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Unveiling fatty acids profile using gas chromatography (GC) and genetic pattern of *Wodyetia bifurcata* family Arecaceae using Random Amplified Polymorphic-DNA (RAPD) technique.

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ABSTRACT

The DNA profiling using random amplified polymorphic DNA (RAPD) technique of aerial parts of *Wodyetia bifurcata* (family Arecaceae) collected in Egypt known as foxtail palm resulted in totally 85 amplified DNA fragments as the primers OPC-12, OPE-01, OPB-02 and OPG-12 was dominating for *Wodyetia bifurcata*. The lipoidal matter obtained from *Wodyetia bifurcata* showed that unsaponifiable fraction revealed that eicosane was the major unsaponifiable fatty acid, while the main sterol was campesterol. The saponifiable fraction revealed that Oleic acid was saponifiable fatty acid.
Keywords: *Wodyetia bifurcata,* Arecaceae, RAPD, DNA, lipoidal matters.

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INTRODUCTION

Family Arecaceae is one of the biggest vegetal families in the world and by its morphological aspects is the most characteristic of tropical flora [1]. It comprises more than 2,700 species in 202 genera[2].

Wodeytia is a genus of trees belonging to family Arecaceae. *Wodyetia bifurcata* is fast growing, large sized evergreen tree, up to 10 m tall. *Wodyetia bifurcata* L is endemic to Australia and it is found in north Queensland [3].

Evaluation of fatty acids composition has a great metabolic and structural importance[4]. The major unsaturated fatty acids are oleic acid (OA), linoleic acid (LA) and alfa -linolenic acid (ALA). Fatty acids with even numbers of carbon atoms, from 16 to 18, with a single carboxyl group, are the most common fatty acids present in vegetable oils[5,6]. DNA-based molecular markers have utility in the fields like taxonomy, physiology, embryology, and genetics.

The chromatographic techniques and marker compounds used to standardize botanical preparations has limitations due to their variable sources and chemical complexity. The DNA-based techniques have been widely used for authentication of plant species of medicinal importance [7]. Recently, Random amplified polymorphic DNA technique (RAPD-DNA) constitutes a useful technique for the study of genetic polymorphism of DNA. It involves the amplification of random segments of genomic DNA by polymerase chain reaction (PCR), using short single primers of arbitrary sequence to develop DNA markers [8]. DNA markers have proven to be efficient in evaluation and selection of plant material because, these markers are not affected by the environment as morphological markers [9]. Low expense, efficiency in developing large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable for DNA profiling[10].

MATERIAL

Plant material:

A sample of *Wodyetia bifurcata* was purchased from El-Orman Botanical Garden, Cairo, Egypt in July 2011 and the plant was kindly identified by agricultural engineer Terease Labib, El-Orman Botanical Garden.

Material for DNA profiling:

Buffer solutions & Enzymes:

- Extraction buffer: 1% (w/v) N-acetyl-N, N, N-trimethylammonium bromide (CTAB), 0.7 M Nacl, M Tris (pH 7.5), 0.01 M EDTA, 1% (v/v) β-mercaptoethanol (added immediately before use).
- Washing buffer 1: 76% ethanol, 0.2 N sodium acetate.
- Washing buffer 2: 76% ethanol, 10mM ammonium acetate.
- TE buffer: 10 mM Tris (pH 8.0), 1 mM EDTA, 10 x reaction buffer: 100 mM tris (pH 8.3), 500 mM KCl, 0.01% (w/v) gelatin, chloroform/isoamyl alcohol 24:1 (v/v), isopropanol.
- DNTP'S mix (Pharmacia, Sweden).
- Taq DNA polymerase (PerkinElmer/Cetus, USA, Advanced Biotechnologies, UK).
- RNA ase: Boehringer Mannheim.

Primers:

Twelve primers used for Random Amplified Polymorphic-DNA analysis, Operon Technologies Inc., Almeda, California, USA, with the following sequence:

- OPG-05 (5'-CTGAGACGGA-3')
- OPC-12 (5'-TGTCATCCCC-3')
- OPC-16 (5'-CACACTCCAG-3')
- OPA-02 (5'-TGCCGAGCTG-3')

- OPA-12 (5'-TCGGCGATAG-3')
- OPE-01 (5'-CCCAAGGTCC-3')
- OPE-10 (5'-CACCAGGTGA-3')
- OPD-20 (5'-ACCCGGTCAC-3')
- OPH-15 (5'-AATGGCGCAG-3')
- OPB-05 (5'-TGCGCCCTTC-3')
- OPB-02 (5'-TGATCCCTGG-3')
- OPG-12 (5'-CAGCTCACGA-3)

Agarose gel:

Stationary phase for electrophoresis, 1.4% with running buffer TE buffer.

