Australian Journal of Basic and Applied Sciences, 5(8): 606-614, 2011
ISSN 1991-8178

Estradiol, Esteriol, Estrone and Novel Flavonoids from Date Palm Pollen

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Abstract: Column chromatography of the Egyptian date palm pollen, resulted in the isolation of estradiol, estrone among ten compounds from the hexane fraction. Also, besides rutin, additional four flavonoids from the ethyl acetate extract were isolated. HPLC analysis of the hexane fraction (using RF- BDS C_{18} – isocratic acetonitrile /water 58:42) revealed the presence of estrone, estradiol and estriol compared with R_t of reference standards. The Antioxidant activity by the DPPH method indicated that the ethyl acetate fraction possess a relatively strong antioxidant against DPPH radicals with value ($SC_{50} \geq 16.51$ µg / ml).

Key words: Date palm pollen (*Phoenix dactylifera* L.), palmae, estradiol, esteriol, estrone, HPLC, cholesterol, flavonoids, antioxidant activity.

INTRODUCTION

Date palm (*Phoenix dactylifera* L., Palmae) is native to the Middle East region over centuries ago (Copley *et al*., 2001). In Folkloric practice, date represents an essential meal in some Arab area (Miller *et al*. 2003) and (Al-Qarawi *et al*. 2003). Extracts of fruits, pits and edible kernels showed improvement of vital activities and increased the hormonal concentration in rat and the pollen has been used by Egyptians to improve fertility in women (Vayalil, 2002); (Bauza, 2002); (Schliemann *et al*., 2002); (El-Mougy *et al*., 1991); (Amin *et al*., 1969); (Elgasim *et al*.,1995); (Ali *et al*., 1999). Some reports on the previous phytochemical studies on the Egyptian palm pollen indicated the presence of cholesterol, estrone, diosgenin, β- amrin, β- sitosterol, rutin, quercetin (identified by mp,mmp, co tlc and ir) (Mahran *et al*., 1985). This work represents the first isolation of estradiol, hplc detection of esteriol and first spectral data for several compounds from date palm pollen.

MATERIAL AND METHODS

Plant material:

Pollen grains of date palm (*Phoenix dactylifera* L.) was collected from El- Kassaseen region (30 Km western Ismailia, Egypt) in March 2008 and kept in a refrigerator at 4^0C.

General:

Melting points were determined on SMP3 (Stuart Scientific, U.K.) and are uncorrected; IR spectra were recorded on a FT/R-6100 type A; NMR spectra were recorded on a Bruker, Avance 11 (600 MHz for ^{1}H- and 150 MHz for ^{13} C-NMR); Chemical shifts are given on a δ (ppm) scale with TMS as internal standard. Mass spectra with high resolution double focusing magnetic sector (GC MS DFS)-THERMO. HPLC analysis: The analyses were performed on Agilent-1100 model (USA), equipped with UV standard cell 61314-60086 with pressure limit 4Mpa at wave length: 205 nm; under the following conditions: column: thermo-BDS,C-18, 5 µ (250 X 4.6 mm), mobile phase: water-acetonitril (58:42), flow rate: 1.5 ml / min, temp 40 ^{0}C; TLC was performed on precoated kieselgel 60 F_{254} plates (Merck) using the following solvents: solvent 1 (for the identification of sterols); $CHCl_3$-MeOH (9:1,v/v), solvent 2 (for the identification of flavonoids); $CHCl_3$-MeOH-H_2O (13:7:1, v/v). Detection of steroidal compounds was made by spraying *p* anisaldehyde/ sulphuric acid followed by heating for 5' at 105 ^{0}C; while detection of flavonoids was made by UV lamp, ammonia vapor and 50 % sulphuric acid as visualizing agents.

Extraction and Isolation:

Pollen grains of date palm (*Phoenix dactylifera* L., 0.5 Kg) were extracted 3 ×2 L Me OH (each for 2 days) at room temperature. The combined extracts were evaporated to dryness in *vacuum* to afford 50 g residue. This residue was suspended in 300 ml H_2O and then extracted successively with n- hexane, pet ether, $CHCl_3$, ether, and EtOAc to yield 5 corresponding fractions (33.7, 0.9, 0.7,, 0.4 and 3 g, respectively).

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Investigation of N-hexane Fraction:

About 10 grams of n- hexane fraction were subjected to column chromatography on silica gel (300 g, 200-300 mesh, 100 x 3.5 cm), eluted with petroleum ether, chloroform and finally with methanol, fractions(250 ml each) were collected and examined (tlc, solvent 1). Similar fractions were combined, crystallized to yield compounds 1-10.

Compound 1:

(clionasterol acetate, 200 mg), colorless crystals, mp: 143-144 ^{0}C,R_f=0.88 (system1); ms: m/z 396 (100%, M^+ - OAc), m/z 382, 286, 273, 255 and 213. Selected ^{1}H-NMR: (600 MHz,$CDCl_3$) δ 2.1 (3H,s, CH_3C=O), 6 methyls at δ 0.69, 0.85, 0.88, 0.81, 0.93, 0.97, one olefinic at δ 5.38; ^{13}C-NMR: (150 MHz, $CDCl_3$) 29 carbons plus δ 21 and 173 for CH_3C=O, 140 (C-5), 122 (C-6).

