2**GC-MS analysis of the unsaponified compounds of petroleum ether extract of *P. roebelenii* leaves .

Leaves	Relative retention time*	Molecular weight	% Area	Fruits	Relative retention time**	Molecular weight	% Area
Hexadecane	0.58	226	1.80	Tetradecane	0.66	198	2.10
<i>n</i> -Octylcyclohexane	0.60	196	0.57	3-Methyltridecane	0.70	198	0.56
2,6,10-Trimethyltetra-decane	0.63	240	0.39	1-Eicosanol	0.72	298	0.58
Octadecane	0.65	254	6.94	Hexadecane	0.73	226	5.78
Cyclotridecane	0.68	182	1.28	Octadecane	0.75	254	0.93
2-Methyl pentadecane	0.69	240	0.86	Heptadecane	0.78	240	3.80
2,6-Dimethyloctade-cane	0.69	282	0.88	Pentatriacontae	0.79	492	1.50
Isopropylcyclo-undecane	0.70	196	0.81	Docosane	0.82	310	0.57
2,2,3,3,5,6,6 Heptamethyl-heptane	0.71	198	8.12	2-Methyl-Octadecane	0.83	268	0.50
2,2,4,9,11,11-Hexamethyl-dodecane	0.74	254	1.07	1-Nonadecene	0.84	266	2.48
<i>n</i> -Decylcyclo-hexane	0.75	224	0.40	Tricosane	0.85	324	1.00
2-Methyl hexadecane	0.75	282	0.49	Nonacosane	0.90	408	1.40
<i>n</i> -Nonadecane	0.77	268	3.63	2,2 Dimethyl-eicosane	0.94	310	0.69
2,6-Dimethyl heptadecane	0.78	268	1.45	1-Docosene	0.95	308	5.05
9- <i>n</i> -Hexyl heptadecane	0.81	324	0.47	Eicosane	0.95	282	4.46
5- <i>n</i> -Butyl hexadecane	0.82	282	0.54	(3E)-3-Icosene	0.96	280	0.74
(3E)-3-Icosene	0.83	280	1.99	<i>n</i> -Octadecanol	1.00	270	9.04
Docosane	0.92	310	0.56	<i>n</i> -Heneicosane	1.01	296	0.55
1-Eicosanol	0.93	298	3.83	Phytol	1.02	296	2.40
Pentacosane	0.94	352	3.53	Docosane	1.04	310	0.80
<i>n</i> -Octadecanol	0.98	270	7.54	1-Nonadecanol	1.05	284	4.98
Triacontane	0.99	422	0.56	2,6,10,14-Tetramethyl-heptadecane	1.05	296	3.10
Phytol	1.00	296	10.64	2-Ethyl-1-decene	1.06	168	0.76
7- <i>n</i> -Hexyl eicosane	1.02	366	0.60	Heptacosane	1.14	380	1.51
Eutanol G	1.03	298	3.63	Octacosanol	1.23	410	1.56
<i>n</i> -Heneicosane	1.03	296	2.23	17-Pent triacontene	1.26	490	0.55
2-Ethyl-1-dodecene	1.04	196	0.59	Cyclohexa-decane	1.31	224	0.68
Lignoceric alcohol	1.12	354	2.03	Squalene	1.32	410	0.53
	1.13	506	0.95	-Sitosterol	1.50	414	0.54

Relative retention time\*: Relative to phytol = 36.91 min; Relative retention time\*\*: Relative to *n*-Octadecanol = 36.33 min.**Table 3**GC-MS analysis of the saponified compounds of petroleum ether extract of *P. roebelenii* fruits .