Molecular size marker:

100 ladder, Promega Corporation, Madison, USA.

Apparatus:

Apparatus for DNA profiling

- DNA thermocycler (Hybaid PCR Express).
- A Perkin Elmer Cetus 480 used for the amplification of DNA
- Agarose gel electrophoresis tool (Biorad Wide Mini Sib Cell) for separation of RAPD fragments according to size.
- UV Polaroid camera type 57 (ASA 3000) for visualization of RAPD fragments.
- Data analyser software: Gel- Pro Analyser V.3.1, USA.

Apparatus for fatty acid:

GLC instrument (Hewlett Packard HP 6890 series GC system) for saponifiable and unsaponifiable matter in GC laboratory (central cervices laporatory), National Research Center, Dokki, Giza.

METHODS

Method for DNA profiling [11]:

DNA extraction and quantification

DNA was extracted using the CTAB (1% (w/v) N-cetyl-N, N, N-trimethylammonium bromide) method (Doyle, J. and Doyle, J. L., 1987). The frozen leaves (50 mg of each sample) were powdered in liquid nitrogen, separately extracted with 0.8 ml CTAB, precipitated with isopropanol, washed in 70% ethanol and dissolved in deionized water.

Amplification of RAPD markers

Twelve oligonucleotide primers of arbitrary sequences were used in this study. The polymerase chain reactions were carried out with 100 ηgm of genomic DNA template following a thermal cyclic program. Amplified products were analysed by electrophoresis in 1.8 % agarose gels and finally stained with ethidium bromide. A molecular size marker was used as standard marker.

Analysis of RAPD data

RAPD bands were treated as presence or absence, without considering their percentage. For estimating genetic distance among the tested samples; each of the DNA bands was treated as a unit character.

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Method for fatty acid:

Extraction of the unsaponifiable matter and fatty acids:

The lipoidal matter obtained by the extraction of (60g) of the air dried aerial part of *Wodyetia* bifurcata with light petroleum ether (b.p.60-80º) were evaporated to yield : (2.4 gm) representing (4%) of the dried plant material, the residue was kept for preparation of unsaponifiable matter and fatty acids.

Saponification process:

About (1.0 g) of the light petroleum ether residue was saponified by refluxing with 30% alcoholic potassium hydroxide for 5 hours [12]. After distillation of the alcohol and dilution with 20 ml water, the unsaponifiable matter (USM) was extracted with ether (4×50ml). The combined ethereal extract was washed several times with distilled water till completely free alkalinity, then dehydrated over anhydrous sodium sulphate and filtered. Residue left after evaporation of ether (0.02 g) was subjected to GLC identification of sterols and hydrocarbons content, tentative identification of hydrocarbons and sterols was accomplished by comparing the relative retention times to those of reference materials run under the same conditions. The aqueous mother liquor left after removal of the USM was acidified with 10 % hydrochloric acid to liberate the corresponding free fatty acids. The liberated fatty acids were extracted with petroleum ether (4×50ml). The combined ethereal extract was washed with water then dehydrated over anhydrous sodium sulphate. It was evaporated to give (0.05g).

Methylation of fatty acids

The fatty acids residue was methylated and analyzed by GLC. Identification of fatty acids of *Wodyetia* bifurcata was accomplished by comparing the relative retention times to those of available references analyzed under the same conditions.

5. RESULTS AND DISCUSSION

DNA profiling

Wodyetia bifurcata was subjected to RAPD assay of its genomic DNA. This was performed using different primers as shown in table (1). The number of RAPD-PCR fragments indicates that the primers were reproduced as shown in figure (1). The DNA amplified with RAPD technique using *OPC-12* (5'-TGTCATCCCC-3') and *OPE-01* (5'-CCCAAGGTCC-3') and *OPB-02 (*5'-TGATCCCTGG-3') and OPG-12 (5'-CAGCTCACGA-3') primers are the most characteristic showing 9 fragment while prime *OPD-20* (5'-ACCCGGTCAC-3') is the least characteristic showing 4 fragments. It is noteworthy that primer *OPA-12* showed good dominaton for *Wodyetia bifurcata* producing 8 amplified DNA fragments while primers *OPG-05, OPE-10 and OPB-05* showed moderate dominations producing 8 amplified DNA fragments. Whereas primers *OPA-02* and *OPH-15* produced only 5 amplified DNA fragments. The primers used for RAPD-DNA analysis produced totally 85 amplified DNA fragments as shown in table (2). Therefore primers *OPC-12* and *OPE-01* and *OPB-02* and OPG-12 was the best sequence for dominating *Wodyetia bifurcata* cultivated in Egypt producing the highest hits.

