Journal of Applied Pharmaceutical Science Vol. 6 (12), pp. 103-109, December, 2016
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2016.601214
ISSN 2231-3354

Phytochemical investigation of *Phoenix canariensis* Hort. ex Chabaud leaves and pollen grains

Mohamed S. Hifnawy, Amr M. K. Mahrous, Rehab M. S. Ashour*

Pharmacognosy Department, Faculty of pharmacy, Cairo University, Kasr El-Aini 11562, Egypt.

ARTICLE INFO

Article history:
Received on: 08/09/2016
Revised on: 09/10/2016
Accepted on: 22/11/2016
Available online: 28/12/2016

Key words:
Phoenix canariensis, Lipids, Headspace volatiles, Flavonoids, Phenolics, Steroids.

ABSTRACT

Phoenix canariensis is a commonly grown, yet understudied, palm plant. The phytochemical screening of leaves and pollens revealed the presence of flavonoids, saponins, tannins, sterols and/or triterpenes. Quantitative estimation of constituents, revealed that the total polyphenolics were higher in the leaves (69.9) than in pollens (29.98) expressed in mg gallic acid equivalent/g d.wt, the total flavonoids calculated as rutin equivalent were (23.86 mg/g) in leaves and (17.20 mg/g) in pollens, the total tannins content were 55.18 and 3.31 mg tannic acid equivalent/g fresh wt, while the total steroids content were 2.6 and 12.4 mg *β*-sitosterol equivalent/g d.wt, in leaves and pollens, respectively. Eighteen phenolic compounds and ten flavonoids were identified by HPLC. GLC analysis of lipids, revealed the identification of phytosterols (4.93 and 28.90%), saturated (35.35 and 40.56%) and unsaturated (62.42 and 59.01%) fatty acids in leaves and pollens, respectively. Proximate analysis revealed a total moisture content of (6.4 and 7.7 %), crude fiber (32.22 and 39.50%), total ash (12.1 and 8.1%) and acid insoluble ash (4.7 and 7.6 %) for leaves and pollens, respectively. Moreover, spathe headspace volatile analysis combined with GC-MS revealed the presence of fifty-two compounds constituting 72.84% of the total oil composition where α- copaene predominates (18.72%).

INTRODUCTION

Palms are among the best known and extensively cultivated plant families and they are of great economic importance. They all belong to family Arecaceae (Johnson, 1998). Palms have been cultivated in the Middle East and North Africa for at least 5000 years (Zohary and Hopf, 2000). The genus *Phoenix* (Family Arecaceae) comprises about 19 species, distributed in tropical and subtropical areas southern Asia and Africa (Adawy *et al.*, 2002, Mohtasheem *et al.*, 2005) *Phoenix canariensis*, the Canary date palm, is native to the Canary Islands. It is widely planted as ornamental in the US and the Mediterranean area (Krueger, 2001). It can be identified by its single, upright, thick trunk topped with a crown of leaves in 2.5 to 4.5 meters long. The stalks of inconspicuous flowers are replaced with clusters of one inch diameter, orange yellow, date-like, ornamental fruits, which ripen in summer (Gilman and Watson, 2006). The inflorescence of most palm trees in its early stage of growth is enclosed in hard covering envelop known as spathe, which splits open as the flowers reach maturation (Zaid and Arias-Jimenez, 2002).

Earlier studies have reported the great medicinal use of different parts of genus *Phoenix*, as antioxidant (Kchaou *et al.*, 2013), anti-inflammatory, antitumor (Rahmani *et al.*, 2014), antibacterial (Al-Zoreky and Al-Taher, 2015), antifungal, neuroprotective (Pujari *et al.*, 2014) and hepatoprotective agents (Singab et al, 2015). The fruits of *Phoenix dactylifera* Linn. have been traditionally used to treat various disorders including diabetes and hypertension.

In Egypt, pollens were believed to enhance fertility and to be an aphrodisiac. Several constituents have been identified and isolated from the genus including lipids, phenolic acids, flavonoids, procyanidins, saponins, triterpenes, sterols and carbohydrates (Elgindi *et al.*, 2015).