Compound 2:

(β- sitosterol acetate, 135), colorless crystals, mp: 122 -124 ^{0}C,R_f = 0.84 (system 1); ms: m/z 396 (M^+ - OAc), 381 (9), 288 (5), 273 (4), 255 (8) and 213 (6). Selected ^{1}H-NMR: (600 MHz,$CDCl_3$): 6 methyls at δ 0.69—1.03, δ 2.1 (3H, s, CH_3 C=O), 4.62 (1H, m, H-3), 5.38 (1H, d, *J* = 3.5 Hz, H-6). Selected ^{13}C-NMR (150 MHz, CD Cl_3): δ 76.6 (C-3), 140 (C-5), 122 (C-6), 21.0 (CH_3 C=O) and 171 (CH_3 C=O).

Compound 3:

(β-sitosterol caproate, 150 mg), needle crystals, mp: 90-92^{0}C, R_f =0.80 (system 1); ms: m/z 568 (M^+,2), 553 (M^+-15,4), 525 (M^+-43,1), 511(2), 426 (4), 412 (17), 396 (M^+- caproate), 382 (35), 367 (8),, 255(20), 213 (20). Selected ^{1}H-NMR(600 MHz,$CDCl_3$): δ 2.2 (2H, d, *J* = 7.6 Hz, CH_2-2'), 4.6 (1H, m, H-3), 5.3 (1H, d, *J* =3 Hz, H-6). Selected ^{13}C –NMR (150 MHz, $CDCl_3$): δ 76.7 (C-3), 144 (C-5), 122.5 (C-6), 173 (C-1'), 34.7 (C-2'), 29.3 – 29.69 (C-3' – C-7'), 30.9 (C-8'), 22.6 (C-9'), 14.1 (CH_3-).

Compound 4:

(cerotic acid, 300 mg), amorphous powder, mp: 89^{0}C, R_f =0.77 (system 1); ms: 396 (M^+)

Compound 5:

(lignoceric acid,350 mg), amorphous powder, mp: 83^{0}C,R_f = 0.75 (system1); ms: 368 (M^+)

Compound 6:

(behenic acid, 200 mg): amorphous powder mp : 79^{0}C, R_f =0.71 (system1); ms : 340 (M^+)

Compound 7:

(β- sitosterol ,100mg) : colorless needles, mp: 140 ^{0}C, R_f = 0.66 (system 1); IR (KBr): 3424, 2938, 2867, 1644, 1462, 1376, 1057. ms : m/z 414 (M^+, 100), 399 (45), 396 (45), 381 (38), 329 (20), 275 (10), 273 (49), 255 (58), 246 (10), 231 (38), 229 (10) and 213 (78). Selected ^{1}H-NMR (600 MHz, $CDCl_3$) : δ 3.5 (1 H , br, s, H-3) and 5.3 (1H, br, s, H-6). Selected ^{13}C-NMR (150 MHz, $CDCl_3$): δ 71.7 (C-3), 140.7 (C-5), 121.6 (C-6).

Compound 8:

(cholesterol, 150 mg), fine colorless needles, mp: 149 ^{0}C, R_f =0.60 (system1); ms: m/z 386; (M^+, 100), 368 (60), 353 (45), 301 (47), 275 (75), 273 (12), 260 (4), 255 (30), 246 (20), 231 (14), 229 (6) 213 (25), 173 (21), 161 (4), 145 (30), 107 (28) and 81 (22), Selected ^{1}H-NMR (600 MHz,$CDCl_3$): five methyl signals (δ 0.7 – 1.0), 3.5 (1H, br, s, H-3), 5.3 (1H, br, s, H-6). Selected ^{13}C-NMR (150 MHz, $CDCl_3$): δ 71.8 (C-3), 140.8 (C-5), 121.6 (C-6)

Compound 9:

(estrone ,70 mg), fine colorless needles, mp: 254^{0}C, R_f =0.56 (system 1); IR : 3343 (O-H), 3066 (C-H aromatic stretching), 2943 - 2865 (C-H), 1718 (C=O), 1619-1582, 1462, 1356, 1287 -1248 ; ms: m/z 270 (M^+), ions at 242 (M^+ - C_2H_4), 237 (M^+ -CH_3 +H_2O), 213 (M^+ - C_3H_5 O), 185 (M^+ - C_5H_9 O), 172 (M^+ - C_6H_{10}O), 159 (M^+ - C_7H_{11}O), 146 (M^+ - C_8H_{12}O), 133 (M^+ - C_9H_{13}O), 107 (M^+ - $C_{11}H_{15}$O). Selected ^{1}H-NMR: (600 MHz,$CDCl_3$):δ 0.92 (3H, s, CH_3 -18), 1.46 (1H, m, H-12 α), 1.67 (1H, m, H -15 β), 1.9 (1H, m, H 15-α) 2.0 (1H, m, H-16 β), 2.2 (1H, dt, *J* =10 Hz, H -12 β) 2.41 (1 H, m, H -14) 2.15 (1H, m, H-8), 2.9 (1H, m, H -16 α), 4.9 (1H, s, OH), 6.6 (1H, d, *J* =2 Hz, H-4), 4.66 (1H, dd, *J* = 8 & 2 Hz, H-2) and 7.1 (1H, d , *J* =8

Hz, H-1), ^{13}C-NMR : (150 MHz, $CDCl_3$): δ 126.5 (C-1), 112.8 (C-2), 153.5 (C-3), 115.2 (C-4), 138 (C-5), 29.5 (C-6), 26.4 (C-7), 36.5 (C-8), 43.9 (C-9), 132 (C-10), 25.9 (C-11), 38.3 (C-12), 48 (C-13), 50.4 (C-14), 21.5 (C-15), 31.5 (C-16), 207.1 (C-17), 13.8 (C-18)

