

GC-MS Analysis of *n*-hexane Extract from Seeds and Leaves of *Phoenix dactylifera* L.

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Summary: *n*-hexane extract of *Phoenix dactylifera* L. (Date Palm) seeds and leaves was analyzed through capillary GC-MS. The compounds were identified by comparison of GC-MS spectrum with library searches. A total of forty one compounds were identified from date palm seeds and leaves. Fatty acid methyl esters (FAME) were found to be the major components from seeds while leaves extract contain fatty acid ethyl ester (FAEE) along with an isopropyl ester of hexadecanoic acid. The seeds extract was also found to contain some steroids and terpenoids. It is the first report of isopropyl and ethyl esters of fatty acids found in date palm leaves. Altogether twenty seven compounds are reported for the first time from date palm.

Introduction

Date palm (*Phoenix dactylifera* L., Palmae) known as Khajoor in Urdu, has been cultivated in the Middle East over at least 6000 years ago. For the natives in this region, dates are considered staple carbohydrate food. Date palm kernels have been shown to exhibit antiaging properties and significant reduction in skin wrinkles in women. Natural fats from date palm reported to prevent irritant contact dermatitis. In animals, the pits have been included in the diet of chicken, sheep, fish and rats and have been shown to enhance growth in this species [1].

The flesh of the fruit of the date palm, *Phoenix dactylifera* L. contains very low percentage of oil (0.2-0.5 %), whereas the seeds contain 7.7-9.7 % oil. The weight of the seed is 5.6-14.2 % of the date and as such represents a potential source of edible oil. A range of saturated and unsaturated fatty acids are present in the flesh and seeds of date. The saturated fatty acids include capric, lauric, myristic, palmitic, stearic, margaric, arachidic, heneicosanoic, behenic and tricosanoic acid. Unsaturated fatty acids include myristoleic, palmitoleic, oleic, linoleic and linoleic acid. [2-7]

Results and Discussion

The GC-MS analysis of *n*-hexane extract of seeds and leaves of *Phoenix dactylifera* afforded number of compounds which were found to be

different from the previously published data [2-7]. Altogether forty one components were identified from date palm seeds and leaves, fifteen of these were identified as fatty acid ethyl ester (FAEE), thirteen as fatty acid methyl ester (FAME) and five as free fatty acids (FFA). Four steroids were also identified along with 3-eicosyne (a long chain alkyne), isopropyl hexadecanoic acid and two terpenoids lupenone and Ursadienone.

The date palm seeds were found to contain fourteen FAME, six of these have been reported previously in the literature [2-7]. The most abundant being 9-octadecenoic acid methyl ester followed by hexadecanoic acid methyl ester. FAME reported for the first time from date palm seeds included 10-methyl undecanoic acid methyl ester, nonanedioic acid dimethyl ester, docosenoic acid methyl ester, tetracosanoic acid methyl ester, hexacosanoic acid methyl ester, octacosanoic acid methyl ester and triacontanoic acid methyl ester. β -sitosterol was found to be the most abundant of steroids, followed by cyclopropa-5, 6-stigmasta-22-en-3-one and stigmasta-4-en-3-one, while lup-20(29)-en-3-one was the only triterpenoid, found to be present in date seeds.

From column chromatography of *n*-hexane extract of date palm leaves, different fractions (Fraction B and C) obtained were found richer in

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FAEE. Altogether fifteen FAEE were present in the leaves extract. Hexadecanoic acid ethyl ester, 9-octadecenoic acid ethyl ester and octadecanoic acid ethyl ester were the major constituents of leaves extract while considerable amount of higher FAEE were also found to be present. A total of twenty four components were identified from *n*-hexane extract of leaves.

Eight major peaks from the MS-data of five compounds, which were resolved partially, are also given (see experimental). These included a monoacylglycerol but its mass spectrum significantly differs from the reported C-18:0, C-18:1 and C-18:2 monoacylglycerol [8], similarly a lupene skeleton was also found but it was neither lupeol (lup-20(29)-en-3-ol) nor lup-20(29)-en-3-one (Table-1).

Experimental

Plant Material

Samples of date palm leaves and seeds were collected during Aug-Sep 2006 from suburbs of Karachi and the identity was confirmed by anatomical examination. The specimen of the recollection (Jul-2008) from the same site was identified by Jan Alam, Senior Taxonomist, Herbarium, Department of Botany, University of Karachi and has been deposited with voucher and herbarium numbers MR-1 and 71517, respectively.