* *Corresponding Author*
Rehab M.S. Ashour, Pharmacognosy Department, Faculty of pharmacy, Cairo University, Kasr El-Aini 11562, Egypt.
Email: rehab.ashour @ pharma.cu.edu.eg

To the best of our knowledge, no published data was found concerning the pollen grains and spathe of the plant while only few reports were found concerning the isolation of flavonoids, phenolic acid and steroidal saponin from the leaves (Garcia *et al.,* 1981; Asami *et al*., 1991) and isolation of an alkaloid from the seeds as well as seed oil chemical composition (Hammami *et al.,* 2010; Nehdi *et al.,* 2011). This study includes the determination of pharmacopoeial constants, phytochemical screening, spectroscopic analysis of different classes, HPLC analysis of polyphenolics and GLC analysis of lipids for leaves and pollen grains of *Phoenix canariensis* in addition to its spathe volatile analysis.

MATERIAL AND METHODS

Plant material

Phoenix canariensis hort. ex Chabaud leaves and pollens were collected over the years 2013-2015 from El-Orman Botanical Garden, Egypt. The plant was identified by Dr. Mohamed el Gebaly (Taxonomist) and Mrs. Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture. Voucher specimens numbered (5.10.2015), were deposited at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Spathe used for volatiles analysis was collected in summer.

Phytochemical screening

The powdered air dried leaves and pollen grains were tested for the presence of steam volatile substances (Wagner *et al.,* 1983), flavonoids (Geissman, 1962, Peach and Tracey, 1955), crystalline sublimate (Claus and Tyler, 1967), tannins (Evans, 2002), cardiac glycosides (Fieser and Fieser,1959), sterols and /or triterpenes, alkaloids and / or nitrogenous bases, anthraquinones, saponins, carbohydrates and/or glycosides (Tiwari *et al.,* 2011).

Proximate analysis

Moisture, crude fiber, total ash and acid insoluble ash were determined (AOAC, 2000). Each value was an average of 3 determinations.

Quantification of different chemical classes of plant constituents

Spectrophotometric determination of total polyphenolic content

The total polyphenolic content was performed using Folin-Ciocalteu reagent. A calibration curve (R^2=0.9987) was prepared using gallic acid standard (Sigma Co., St. Louis, MO, USA) at concentrations of 50 μg to 0.9 mg/ml. Total content of phenolic compounds was calculated as gallic acid equivalents (GAE) (Singleton et al.,1999). For each concentration, three replicates were carried out.

Spectrophotometric determination of flavonoid content

Spectrophotometric determination of total flavonoid content was based on measuring the intensity of the yellow colour developed when flavonoids were complexed with aluminum chloride reagent at 420 nm using UV spectrophotometer expressed as rutin equivalent. A standard calibration curve (R^2 = 0.9984) was prepared using different aliquots of standard rutin (Sigma Co., St. Louis, MO, USA) equivalent to 20-180 µg/ml (Geissman, 1962). Three replicates were carried out, for each concentration.

Spectrophotometric determination of total tannin content

Tannin content was estimated by Folin- Denis reagent using tannic acid as standard (Sigma Co., St. Louis, MO, USA) (Earp *et al.,* 1981). A standard calibration curve (R^2 = 0.9998) was prepared using tannic acid concentrations ranging from 0 - 100μg/ml. The values were expressed in micrograms (μg) of tannic acid equivalents (TAE) per gram fresh weight. Three replicates were carried out, for each concentration.

Spectrophotometric determination of total steroidal content

The determination of total steroidal content of leaves and pollen grains was based on measuring the intensity of the green colour developed when steroidal compound complexes with Libermann-Burchard's reagent using β-sitosterol as a standard. A standard calibration curve (R^2=0.9981) was prepared using different aliquots of β-sitosterol solution (E-Merk, Darmstadt, Germany) equivalent to 0.1 to 0.5 mg/ml. The concentration of the total steroids was calculated as β-sitosterol equivalent, Daksha *et al.,* 2010. The results were average of three determinations.