Compound 10:

(estradiol, 100 mg), fine colorless needles, mp: 174- 176 ^{0}C, R_f =0.51 (system 1); IR (KBr): 3755, 3417, 3019, 2934, 2868, 2861, 1867, 1697, 1618, 1581, 1497, 1457, 1383, 1346, 1317, 1270, 1231, 1183, 1150, 1096, 1048, 1005, 963, 922, 871, 818, 783, 729, 608, 504 and 443; ms : m/z 272 (3), 271 (8), 254 (25), 213 (25), 186 (30), 172 (45), 160 (35), 159 (63), 158 (35), 146 (50), 133 (45), 107 (15), 85 (55), 81 (3), 57 (47). Selected ^{1}H-NMR: (600 MHz,$CDCl_3$) : δ 0.67 ((3H, s, CH_3-18), 3.75 (1H, br. s, H-17 α), 4.5 (1H, s, OH), 6.58 (1H, d, *J* = 2 Hz, H-4), 6.65 (1H, dd, *J* = 8 & 2 Hz, H-2), 7.17 (1H, d, *J* = 8 Hz, H-1), ^{13}C-NMR (150 MHz, $CDCl_3$): δ 126.4 (C-1), 112.6 (C-2), 155.8 (C-3), 115.2 (C-4), 138 (C-5), 29.5 (C-6), 27.5 (C-7), 38.8 (C-8), 43.9 (C-9), 132 (C-10), 27.1 (C-11), 36.7 (C-12), 43.9 (C-13), 50.9 (C-14), 23.1 (C-15) , 30.6 (C-16), 81.9 (C-17), 11 (C-18)

Chromatography of the Ethyl Acetate Fraction:

Two grams of the ethyl acetate fraction were chromatographed on silica gel column (100 g, 200-300 mesh, 70 x2.5 cm). Eluted with chloroform and methanol; fractions 100 ml each were collected and examined (TLC, system 2) and UV, ammonia vapors and 50 % sulphuric acid as visualizing agents to afford compounds 11-15.

Compound 11:

(isorhamnetin – 3 –O- glucoside, 30 mg): fine yellow needles, mp 195^{0}C, R_f =0.7 (system 2); yellow color with NH_4OH and positive Molisch's test ; ms: m/z (% rel. int. %) 316 (M^+ - sugar,100), 301 (5), 287 (8), 273 (4), 151 (4), 149 (1), 108 (3) and 69 (3). ^{1}H-NMR (600 MHz, CD_3OD) : δ 7.9 (1H, s, H -2'), 7.6 (1H, d, *J*=8 Hz, H 6'), 6.9 (1H, d, *J* = 8 Hz, H- 5'), 6.4 (1H, s, H-8), 6.2 (1H, s, H -6), 5.4 (1H, d, *J* = 6.6 Hz, H -1"), 3.9 (3H, s, OCH_3), 3.7 -3.2 (H-2 "-H- 6 "). ^{13}C-NMR (150 MHz, CD_3OD): δ 158.7 (C-2), 135.4 (3), 179.5 (C-4), 163. 2 (C-5), 100 (C-6), 166.5 (C-7), 94.6 (C-8), 158.6 (C-9), 105.9 (C-10), 123.2 (C-1'), 116.1 (C-2'), 150.9 (C-3'), 148.5 (C-4'), 114.4 (C-5'), 123.9 (C-6'), 103.7 (C-1"), 76 (C-2"), 78.2 (C-3"), 71 (C-4"), 78.7 (C-5"), 62.6 (C-6"), 56.9 (O-CH_3).

Compound 12:

(apigenin, 30 mg), mp 349 -351 ^{0}C, R_f =0.63 (system 2); ms : m/z 270 (M^+). ^{1}H-NMR : (600 MHz, CD_3OD) : δ 7.8 (2H, d, *J* =8.4 Hz, H -2' , H 6'), 6.9 (2H, d, *J* =8.4 Hz, H-3', H-5'), 6.6(1H, s, H-3), 6.4 (1H, s, H-8), 6.2 (1H, s, H-6) . ^{13}C –NMR (150 MHz, CD_3OD): δ 164.1 (C-2), 102.8 (C-3), 181.8 (C-4), 161.5 (C-5), 98.8 (C-6), 163.7 (C-7), 93.9 (C-8), 157.3 (C-9), 103.7 (C-10), 121.2 (C-1'), 128.4 (C-2'), 115.9 (C-3'), 161.1 (C-4'), 115.9 (C-5'), 128.5 (C-6').

Compound 13:

(luteolin- 7- O- glucoside, 20 mg), mp 240 -242 ^{0}C, $R_{f=}$ 0.55 (system 2); ms: m/z 286 (M^+ for aglycone). ^{1}H-NMR :. (600 MHz, CD_3OD): δ 7.43 (1H, d, *J* =2.2 Hz, H- 2'), 7.41 (1H, dd, *J* =2.5 &8.5 Hz, H- 6'), 6.9 (1H, d, *J*=8.5 Hz, H -5'), 6.8 (1H, d, *J* = 2.5 Hz, H-8), 6.6 (1H, s, H -3), 6.5 (1H, d, *J* =2.5 Hz, H -6), 5.0 (1H, d , *J* =8.2 Hz, H -1") and 3.9 -3.4 (H -2" - H- 6"). ^{13}C-NMR (150 MHz, CD_3 OD): δ 164.5 (C-2), 103.3 (C-3), 182 (C-4), 161 (C-5), 99.6 (C-6), 163 (C-7), 95 (C-8), 157.1 (C-9), 105.4 (C-10), 121.6 (C-1'), 113.7 (C-2'), 145.7 (C-3'), 149.8 (C-4'), 116 (C-5'), 119.3 (C-6'), 100 (C-1"), 73.1 (C-2"), 76.6 (C-3"), 69.6 (C-4"), 77.2 (C-5"), 60.6 (C-6").