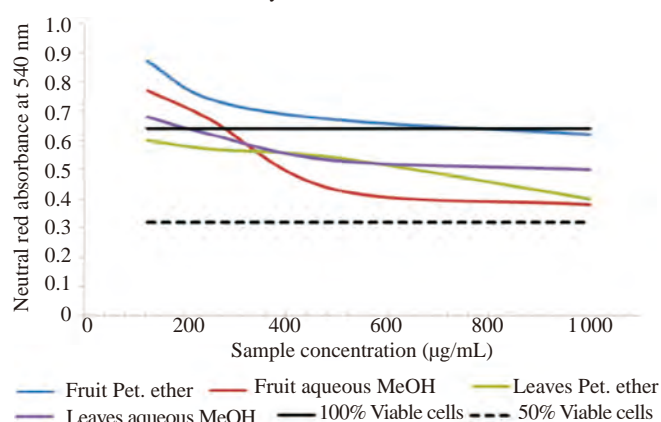
Leaves	Relative retention time*	Molecular weight	% Area	Fruits	Relative retention time**	Molecular weight	% Area
Methyl palmitate	0.66	270	7.72	Methyl 14-methyl-Pentadecanoate	0.80	270	1.88
Methyl 14-methyl hexadecanoate	0.74	284	0.18	Methyl 2-isopropyl-methyl-5-oxo-hexanoate	0.96	200	0.14
Methyl trans-9, trans-11-octadecadienoate	0.80	294	0.58	Methyl linolelaidate	1.00	294	33.61
Methyl linoleate	0.82	294	11.68	Methyl stearate	1.02	298	2.36
Methyl oleate	0.83	296	12.35	Methyl trans-9,10-epoxystearate	1.03	312	0.11
Methyl stearate	0.84	298	5.74	Dimethyl pentadecanedioate	1.10	300	24.41
Methyl linolelaidate	0.85	294	1.01	Methyl 2'-hexyl-[1,1'Bicyclopropyl]-2-octanoate	1.14	322	0.19
Methyl trans-10, cis-12-octadecadienoate	0.87	294	0.71	Methyl cis-12,13-epoxistearate	1.16	312	0.13
Methyl dihydrosterulate	0.90	310	0.33	Methyl(9E)-9-dodecenoate	1.18	212	0.39
Methyl 10-undecenoate	0.97	198	0.07	Methyl 12-hydroxy-9-octadecenoate (methyl ricinoleate)	1.19	312	1.47
Methyl ricinoleate	1.00	312	51.12	Methyl 9,10-dihydroxystearate	1.19	330	2.52
Methyl arachisate	1.01	326	0.19	Methyl Trans-11-(3-pentyl-2-oxiranyl) undecanoate	1.21	312	0.27
Methyl undecenoate	1.02	212	0.41	Methyl 3-hydroxyoctadec-anoate	1.28	314	0.16
Methyl 6,7-Methylene Octadecanoate	1.08		0.05				

Relative retention time\*: Relative to methyl ricinoleate = 21.65 min; Relative retention time\*\*: Relative to methyl linolelaidate = 17.75 min.

(PLAME, PFAME respectively) exhibited a free radical scavenging activity against DDPH at  $SC_{50}$  values 60.25 and 51.7  $\mu\text{g/mL}$  respectively, while their petroleum ether extracts PLPEE and PFPEE at  $SC_{50}$  values 6.3 and 21  $\mu\text{g/mL}$  respectively relative to ascorbic acid ( $SC_{50}$  = 12.66  $\mu\text{g/mL}$ ).

### 3.3.2. Hepatotoxicity

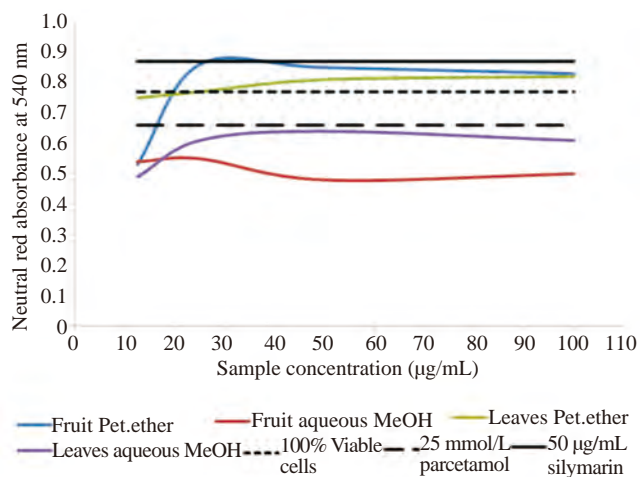
The viability assay was applied with a broad range of concentrations of the aqueous methanol and petroleum ether extracts of *P. roebelenii* O'Brien leaves and fruits (from 125-1000  $\mu\text{g/mL}$ ) on monolayer of rat hepatocytes. The assay revealed that the extracts (PLAME, PFAME, PLPEE and PFPEE) hepatotoxicity were at an  $IC_{50}$  > 1000  $\mu\text{g/mL}$  (Figure 3).