HPLC analysis of polyphenolics

Identification of individual polyphenolic compounds in 70% ethanolic extracts of leaves and pollen grains was performed using HPLC (Goupy *et al.,* 1999). Analysis was performed by reversed phase HPLC /diode array detection (DAD) (Hewlett Packard 1050) using a column Alltima C18, 5mm (150mm x 4.6mm id) with a guard column Alltima C18, 5mm (Alltech). Gradient elution of A (acetic acid 2.5%), B (acetic acid 8%) and C (acetonitrile) was used. Best separation was at 0min, 5% B; at 20min, 10% B; at 50min, 30% B; at 55min, 50% B; at 60min, 100% B; at 100min, 50% B and 50% C; at 110min, 100% C until 120min. Solvent flow rate was 1ml/min and separation was performed at 35 °C.

The volume injected was 10µl. Phenolic compounds were assayed by external standard calibration at 280nm and expressed in mg/g dry matter of equivalent (+)-catechin for flavan-3-ols, equivalent coumarin for apolar aromatic compounds and equivalent quercetin-3-rutinoside for flavonols.

Identification of the major phenolic components were performed by comparing their retention times to those obtained for the standards (prepared as 50-600 ug/ml solutions in methanol). The concentration of individual phenolic component was calculated on the basis of peak area measurements of both standards and samples by adopting the external standard method. Results were the average of triplicate experiments. All standards used were obtained from Sigma.

HPLC analysis of flavonoids

The 70% ethanolic extracts of leaves and pollen grains were subjected to HPLC, adopting the conditions described in (Mattila *et al.*, 2000)**.** Flavonoid analysis was done on Inertsil (GL Sciences, Inc., Japan) using ODS-3 (4.0×150 mm, 3 *u*m) column with a C-18 guard column. Temperature of the column oven was set at 35 °C. Gradient elution was employed for flavonoids with a mobile phase consisting of 50 mM H_3PO_4, pH 2.5 (solution A) and acetonitrile (solution B) as follows: Isocratic elution 95% A/5% B, 0-5 min; linear gradient from 95% A/5% B to 50% A/ 50% B, 5-55 min; isocratic elution 50% A/50% B, 55-65 min; linear gradient from 50% A/50% B to 95% A/5% B, 65-67 min; post-time 6 min before next injection. The flow rate of the mobile phase was 0.7 mL/min, and the injection volumes were 10 *u*L of each the standards and sample extracts. Identification of major flavonoids was done by comparing their retention times to those obtained for the standards. Peak areas were used for quantification of both standards and samples using the external standard method. Standard flavonoids calibration curves were made by diluting stock standards in methanol to yield 2-20 *u*g/mL. Results were the average of triplicate experiments. Standards of flavonoid aglycones were obtained from different manufacturers and must be in HPLC purity grade.

All standards were prepared as stock solutions at 5 mg/50 mL in MeOH, except for luteolin and apigenin (5 mg/50 mL in DMF/MeOH, 1:6, v/v), and isorhamnetin and rhamnetin (5 mg/50 mL in DMF/MeOH, 1:10, v/v).

Investigation of lipid content

100 g of each of the air-dried powdered leaves and pollen grains were, separately, extracted with petroleum ether till exhaustion. The extract was evaporated under vacuum to give 0.84g and 0.38g residue, respectively. The unsaponifiable and saponifiable fractions were obtained from the petroleum ether fraction and the liberated fatty acids were methylated according to Finar, 1973 and Vogel, 1967. GLC conditions for the analysis of unsaponifiable matter (USM) was carried out on a capillary column (30 m × 0.32 mm I.D. × 0.25 *u*m film) and packed with HP-5 (5% phenyl methyl siloxane), the injected volume was 2 *u*l. The analysis was carried out at a programmed temperature. Initial temperature was 80˚C for 5 min. then increased to 280˚C by the rate of 8˚C/min., injector and detector (FID) temperatures were 240˚C and 300˚C, respectively.