Compound 14:

(Naringin,45 mg): yellow granules, mp171 ^{0}C, R_f =0.50 (system 2); yellow color with NH_4OH and a positive Molisch's test; ms :m/z 272 (M^+ - Rha. +Glu., 25), 257 (10), 205 (5), 192 (1), 181 (25), 154 (95), 153 (80), 121 (63), 97 (25), 85 (83) and 71 (50). ^{1}H-NMR (600 MHz, CD_3OD) : δ 7.33 (2H, d, *J* =7.8 Hz, H-2', H-6'), 6.8 (2H, d, *J* = 7.8 Hz, H -3', H-5'), 6.19 (1H, s, H-6), 6.17 (1H, s, H-8), 5.39 (1H, d, *J* =7.6 Hz, H-1"), 5.25 (1H, d, *J* =1.2 Hz, H-1'"), 5.1 (1H, t, *J* =13 Hz, H-2), 3.9 -3.3 (9H, H -2" - 6", H -2'"-5'"), 3.18 (1 H, dd, *J* = 17 &13 Hz, H-3), 2.77 (1H, d, *J* = 13 Hz, H-3) and 1.29 (3H, d, *J* =6 Hz, Rha - CH_3).^{13}C-NMR (150 MHz, CD_3OD) : δ 78.2 (C-2), 44.1 (C-3), 198.6 (C-4), 164.7 (C-5), 96.8 (C-6), 166.7 (C-7), 96.8 (C-8), 129.3. (C-1'), 130.9 (C-2'), 116..4 (C-3'), 159.2 (C-4'), 116.4 (C-5'), 130. 8 (C-6'), 102.7 (C-1"), 71.3 (C-2"),

79 (C-3"), 62.3 (C-4"), 79.1 (C-5"), 62.3 (C-6"), 105 (C-1 "'), 70.1 (C-2"'), 72.2 (C-3"'), 78.2 (C-4"'), 70.1 (C-5"'), 18.3 (C-6"').

Compound 15:

(Rutin, 60 mg) as yellow granules, mp183^{0}C, R_f =0.45 (system 2); ms : m/z 302 (M^+ - Rha. + Glu. 100), 301 (25), 287 (5), 273 (10), 258 (4), 242 (8), 229 (8), 200 (5), 173 (5), 153 (12), 137 (17), 128 (15), 85 (9), 69 (14) and 63 (7). ^{1}H-NMR (600 MHz, CD_3OD) : δ 7.67 (1H, d, *J* =1.8 Hz, H -2'), 7.63 (1H, dd, *J* = 2.4, 8.4 Hz, H -6'), 6.88 (1H, d, *J* = 8.4 Hz), 6.41 (1H, d, *J* = 1.8 Hz, H -8), 6.2 (1H, d, *J* =1.8 Hz, H- 6), 5.1 (1H, *J* = 7.2 Hz, H -1"), 4.5 (1H, s, H-1"'), 3.8-3.2 (9H, H2"-6", H 2"'-5"') and 1.13 (1H, d , *J* = 6.6 Hz, Rha –CH_3).^{13}C-NMR (150 MHz, CD_3OD): δ 156.4 (C-2), 135.7 (C-3), 179.5 (C-4), 163.1 (C-5), 100 (C-6), 166.2 (C-7), 95 (C-8), 158.6 (C-9), 105.7 (C-10), 123.2 (C-1'), 116.1 (C-2'), 145.9 (C-3'), 149.9 (C-4'), 117.8 (C-5'), 123.6 (C-6'), 104.8 (C-1"), 75.8 (C-2"), 77.3 (C-3"), 71.5 (C-4"), 78.3 (C-5"), 68.5 (C-6"), 102.5 (C-1"'), 72.2 (C-2"'), 72.5 (C-3"'), 74.0 (C-4"'), 69.8 (C-5"'), 18.0 (C-6"').

HPLC analysis:

The analyses were performed on Agilent-1100 model (USA), equipped with UV standard cell 61314-60086 with pressure limit 4Mpa at wave length: 205 nm; under the following conditions : column : thermo-BDS,C-18, 5 µ (250 X 4.6 mm), mobile phase : water-acetonitril (58:42), flow rate : 1.5 ml / min, temp 40 ^{0}C. The samples were dissolved in methanol and sonicated then injected; and the standards were dissolved in methanol at the required concentrations .