Table (1): Molecular size in base pairs of amplified DNA fragments produced by ten decamer primers in *Wodyetia bifurcata*.

Band #	Approx. band size in bp	OPG-05	OPC-12	OPA-02	OPE-01	OPE-10	OPD-20	OPH-15	OPB-05	OPC-16	OPA-12	OPB-02	OPG-12
1.	1700	-	-	-	-	-	-	-	+	-	-	+	-
2.	1600	+	-	-	-	-	-	-	-	-	-	-	+
3.	1500	-	-	-	-	-	-	-	-	-	+	+	-
4.	1400	+	+	-	-	-	-	-	-	-	-	-	-
5.	1300	-	-	-	-	-	-	-	-	-	+	-	-
6.	1200	-	+	-	-	-	-	-	-	+	-	-	-
7.	1100	-	+	-	-	-	-	-	-	-	+	-	-

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8.	1000	-	+	-	-	+	+	-	-	-	-	-	-
9.	950	+	+	-	-	-	-	+	-	-	+	+	+
10.	900	-	-	-	+	-	-	-	-	+	-	-	-
11.	850	-	-	-	+	-	-	-	-	-	-	-	-
12.	800	-	-	-	-	-	+	+	+	-	-	-	+
13.	750	-	+	-	-	+	-	-	-	+	+	-	-
14.	700	-	-	+	+	-	-	+	+	-	-	+	+
15.	650	-	+	-	-	-	-	-	-	-	+	-	-
16.	600	-	-	-	+	-	-	+	+	+	-	+	+
17.	550	-	-	-	-	-	+	-	-	-	-	+	
18.	500	+	+	-	+	+	-	-	+	-	+	+	+
19.	450	-	-	-	-	+	-	-	-	-	-	+	
20.	430	-	-	-	-	-	-	-	-	-	-	-	+
21.	400	+	-	-	+	+	+	-	-	-	-	+	+
22.	370	-	+	+	-	-	-	-	-	-	+	-	-
23.	350	-	-	-	-	-	-	-	+	-	-	-	+
24.	300	-	-	+	+	-	-	-	+	-	-	-	-
25.	270	-	-	-	-	+	-	-	-	-	-	-	-
26.	250	-	-	-	-	-	-	+	-	-	-	-	-
27.	200	-	-	-	-	+	-	-	-	+	-	-	-
28.	180	-	-	-	+	-	-	-	-	-	-	-	-
29.	150	-	-	+	-	-	-	-	-	+	-	-	-
30.	100	-	-	+	+	-	-	-	-	-	-	-	-
Total		7	9	5	9	7	4	5	7	6	8	9	9