Flow rate of nitrogen was 20 ml/min. Fatty acid methyl ester was analysed on the same column used for USM with the same carrier gas but at flow rate of 30ml/min. The initial temperature was 120˚C increased to 240˚C by the rate of 4˚C/min. the injector and detector (FID) temperatures were 250 ˚C and 280 ˚C, respectively. Identification of compounds was carried out by comparing the retention times of their peaks with those of the available authentic compounds (Sigma Chemical Co. St. Louis, MO, USA) similarly analysed. Quantification was based on peak area measurement using a computing integrator.

Headspace GC/MS analysis of spathe volatiles

Headspace volatiles analysis using solid phase micro-extraction (SPME) was adopted from (Farag and Wessjohann, 2012) with slight modification. Frozen spathe sample (3 g) was cut into small pieces and transferred to SPME screw cap vials (20 ml). Vials were then capped and SPME fiber left to sample then the head space heated above the sample at 50 °C for 30 min, the fiber withdrawn into the needle and transferred to the injector port of the GC/MS instrument. A system blank containing no plant material was run as a control.

GC/MS parameters

SPME fibers were desorbed at 210˚ C for 1 min in the injection port of a shimadzu model GC-17A gas chromatograph interfaced with a shimadzu model QP-5050 mass spectrometer. Volatiles were analysed on a SLB-5 MS column (30 m, 0.25 mm I.D., 0.25µm film) (Supelco, Oakville, ON, Canada). Injections were made in the splitless mode for 30s. the gas chromatograph was operated under the following conditions: injector 220˚ C, column oven 38˚ C for 3 min, then programmed at a rate of 12˚ C/min to 180˚ C, kept at 180˚ C for 5 min and finally ramped at a rate of 40˚ C / min. the transfer line and ion source temperatures were adjusted at 230 and 180˚ C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at m/z 40-500. Volatile components were identified using the procedure described in (Farag and Wessjohann, 2012) and peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) relative to n- alkanes (C6-C20), mass spectrum matching to NIST, WILEY library database and with authentic standards when available.

RESULTS AND DISCUSSION

Phytochemical screening

In the present investigation, preliminary phytochemical screening of leaves and pollens of the plant revealed the presence of flavonoids, saponins, tannins, carbohydrates and/or glycosides, sterols and / or triterpenes.

Proximate analysis

Pharmacopoeial constants for both organs were determined viz. total moisture content (6.4% and 7.7 % in leaves and pollens, respectively), crude fiber (32.22% and 39.50%, respectively), total ash (12.1% and 8.1% in leaves and pollens, respectively) and acid insoluble ash (4.7 and 7.6% in leaves and pollens, respectively). These constants can serve as standards for the purity of leaves and pollens as well as for the differentiation from other *Phoenix* species.

Spectrophotometric determination of different classes of the plant

The total polyphenolic content were highest in the leaves (69.9 mg/g) than the pollens (29.98 mg/g) expressed in GAE per

gram dry weight; the concentration of total flavonoids calculated as rutin was higher in leaves (23.86 mg/g) than in pollens (17.20 mg/g). Also the total tannins content, were 55.18 mg and 3.31 mg of TAE per gram fresh weight in leaves and pollens, respectively. While the total steroids content, were 2.6 and 12.4 mg/g per gram dry weight expressed as *β*-sitosterol equivalents in leaves and pollens, respectively. The results showed that the leaves were rich in polyphenolics, flavonoids and tannins as compared to pollens while pollens were rich in steroids (Table 1). The total polyphenolic content in 21 Egyptian date fruit varieties of *Phoenix dactylifera* was previously recorded to vary from 2.33 to 18.97mg/g dry weight (Farag *et al.,* 2014). While the phenolic profile of the Algerian type date palm fruit range from 2.49-8.36 mg/100 g fresh weight GAE (Mansouri *et al.,* 2005). The total phenolic content of seeds of seven different varieties ranges between 1.98 to 4.65 mg/g GAE (Al-Juhaimi *et al.,* 2012)

Table 1: Quantification of different chemical classes of plant constituents.