Antioxidant activity:

The antioxidant activity was determined using the DPPH method (Ratty *et al*.,1988). Serial dilutions of the ethyl acetate fraction. An aliquot of 20 µl of diluted sample was added to 180 µl DPPH solution in methanol and vortexed. After one hour the decrease in the absorbance was determined at 515 nm by microplate ELISA reader. The reduction of the purple color reflects the extent of radical scavenging according to the equation:

$$DPPH\ inhibition\ (\%) = DPPH_{bl} - DPPH_{test} / DPPH_{bl} \times 100$$

Where $DPPH_{bl}$ is the peak area of solution corresponding to blank DPPH and $DPPH_{test}$ is the peak area of the solution corresponding to DPPH + sample. A curve for sample concentration versus DPPH % inhibition was plotted. The half maximal scavenging capacity (SC_{50}) values was calculated

RESULTS AND DISCUSSION

The methanolic extract of date pollen was fractionated into hexane, ethyl acetate and water. The hexane fraction on column chromatography afforded ten compounds (1-10) from which estradiol, cholesterol and estrone were prominent; while hplc analysis using RP C-18 proved the presence of esteriol for the first time.

Compound 1:

as colorless crystals, mp143-144^{0}C, MS m/z 396 (M^+ - O Ac,100%) for C_{31} $H_{52}O_2$, and fragments at m/z 382, 286, 273, 255, 213 typical for steroids (Brooks, 1970). The ^{13}C - and ^{1}H-NMR showed 29 carbons and signals for acetyl group at δ 173, δ 21 (δ 2.1, 3H, s, CH_3C=O), one olefinic at δ 140 (C-5), 122 (C-6) (1H, δ 5.38) in addition to 6 methyl signals at δ 0.69 - 0.97. The chemical shift values of C-1 to C-29 were similar to that of clionasterol acetate (Brooks, 1970). This is the first report on isolation of this compound from the pollen.

Compound 2:

as colorless crystals, mp 122 -124 ^{0}C, MS m/z 396 (M^+ - OAc) for C_{31} H_{52} O_2, other fragments at m/z 381, 288, 273, 255, 213 indicating a steroidal compound (Brooks, 1970). ^{1}H-NMR showed a singlet for an acetyl proton at δ 2.1 (3H, s, CH_3 C=O), one olefinic proton at δ 5.38; with ^{13}C-NMR of 29 carbons for sterol beside two signals at δ 21 and 171 for acetyl group. The chemical shift values of C-1 to C-29 were similar to that of β-sitosterol acetate (Brooks, 1970).

Compound 3: as white needle crystals, mp 90-92 ^{0}C; M^+ at m/z 568 calculated for C_{39} H_{68} O_2, a prominent

peak at m/z 396 for (M^+- $C_{10}H_{20}O_2$), other fragments at m/z 382, 381, 288, 255, 213 for a steroidal compound (Brooks, 1970). ^{13}C –NMR showed 29 carbons and signals at δ_c at 76.7 (C-3), 144 (C-5), 122.5 (C-6) for sterol (Brooks, 1970) beside signals at δ_c, 173 (C-1'), 34.7 (C-2'), 29.3 – 29.69 (C-3' – C-7'), 30.9 (C-8'), 22.6 (C-9'), 14.1 (CH_3-10') for the presence of C-10 long chain ester, deducing the compound to be β-sitosterol caproate. This is the first report on isolation of this ester from the pollen.

Compound 4:

as amorphous powder, mp 89 ^{0}C ; IR 1704 (COOH) and long chain 2918, 2849, 1467, 1433, 724 cm^{-1}; M^+ ion m/z 396 for C_{26} H_{52} O_2 and fragments with 14 mass units differences. This compound 4 was identified as hexacosanoic acid (cerotic acid) (Leland *et al*., 2006).

Compound 5:

as amorphous powder, mp 83^{0}C, IR similar to compound 4. M^+ ion at m/z 368 corresponding to C_{24} H_{48} O_2. This compound 5 was identified as tetracosanoic acid (lignoceric acid) (Leland *et al*., 2006).

Compound 6:

as amorphous powder, mp 79^{0}C, IR spectrum similar to compounds 4 and 5. M^+ ion at m/z 340 for C_{22} $H_{44}O_2$ confirmed that compound 6 is docosanoic acid (behenic acid) (Leland *et al*., 2006).

Compound 7:

as fine needles was identified as β-sitosterol by comparison of its spectral data with authentic sample (mp, co-tlc) and literature (Brooks., 1970).

Compound 8:

as fine needles was identified as cholesterol by comparing the spectral data with those reported for cholesterol (Brooks., 1970).

Compound 9:

as fine needles, mp 254 ^{0}C, IR spectrum at 3343 (O-H), 3066 (Ar-H), 2943 - 2865 (C-H), 1718 (C=O), 1619-1582 (C=C), 1462 - 1356 (CH_3 –C), 1287 – 1157. MS showed M^+ ion at m/z 270 calculated for C_{18} $H_{22}O_2$ and other fragments at 242 (M^+ - C_2H_4), 237 (M^+ -CH_3 +H_2O), 213(M^+ - C_3H_5 O),185 (M^+ - C_5H_9 O), 172 (M^+ - $C_6H_{10}O$), 159 (M^+ - $C_7H_{11}O$), 146 (M^+ - $C_8H_{12}O$), 133 (M^+ - $C_9H_{13}O$), 107 (M^+ - $C_{11}H_{15}O$). ^{1}H-NMR revealed the presence of one tertiary methyl group at δ_H 0.92 (3H, s, CH_3 -18), three aromatic protons at δ_H 7.1 (1H, d, J =8 Hz, H-1), 6.66 (1H, dd, J = 8 & 2 Hz, H-2), 6.6 (1H, d, J =2 Hz, H-4) and one singlet at δ_H 4.9 (1H, s, OH). ^{13} C-NMR exhibited 18 carbons including aromatic signals at δ_C 126.5, 112.8, 153.5, 115.2, 138, 132 and signals at δ_C 29.5, 26.4, 36.5, 43.9, 25.9, 38.3, 48, 50.4, 21.5 and 31.5 for aliphatics, while signal at 13.8 for C-18 CH_3 and δ_C 207.1 for C-17 C=O. Compound 9 was identified as estrone by comparing the above data with literature data (Klaus, 1983). To our knowledge, this is the first spectroscopic data of estrone isolated from the date pollen.