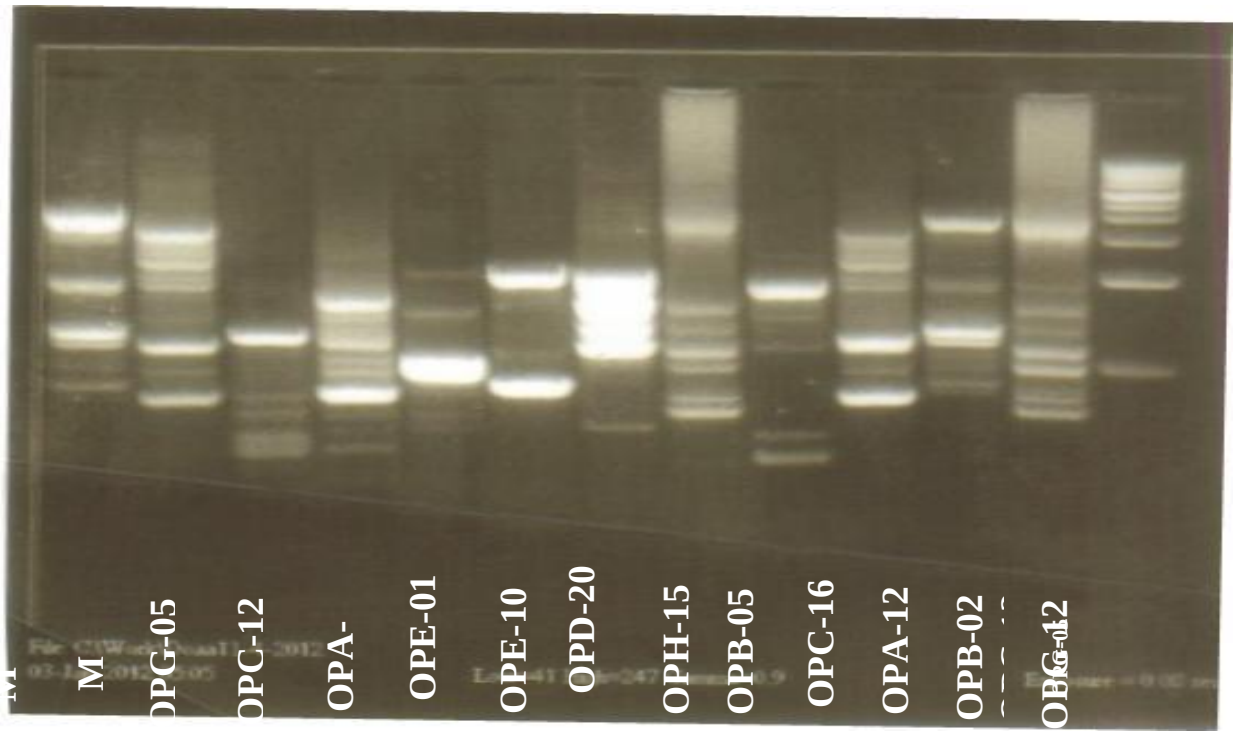

Figure (1): The obtained RAPD-PCR products for *Wodyetia bifurcata*. using ten decamer primers

Table(2): Total numbers of RAPD Fagments in *Wodyetia bifurcata*

Primer Code	OPG-05	OPC-12	OPA-02	OPE-01	OPE-10	OPD-20	OPH-15	OPB-05	OPC-16	OPA-12	OPB-02	OPG-12	Total
RAPD Fragments	7	9	5	9	7	4	5	7	6	8	9	9	85

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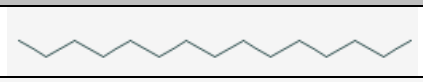
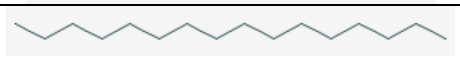
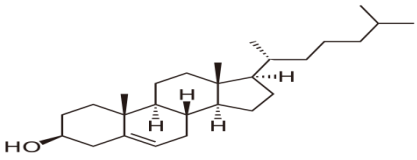
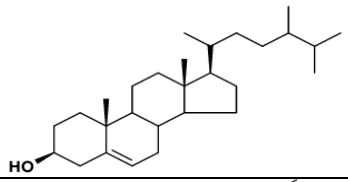
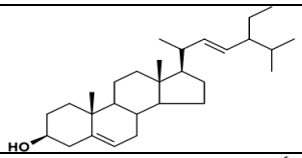
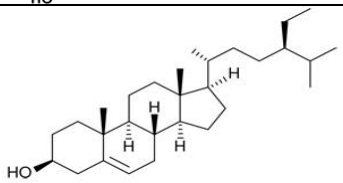
Lipoidal matters:

The results of the fatty acid analysis of aerial parts of *Wodyetia bifurcata* unsaponifiable fraction are shown in table (3) revealed the presence of series of *n*-alkanes ranging from Pentadecane (C15) to hexacosane (C26) representing (24.17%) of (USM), and the most predominant homolog was eicosane (C-20) (9.71%). Also revealed sterols and campesterol represent (4.71%).

Table (3): GLC analysis of hydrocarbons and sterols in Unsaponifiable matters of *Wodyetia bifurcata*:

Peak no.	RR_t	Percentage	Carbon no.	Component name	Structure
1	0.675	0.06469	c-15	Pentadecane	
2	0.791	0.43328	c-16	Hexadecane	
3	0.827	0.56436	c-17	Heptadecane	
4	0.878	0.83634	c-18	Octadecane	
5	0.919	0.53456	c-19	Nonadecane	
6	1	9.71715	c-20	Eicosane	
7	1.080	3.33135	c-21	Heneicosane	
8	1.132	2.01282	c-22	Docosane	
9	1.180	0.75598	c-23	Tricosane	
10	1.215	1.04224	c-24	Tetracosane	
11	1.271	2.01008	c-25	Pentacosane	
12	1.310	2.87999	c-26	Hexacosane	
13	1.592	1.91954	-----	Cholesterol	
14	1.638	4.71714	-----	Campesterol	
15	1.704	1.22178	-----	Stigmasterol	
16	1.747	0.96514	-----	β –sitosterol	

RRT=Relative retention time to Eicosane.