Extraction

Seeds (3 Kg) were soaked in 3L of *n*-hexane for a period of 15 days at room temperature, the extract was then concentrated under reduced pressure to obtained crude fatty substance (156 g). Small portion (5 g) of this crude fat (Fraction A) was then subjected to GC and GC-MS analysis. A total of forty components were present and twenty nine have been identified (Table-1).

The freshly collected leaves (5 Kg) of *Phoenix dactylifera* were shade dried, chopped and were soaked in 10 L of *n*-hexane for a period of 15 days at room temperature. The *n*-hexane extract was collected after 15 days and then concentrated under reduced pressure. The green gummy mass (250 g) thus obtained was then subjected to column chromatography over silica gel 60; (70-230 mesh size, Merck) and the column was then eluted with

n-hexane-chloroform and chloroform-methanol gradient systems. Total nine fractions were collected. Fraction B (10 g) eluted from *n*-hexane-chloroform (99:1) eluent was re-chromatographed over silica gel 60; (70-230 mesh size, Merck) and the column was then eluted with *n*-hexane-EtOAc and EtOAc-methanol gradient systems. Fraction 'C' (7 g) eluted from hexane: EtOAc (99:1) was obtained. Small portion (3 g) of fraction C was then submitted to GC and GCMS analysis indicating thirty nine components of which twenty four were identified (Table-1).

Similarly small amount (1g) of fraction 'B' was also submitted for GC and GCMS analysis which resulted in the identification of twenty two out of thirty four components (Table-1).

GC and GC-MS Analysis

For GC and GC-MS, SPB-5[®] and DB-5MS[®] columns (30 m length, 0.32 mm ID and 0.22 μ m df) were used respectively. Both the GC and GC-MS analysis were performed with the identical gradient thermal ramping and temperatures, as follows: the column was kept at 50 °C for 2 min, and then ramped to 260 °C at a rate of 5 °C. In case of GC, injector and detector were kept at 260 °C. For GC-MS injector was kept at 260 °C, transfer line at 280 °C and EIMS was operated at 70 eV, at 250 °C. He was used as carrier with a flow of 1.8 ml / min. 5 μ l of filtered sample was injected at a split ratio of 1:40.

GC-FID was performed on Shimadzu (Japan) GC-17A coupled with Class GC-10 software while GC-MS was performed on an Agilent (USA) GC 6890 coupled with Jeol (Japan), 600H MS. For the spectral library search, NIST 2005 was used [9]. The quantization was performed using area normalization method on Class GC-10 software.

GC-MS Data of Partially Identified Compounds [*m/z* (% abundance)]

Some fatty acid methyl ester at scan No. 397 in seeds: 155(34), 143(57), 111(52), 87(74), 83(44), 74(100), 55(50), 43(36).

Some oxo fatty acid methyl ester at scan No. 641 in leaves: 123(12), 109(22), 95(32), 85(34), 71(52), 69(32), 58(100), 55(44).

Table-1: GC and GC-MS analysis of seeds and leaves extract of *Phoenix dactylifera* L.