Natural compounds	Leaves (in mg/g)	Pollen grains (in mg/g)
Polyphenolics	69.90	29.98
Flavonoids	23.86	17.20
Tannins	55.18	3.31
Steroids	2.60	12.40

GLC analysis of lipoidal content

The percentage of the USM represents 29.98% and 13.04% of the total lipids of leaves and pollens, respectively. The GLC analysis of the unsaponifiable fraction resulted in the identification of 83.47% and 84.68% of the USM of the leaves and pollens, respectively. The percentage of total hydrocarbons identified was 58.68% and 47.26% in the leaves and pollens, respectively. N-docosane (C22) was the major hydrocarbon (37.54%) of the leaves while n-tricosane (C23) was the major hydrocarbon in the pollens (13.43%). On contrary phytosterols percent was much higher in the pollens (28.90%) than in the leaves (4.93%). Concerning the leaves, *α*-amyrin was the major triterpene (19.61%), while β-sitosterol and campesterol were the main phytosterols (2.82% and 1.30%, respectively). In the pollens β-amyrin was the main triterpene (5.30%) while the main phytosterols were campesterol and β-sitosterol (10.45% and 9.96%, respectively) (Table 2). The percentage of fatty acids represents 70.02% and 86.96% of the total lipids of leaves and pollens, respectively.

Table 2: GLC analysis of the identified USM of the leaves and pollen grains.

Authentic	RRt*	Percent	
		Leaves	Pollen
n-Undecane C-11	0.254	0.79	3.74
n-Dodecane C-12	0.348	2.67	0.47
n-Tridecane C-13	0.437	0.77	_
n- Tetradecane C-14	0.493	0.90	0.38
n- Pentadecane C-15	0.592	1.23	0.82
n- Hexadecane C-16	0.639	1.42	0.73
n- Heptadecane C-17	0.711	0.23	0.96
n- Octadecane C-18	0.771	1.50	1.08
n- Nonadecane C-19	0.837	3.36	3.26
n- Eicosane C-20	0.890	2.47	1.83
n- Henicosane C-21	0.948	--	1.88
n- Docosane C-22	1	37.54	1.81
n- Tricosane C-23	1.059	--	13.43
n- Tetracosane C-24	1.151	2.67	6.08
n- Pentacosane C-25	1.192	1.11	1.63
n- Hexacosane C-26	1.237	--	2.31
n- Heptacosane C-27	1.329	0.51	2.30
n- Octacosane C-28	1.395	0.55	--
n- Triacontane C-30	1.412	0.96	4.55
Cholesterol	1.463	0.71	3.86
Campsterol	1.503	1.30	10.45
Stigmasterol	1.642	0.10	4.63
β-sitosterol	1.736	2.82	9.96
α-amyrin	1.845	19.61	3.22
β-amyrin	1.954	0.25	5.30
% Total identified compounds		83.47	84.68
Percentage of total hydrocarbons		58.68	47.26
Percentage of total phytosterols		4.93	28.90
Percentage of total triterpenes		19.86	8.52

* RRt: Relative retention time to n-Docosane C-22 with Rt= 20.662 min.
-- : not detected

The GLC analysis of fatty acids resulted in the identification of 17 and 19 compounds in leaves and pollens, respectively. Palmitic acid constituted the highest percentage of the saturated fatty acids in the pollens (27.71%) and in the leaves (25.47%). Concerning the unsaturated fatty acids the α-linolenic acid was the major constituent of the leaves (29.12%), while oleic acid was the major unsaturated fatty acid in the pollens (20.52%) (Table 3).

Table 3: GLC analysis of the identified FAME of the leaves and pollen grains.