Compound 10:

as fine needles, mp 174 - 176 ^{0}C, IR 3755 - 3417 (O-H), 3019 (Ar-H), 2943 - 2868 (C-H), 1618-1581 (C=C), 1497 - 1346 (CH_3 –C), 1270 -1183. MS showed M^+ ion at m/z 272 for C_{18} $H_{24}O_2$ and, fragment at m/z 254 (M^+ - H_2 O), 213 (M^+ - C_3H_7 O), 186 (M^+ - $C_5H_{10}O$), 172 (M^+ - $C_6H_{12}O$), 159 (M^+ - $C_7H_{13}O$), 146 (M^+ - $C_8H_{14}O$), 133 (M^+ - $C_9H_{15}O$). Selected ^{1}H-NMR revealed the presence of one tertiary methyl group at δ_H 0.89 (3H, s, CH_3 -18), three aromatic functions at δ_H 7.1 (1H, d, J =8 Hz, H-1), 6.66 (1H, dd, J = 8 & 2 Hz, H-2), 6.58(1H, d, J =2 Hz, H-4), one singlet at δ_H 4.5 (1H, s, OH). and one singlet at δ_H 3.75 (H-17α). ^{13}C-NMR exhibited 18 carbons, signals at δ_C 126.48, 112.8, 155, 115.2, 138 and 132 for aromatics, signals at δ_C 29.5, 27.1, 38.8, 43.9, 27.1, 36.7, 43.9, 50.9, 23.1 and 30.6 for aliphatics and δ_C 81.9 and 11 for C-17 OH and C-18 CH_3, respectively. Based on the above data and authentic sample as well as literature (Klaus, 1983) compound 10 was identified as estradiol. Hplc analysis of the hexane extract supported this finding as the first report on the occurrence and isolation of estradiol from the pollen .

Continuing elution of the ethyl acetate column, in addition to rutin , four flavonoids)11-14 (luteolin -7-O- β -D – glucoside, apigenin, isorhamnetin-3-O- glucoside and naringin were isolated for the first time from the pollen .

Compound 11:

as fine yellow needles, mp 195 ^{0}C, R_f =0.7 (system 2), yellow color with Na OH for flavonoids. MS showed M^+ ion at m/z 316]M $^+$ - sugar [for $C_{16}H_{12}O_7$. ^{13}C-NMR spectrum revealed the presence of 22 carbons ;^{1}H- and ^{13}C- NMR spectra showed four singlets at δ_H 6.2, 6.4, 7.9 and 3.9 assigned for H – 6, H -8, H -2' and OCH_3 (δ_c 100, 94.6, 116.1 & 56.9), respectively; signals at δ_H 6.9 (1H, d, *J*= 8 Hz, H- 5') for ortho coupling with the doublet at δ_H 7.6 (1H, d, *J*=8 Hz, H 6') which was supported by signals at δ_c 114.4 and 123.9, respectively. Acid hydrolysis gave β -D- glucose (tlc, anomeric proton at δ_H 5.4 and δ_C 103.7). Comparing these data with reference sample as well as literature data (Agrawal, 1989) confirmed the structure of compound 11 as isorhamnetin – 3 –O- glucoside .

Compound 12:

as yellow crystals, mp 350 -352 ^{0}C, R_f =0.63 (system 2), bright yellow color with Na OH for flavonoid .^{1}H-NMR exhibited typical spectrum of para substituted aromatic system (Silverstein *et al* .,1974) also three singlets for H-3, H-6, H-8 . ^{13}C –NMR spectrum showed 15 signals suggesting the presence of at least 15 carbon atoms. EIMS showed M^+ ion at m/z 270, a peak at m/z 153 confirmed the hydroxyls at C-5, C-7 (Harborne *et al*., 1975). Compound 12 was identified as 4',5,7- trihydroxy flavone (apigenin). This is the first report on occurrence and isolation from the pollen.

Compound 13:

as yellow sandy crystals, mp 240 -242 ^{0}C; R_f =0.55 (system 2). MS showed a M^+ at m/z 286 (for aglycone $C_{15}H_9O_6$) and the presence of two hydroxyl groups in ring B (peak at m/z 134) in addition to two hydroxyl groups in ring A (peak at m/z 153) (Harborne *et al*., 1995). ^{13}C-NMR data revealed 21 carbons of which six are attributed to β –glucose (Breitmaier and Voelter, 1987) and the other fifteen signals are assigned to the aglycone. ^{1}H-NMR data showed signal of the anomeric proton at δ_H 5 (d, *J* =8.2 Hz). Acid hydrolysis revealed the presence of β - D- glucose (tlc, δ_c 100, δ_H 5.0)d, *J* =8.2 Hz (. Compound 13 was identified as luteolin -7- O- β -D - glucoside by comparison with previously published data (Huang *et al*., 2003); (Yi *et al*., 2009). This is the first report on occurrence and isolation from the pollen and the second report from the genus (Yi *et al*., 2009).