S. No.	Systemic Name	Seeds Extract		Leaves Extract				Refer- ences
		Fraction A		Fraction B		Fraction C		
		Scan	Conc	Scan	Conc	Scan	Conc	
1	Octanoic acid ethyl ester	-	-	233	0.15	234	0.41	2*,3*,5, #
2	Some fatty acid methyl ester	397	0.89	-	-	-	-	PI
3	9-Oxo-nonanoic acid ethyl ester	-	-	447	1.68	451	2.94	#
4	10-methyl Undecanoic acid methyl ester	455	0.98	-	-	-	-	#
5	Nonanedioic acid dimethyl ester	470	0.30	-	-	-	-	#
6	Dodecanoic acid	492	1.04	492	Tr	492	0.14	2*, 3, 5
7	Tetradecanoic acid methyl ester	575	1.09	-	-	-	-	2*
8	Tetradecanoic acid	605	1.38	604	Tr	602	0.13	2*, 3, 5
9	Tetradecanoic acid ethyl ester	612	1.03	614	0.68	614	0.59	#
10	3-eicosyne	-	-	639	0.72	639	0.16	#
11	Some Oxo acid methyl ester	640	Tr	641	1.09	-	-	PI
12	Pentadecanoic acid ethyl ester	-	-	668	0.72	669	1.03	5*, #
13	Hexadecanoic acid methyl ester	688	12.34	684	0.68	685	0.45	2*
14	Hexadecanoic acid ethyl ester	719	2.73	728	37.29	738	49.15	#
15	Hexadecanoic acid	728	1.69	-	-	-	-	2*, 3, 5
16	Hexadecanoic acid isopropyl ester	-	-	748	0.69	744	0.80	#
17	Heptadecanoic acid ethyl ester	-	-	769	1.74	773	2.20	2*,3,5*,#
18	9-Octadecenoic acid methyl ester	774	16.88	-	-	780	Tr	2*, 3, 5*
19	Octadecanoic acid methyl ester	784	2.55	780	Tr	788	Tr	2*
20	9-Octadecenoic acid ethyl ester	801	3.81	805	21.18	809	9.94	#
21	Octadecanoic acid	817	1.10	-	-	818	Tr	2*, 3
22	Octadecanoic acid ethyl ester	-	-	818	5.38	825	9.54	#
23	Nonadecanoic acid ethyl ester	858	1.52	-	-	862	0.56	#
24	Eicosanoic acid methyl ester	872	1.01	872	Tr	872	Tr	2*, 3
25	Eicosanoic acid	895	1.49	-	-	-	-	2, 3*
26	Eicosanoic ethyl ester	-	-	903	0.75	906	1.84	#
27	Some monoglyceride	933	0.98	-	-	-	-	PI
28	Heneicosanoic acid ethyl ester	-	-	945	Tr	947	0.02	2*, 3, #
29	Docosenoic acid methyl ester	945	0.93	-	-	-	-	#
30	Docosanoic acid methyl ester	956	0.98	957	Tr	956	Tr	2*, 3*
31	Docosanoic acid ethyl ester	-	-	986	1.59	991	2.82	#
32	Tricosanoic acid ethyl ester	-	-	1026	0.75	1031	1.90	2*, 3*, #
33	Tetracosanoic acid methyl ester	1035	1.15	-	-	-	-	#
34	Tetracosanoic acid ethyl ester	1063	0.86	-	-	1076	3.00	#
35	Pentacosanoic acid ethyl ester	-	-	1066	1.18	-	-	#
36	Hexacosanoic acid methyl ester	1139	1.33	-	-	-	-	#
37	Some Lupene	1263	0.85	-	-	-	-	PI
38	Ursa-9(11)-12-diene 3-one	-	-	1291	Tr	-	-	#
39	Octacosanoic acid methyl ester	1310	1.08	-	-	-	-	#
40	Some Trimethyl-hexacosanoic acid methyl ester	1383	Tr	-	-	-	-	PI
41	β -sitosterol	1488	1.32	-	-	-	-	3
42	Lup-20(29)-en-3-one	1539	1.35	-	-	-	-	#
43	Cyclopropa-(5,6)-stigmast-22-en-3-one	1550	1.13	-	-	-	-	#
44	Triacotanoic acid methyl ester	1571	1.31	-	-	-	-	#
45	Stigmast-3,5-dien-7-one	1589	0.86	-	-	-	-	#
46	Stigmast-4-en-3-one	1638	1.13	-	-	-	-	3

* = reported earlier as FFA, # = Identified for the first time from the source, PI = Partially Identified,

Tr = Traces < 0.1%, Conc= percentage concentration, Ref= reference number

Statistics

S. No.	Components	Fraction A		Fraction B		Fraction C	
		No.	Conc.%	No.	Conc.%	No.	Conc.%
1	Total Fatty acid methyl ester (FAME)	14	42.82	4	1.77	5	0.45
2	Total Fatty acid ethyl ester (FAEE)	5	9.95	13	67.71	14	85.92
3	Total Free Fatty Acid (FFA)	5	6.70	2	Tr	3	0.27
4	Steroids / Terpenoids / others	5	7.62	3	6.79	2	0.96
5	Total Identified Components	29	67.09	22	76.27	24	87.60
6	Total Unidentified Components	11*	32.91	12*	23.73	15*	12.4

a = Appearing at scan numbers 669, 736, 810, 884, 981, 1021, 1127, 1298, 1360, 1402 and 1669;

b = Appearing at scan numbers 378, 559, 652, 736, 758, 810, 863, 885, 915, 965, 1197 and 1393;

c = Appearing at scan numbers 380, 429, 559, 564, 642, 652, 758, 762, 834, 839, 866, 885, 895, 917 and 1197.