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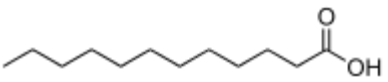
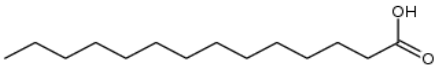
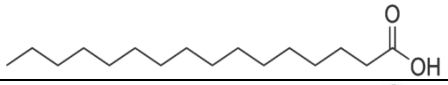
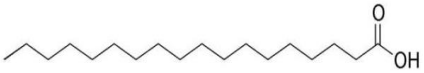
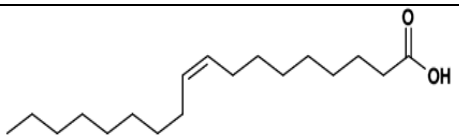
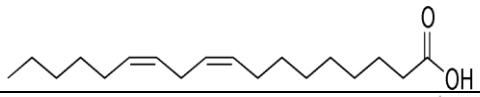
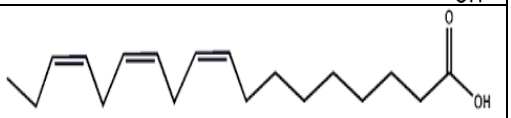
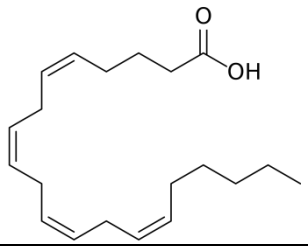
Biological studies showed that eicosane possess antitumour activity against the human gastric SGC-7901 cell line [13]. And could have been responsible for the antibacterial activity [14].

The results of the fatty acid analysis of aerial parts of *Wodyetia bifurcata* saponifiable fraction are shown in Table (4) revealed series of free fatty acids were identified ranging from Dodecanoic acid (Lauric acid) to 5, 8,11,14-all-cis-Eicosatetraenoic acid (Arachidonic acid).

Table (4): GLC analysis of fatty acid esters of Wodyetia bifurcata

Peak no	RR_t^*	Percentage (%)	Carbon no	Component	Structure
1	0.667	11.13208	$C_{(12:0)}$	Dodecanoic acid (Lauric acid)	
2	0.788	1.57590	$C_{(14:0)}$	Tetradecanoic acid (Myristic acid)	
3	0.897	15.93214	$C_{(16:0)}$	Hexadecanoic acid (Palmitic acid)	
4	0.975	1.43804	$C_{(18:0)}$	Octadecanoic acid (Stearic acid)	
5	1	16.92158	$C_{(18:1)}$	*cis*-9-Octadecenoic acid (Oleic acid)	
6	1.012	5.04438	$C_{(18:2)}$	Cis, cis-9,12-octadecadienoic acid (Linoleic acid)	
7	1.048	1.16018	C(18:3)	All cis9,12,15-octadecatrienoic acid (Linolenic acid)	
8	1.169	1.96898	$C_{(20:4)}$	5,8,11,14-all-*cis*-Eicosatetraenoic acid (Arachidonic acid)	

RRT=Relative retention time to Oleic acid.

The monounsaturated fatty acid cis-9-Octadecenoic acid (Oleic acid $C_{18:1}$) was the most abundant fatty acid (16.92%), and the main saturated fatty acid was hexadecanoic acid (Palmetic acid $C_{16:0}$). Oleic acid was reported as an anti-inflammatory fatty acid by influencing Arachidonic acid metabolism which is a precursor of the eicosanoids pro-inflammatory agents [15] as well as playing a role in the activation of different pathways of immune competent cells [16]. Oleic acid is currently thought to exert atheroprotective effects, mostly through a lowering of total LDL and cholesterol [17-19] or through a decrease in coronary risk factors such as hypertension [20,21] diabetes, [22-24] or obesity [25].It is also able to prevent/attenuate Palmitic acid hepatotoxicity [26] through channeling Palmitic acid into TG synthesis, changing the composition of the intracellular fatty acids pool thus protecting the liver cells from Palmitic acid-dependent apoptosis [27]. In addition, it has the ability to reduce the inflammatory effects of long-chain saturated fatty acids in human aortic endothelial cells [28].

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