Compound 14:

as yellow granules, mp 171 ^{0}C; R_f =0.50 (system 2). MS showed M^+ peak at m/z 272 (for aglycone, $C_{15}H_{12}O_5$), m/z 121 for one OH in ring B, m/z 153 for two hydroxyl groups in ring A, indicating a flavanone aglycone with three hydroxyl groups (Harborne *et al*, 1995). ^{13}C-NMR revealed 27 carbons, twelve of them for β –glucose and α –L- rhamnose (Breitmaier and Voelter, 1987) and the other 15 signals were assigned to the aglycone. ^{1}H- and ^{13}C –NMR data were comparable with naringenin (Harborne *et al*., 1982; Fatope *et al*., 2003). Acid hydrolysis revealed the presence of β -D- glucose and α –L -rahmnose (two anomeric signals at δ_H 5.39, 5.25 and δ_C 102.7, 105). Thus, compound 14 was unambiguously identified as naringen by comparison with previously published data (Breitmaier and Voelter, 1990). This is the first report on the occurrence and isolation of naringen from the pollen.

Compound 15:

as yellow amorphous powder, mp 183 ^{0}C, R_f =0.45 (system 2), yellow color with NaOH. MS with M^+ at m/z 302 (for aglycone, C_{15} H_{14} O_7). ^{13}C and ^{1}H-NMR showed identity with those reported for 5,7, 3', 4-'tetradyroxyflavonol glycoside with β- D – glucopyranoside and α -L –rahmnopyranoside moieties (Silverstein *et al*.,1974). The location of sugar moieties was deduced to be at C-3 position from the downfield shift of C-3 signal (δ_C 135.7) compared with that reported for the aglycone quercetin (Mabry *et al*., 1970). Acid hydrolysis revealed the presence of D- glucose and L-rahmnose (tlc, anomeric signals at δ_H 5.1, 4.5 and δ_C 104.8 & 102.5). Thus, compound 15 was identified as rutin against reference sample (tlc, mp) and published data (Mabry *et al*., 1970).

HPLC analysis:

The analysis was carried out on thermo-BDS,C18, proved the presence of estrone (R_t 2.092), estradiol (R_t 7.663) and estriol (R_t 1.442).

Antioxidant activity:

In the present study, the tested extract possessed a strong antioxidant scavenging affinity against DPPH radicals as concluded from its half maximal scavenging capacity value ($SC_{50} \geq$ 16.51 µg /ml).

Table 1: Results of antioxidant activity of the ethyl acetate fraction

Concentration	Absorbance	S.D.
25	55.02	9.54
12.5	97.72	8.28
6.25	114.80	7.38
3.125	137.90	9.10
1.56	145.20	10.35
0.8	151.80	11.88
0	152.10	16.76
SC50	16.51 µg/ml	

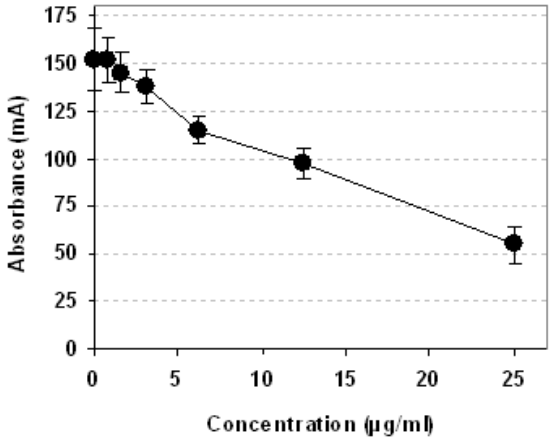

Fig. 1: Antioxidant activity of the ethyl acetate fraction

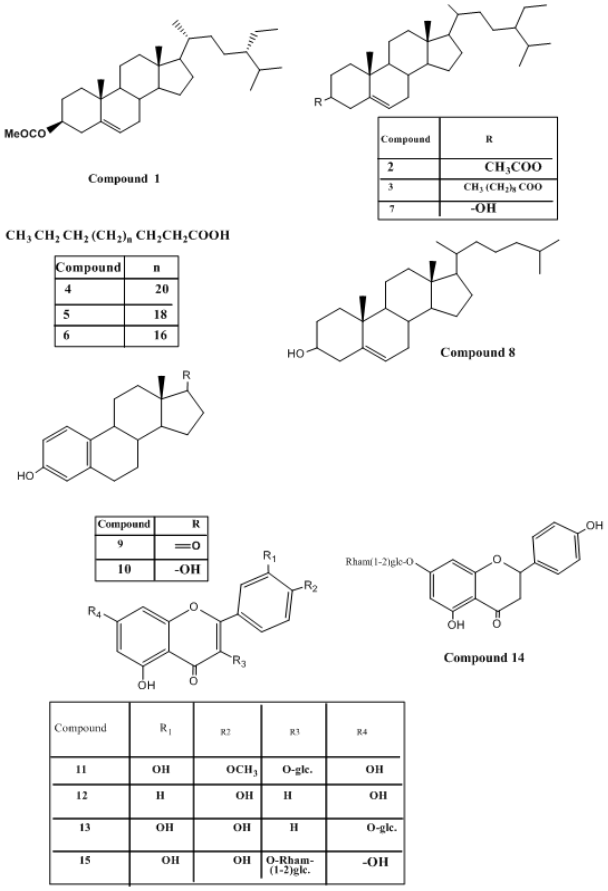

Fig. 2: Compounds isolated from date palm pollen

AKNOWLEDGEMENT

The authors are thankful for the support granted by the University of Zagazig project for scientific research fund stage-4 with the grant #3K-2 /M 4. Also, authors are greatly indebted to Prof Dr Khaled Y. Orabi, Faculty of Pharmacy, University of Kuwait for the NMR and Mass spectral facilities.