**Figure 3.** Viability of monolayer of rat hepatocytes after 2 h treatment with different concentrations of *P. roebelenii* extracts using neutral red colourimetric assay. Each point represents the mean  $\pm$  SD ( $n$  = 3).

### 3.3.3. Evaluation of hepatoprotective activity applying rat hepatocyte monolayer

The hepatoprotective effect of the tested extracts against paracetamol toxic effect using neutral red colourimetric assay showed that PLPEE and PFPEE have exerted hepatoprotective effects at concentrations >12.5 and 15  $\mu\text{g/mL}$ , respectively. While, PLAME and PFAME did not show hepatoprotection activity till the maximum concentration tested (100  $\mu\text{g/mL}$ ) (Figure 4).



**Figure 4.** Viability of monolayer of rat hepatocytes after 2 h treatment with different concentrations of *P. roebelenii* extracts followed by treatment with 25 mmol/L paracetamol for 1h in comparison with 50  $\mu\text{g}$  silymarin as control using neutral red colourimetric assay. Each point represents the mean  $\pm$  SD ( $n = 3$ )

## 4. Discussion

In the DNA profiling analysis of *P. roebelenii* O'Brien, the ten primers used had produced multiple band profiles with a number of amplified DNA fragments ranging from 15 when C01 was used. Whereas, the least number of fragments was 4 being produced by D05. Primers A18 and C19 showed good domination for *P. roebelenii* O'Brien producing 12 and 11 amplified DNA fragments, respectively. Primers G19 and C07 showed moderate domination producing 10 and 9 amplified DNA fragments, respectively. Primers A15 and M02 produced 8 amplified DNA fragments. Whereas primers A04 and A17 produced only 7 amplified DNA fragments. Therefore, primer C01 was the best sequence for dominating *P. roebelenii* O'Brien cultivated in Egypt.

The present study revealed that total phenolics content of *P. roebelenii* is more prominent in leaves than fruits. Ten phenolic compounds were isolated and identified for the first time from *P. roebelenii*. Their structures were established on the basis of chemical methods (acid hydrolysis, enzymatic hydrolysis and  $\text{FeCl}_3$  degradation) and spectral techniques (UV,  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and M+S)[14-16](Figure 2). The compounds spectral data were in good accordance with those previously published ones[23-31]. Referring to the literature, baicalein is not reported before in genus *Phoenix*.

GC-MS spectral data analysis of PLPEE and PFPEE revealed the presence of 59 and 46 phytoconstituents, respectively. For instance, ricinoleic acid (51.12%), oleic acid (12.35%) linoleic acid (11.68%), linolelaidic acid (5.05 %), phytol (10.64%) and *n*-octadecanol (7.54%) are major constituents of PLPEE. Whereas, linolelaidic acid (33.61 %),

*n*-octadecanol (9.04%) and hexadecane (5.78%) are major constituents of PFPEE. The identified compounds possess various biological properties, their potential antioxidant and anti-inflammatory activities play important roles in the antioxidant and hepatoprotective activity of the leaves and fruits petroleum ether extracts[32-45].

Antioxidant assay of PLPEE and PFPEE displayed more prominent free radical scavenging activity against 1, 1-diphenyl-2-picrylhydrazyl than PLAME and PFAME. In addition, *in vitro* hepatotoxicity assay, revealed that all the tested extracts displayed high margin of safety on the hepatocytes. PLPEE and PFPEE conferred an *in vitro* potent hepatoprotective activity with therapeutic index 40 (Hepatotoxicity/therapeutic dose 100) compared to 56 for silymarin (Reference control). Whereas, PLAME and PFAME did not reveal significant hepatoprotective activity. The antioxidant and hepatoprotective activities of the leaves and fruits petroleum ether extracts (PLPEE and PFPEE) could be presumably ascribed in part to the lipophilic constituents revealed by GC-MS analysis of the saponified and usaponified fractions. Therefore, this study provides the rationale for further deep chemical and biological studies on this newly investigated palm tree, which can be used as a source of natural raw material for phytopharmaceuticals.

## Conflict of interest statement

We declare that we have no conflict of interest.

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