No. = number of components

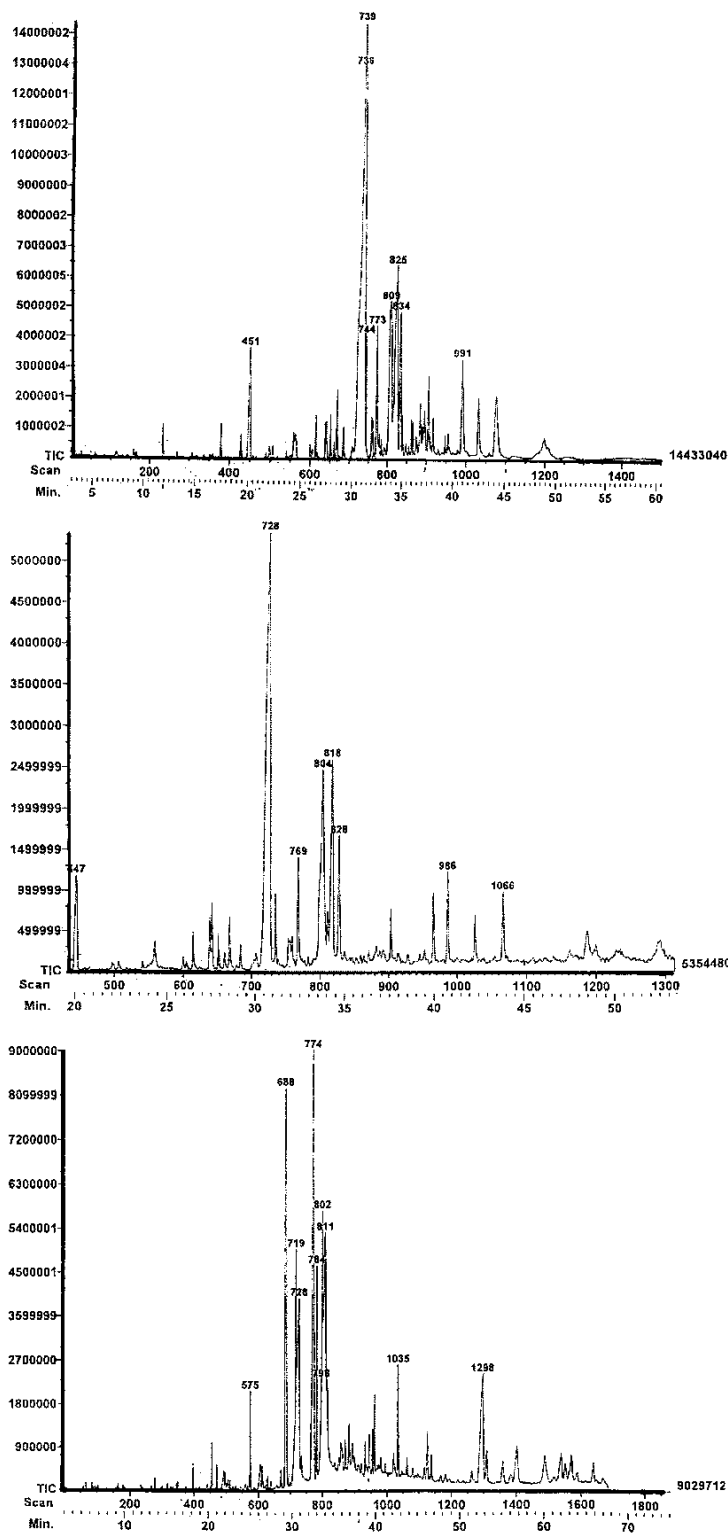


Figure – GCMS Chromatograms: Seeds fraction A (Top) and Leaves fractions B and C (Bottom).

Some monoglyceride at scan # 933 in seeds: 129(100), 116(32), 111(30), 97(46), 83(53), 69(60), 55(85), 43(47).

Some lupane at scan # 1263 in seeds: 410(73), 218(74), 205(74), 109(69), 95(100), 81(64), 69(93), 55(85).

Some trimethyl hexacosanoic acid methyl ester at 1383 in seeds: 452(89), 101(100), 95(16), 88(77), 69(54), 57(95), 55(74), 43(68).

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