REFERENCES

Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids, Elsevier Science Publisher, Amsterdam.

Ali, B.H., A.K. Bashir and G. Al Hadrami, 1999. Reproductive hormonal status of rats treated with date pits. Food Chem., 66: 437-441.

Al-Qarawi, A.A., B.H. Ali, S. Mougy and H.M. Mossa, 2003. Gastrointestinal transit in mice treated with various extracts of date (*Phoenix dactylifera* L.). Food and Chemical Toxicology, 41: 37-39.

Amin, E.S., O. Awad, M. Abdel Samad and M.N. Iskander, 1969. Isolation of estrone from Moghat roots and from pollen grains of Egyptian date palm. Phytochemistry, 9: 295-297.

Bauza, E., 2002. Date palm kernel extract exhibits antiaging properties and significantly reduces skin wrinkles. International Journal of Tissue Reactions., 24: 131-136.

Breitmaier, E and W. Voelter, 1990. Carbon-13 NMR Spectroscopy: High Resolution Methods and Application in organic Chemistry and Biochemistry, 3rd. ed. VCH Publisher, New York.

Breitmaier, E and W. Voelter, 1987. Carbon-13 NMR Spectroscopy, 3rd. ed., VCH Verlagsgesellschaft, Weinheim, Germany, VCH Publishers, New York, USA.

Brooks, C.J.W., 1970. Rodd's Chemistry of Carbon Compounds. 2nd. ed. Vol. IID.P.I. Elsevier, Amesterdam.

Copley, M.S., P.J. Rose, A. Clampham, D.N. Edward, M.C. Horton and R.P. Evershed, 2001. Detection of palm fruit lipids in archaeological pottery from Qasr Ibrim, Egyptian Nubia. Proceedings of the Royal Society, London, 268: 593-597.

El-Mougy, S.A., S.A. Abdel–Aziz, M. Al –Shanawany and A. Omar, 1991. The gonadotropic activity of Palmae in mature male rats. Alexandria J. Pharmac. Sci., 5: 156-159.

Elgasim, E.A., Y.A. Alyoussif and A.M. Homeida, 1995. Possible hormonal activity of date pits and flesh fed to meat animals. Food Chem., 52: 149-150.

Fatope, M.O., S.K.S. AL- Burtomani, J.O. Ochei, A.O. Abdulnour, S.M.Z. Al-Kindy and Y. Takeda, 2003. Muscanone: a 3-*O*-(1′′, 8′′, 14′′- trimethylhexadecanyl naringenin from *Commiphora wightii,* Phytochemistry, 62: 1251.

Harborne, J.B., T.J. Mabry and H. Mabry, 1975. Flavonoids, Chapman and Hall, London.

Harborne, J.B., Mabry, T.J. and H. Mabry, 1995. The Flavonoids, Academic Press, New York, San Fancisco.

Harborne, J.B., T.J. Mabry, 1982. The flavonoids, Advances in Research, London, New York Chapman& Hall.

Huang, Y., Z.H. Ding and J.K. Liu, 2003. A newly highly oxygenated flavones from *Vernonia saligna*, Z. Naturforsch., 58: 347-350.

Klaus, Florey, 1983. Analytical Profiles of Drug Substances, 12 &15, New York, London.

Leland, J., Cseke, Ara Kiraqkosyan, B. Peter, Kaufman, L. Sara Warner, A. James Duke and Harry L Brielmann, 2006. Natural Products from Plants. 2 nd. ed. Taylor & Francis.

Mahran, G.H., S.M. Abdel Wahab, Selim and A.M. Ateya, 1985. Constituents of the Egyptian Date Palm Pollen: Saponin and Lipid Constituents of Pollen Grains First Int. Conf. vol. I, Zag. Univ., 30 March – 1 April.

Miller, C.J., E.V. Dunn, I.B. Hashim, 2003. The glycaemic index of dates and date / yoghurt mixed meals. Are dates " the candy that grows on trees"? European Journal of Clinical Nutrition, 57: 427-430.

Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The Systemic Identification of Flavonoids, Springer, Heidelberg.

Ratty, A.K., J. Sunamoto, N.P. Das, 1988. Interaction of flavonoids with 1,1- diphenyl – 2- picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1. Biochem Pharmacol., 37(6): 989-95 .

Schliemann –Willers, S., W. Wigger – Alberti, P. Kleesz, R. Grieshaber, P. Elsner, 2002. Natural vegetable fats in the prevention of irritant contact dermatitis. Contact Dermatitis, 4: 6-12.

Silverstein, R.N., G.C. Bassler and Mcrill, 1974. Spectrophotometric Identification of Organic Compounds, John Wily and Sons, Inc., N.Y.,L. Sydney, III ed.

Vayalil, P.K., 2002. Antioxidant and antimutagenic properties of aqueous extract of date fruit. Journal of Agricultural Food Chemistry, 50: 610-617.

Yi-Pei Lin, Tai-Yuan Chen, Hsiang-Wen Tseng, Mei-Hsien Lee and Shui-Tein Chen (2009). Neural cell protective compounds isolated from *Phooenix* hanceana var. formosana. Phytochemistry, 70: 1173-1181.