Authentic	RRt*	Percent	
		Leaves	Pollen grains
Hexanoic acid C6 (0)	0.380	0.46	---
Caprylic acid C_8 (0)	0.459	---	0.30
Capric acid C_{10} (0)	0.501	0.48	---
Lauric acid C_{12} (0)	0.646	1.43	0.24
Myristic acid C_{14} (0)	0.813	0.58	0.48
Pentadecanoic acid C_{15} (0)	0.909	0.82	0.39
Palmitic acid C_{16} (0)	1	25.47	27.71
Palmitioleic acid C_{16} (1)	1.045	2.51	8.60
Heptadecanoic acid C_{17}(0)	1.111	1.82	0.57
Heptadecenoic acid C_{17}(1)	1.143	0.50	0.86
Heptadecenoic acid C_{17}(2)	1.158	1.09	---
Stearic acid C_{18} (0)	1.210	3.56	3.43
Oleic acid C_{18} (1)	1.241	7.84	20.52
Linoleic acid C_{18} (2)	1.308	19.52	18.05
α-Linolenic acid C_{18} (3)	1.391	29.12	6.36
γ-Linolenic acid C_{18} (3)	1.483	1.58	---
Arachidic acid C_{20}(0)	1.516	0.73	0.83
Arachidonic acid C_{20}(4)	1.576	0.26	3.71
Ecosapentaenoic acid C_{20}(5)	1.664	---	0.63
Behenic acid C_{22} (0)	1.753	---	4.52
Erucic acid C_{22}(1)	1.833	---	0.28
Tricosanoic acid C_{23}(0)	1.915	---	1.51
Lignoceric acid C_{24}(0)	2.091	---	0.58
Percentage of identified fatty acids		97.77	99.57
Percentage of unsaturated F.A.		62.42	59.01
Percentage of saturated F.A.		35.35	40.56

* RRt: Relative retention time to Palmitic acid C_{16}(0) with Rt= 15.177 min.
--- : not detected.

Several reports were found concerning the fatty acid profile of seeds and fruits genus *Phoenix* while little is known

about the leaves and pollens. Oleic acid was the major fatty acid in most reports of *Phoenix dactylifera* seeds and fruits (Al-Shahib and Marshall, 2003, Nehdi *et al.,* 2010, Ogungbenle 2011, Akbari *et al.,* 2012, Ben Salah *et al.,* 2012), while linoleic acid was the major in fruits grown in Tunisia (Saafi *et al.,* 2008). In *Phoenix theophrasti* fruits palmitic acid predominate (Liolios *et al.,* 2009) while in the leaves of *Phoenix dactylifera* hexadecanoic acid ethyl ester was reported the major (Azmat *et al.,* 2010).

The antioxidant and anti-inflammatory activities of the major identified compounds by GLC; *α*-amyrin, β-amyrin, β-sitosterol and polyunsaturated fatty acids; were previously reported by (Zhao *et al.,* 2005, Richard *et al.,* 2008, Melo *et al.,* 2011, Saeidnia *et al.,* 2014). This might be a guide while performing further biological studies.

HPLC analysis of polyphenolics

The HPLC analysis of the 70% ethanolic extracts of leaves and pollens separately, revealed quantitative variation in phenolic and flavonoids composition (Tables 4 and 5).

Table 4: HPLC analysis of phenolics in *Phoenix canariensis* hort ex. Chabaud, leaves and pollen grains.

No.	Retention time (min)	Phenolic Compounds	Test results of phenolic compounds (ppm)	
			Leaves	Pollen grains
1	7.060	Gallic acid	295.73	62.43
2	7.167	Pyrogallol	2537.37	1306.95
3	8.333	3-OH-Tyrosol	706.15	415.86
4	8.522	Protocatechuic acid	1474.01	36.40
5	9.249	Chlorogenic acid	1232.51	572.87
6	9.536	Catechol	1682.16	362.88
7	9.744	Catechin	1513.21	546.72
8	10.358	Caffeic acid	484.00	238.19
9	10.494	Vanillic acid	876.02	320.56
10	11.808	Para-coumaric acid	430.61	86.31
11	11.977	Ferulic acid	505.68	547.86
12	12.293	Iso-ferulic acid	159.12	31.29
13	12.846	Rosmarinic acid	2198.51	448.70
14	12.849	Reversetrol	564.14	131.48
15	13.067	Ellagic acid	220.44	266.82
16	13.146	Ethyl vanillic acid	35008.54	11624.22
17	13.553	Alpha-coumaric acid	5486.41	1815.54
18	14.456	Salycilic acid	3891.13	822.15

Table 5: HPLC analysis of flavonoids in *Phoenix canariensis* hort ex. Chabaud, leaves and pollen grains.

No.	Retention time (min.)	Flavonoids	Concentration (ppm)	
			Leaves	Pollen grains
1	12.233	Luteolin	622.27	190.94
2	12.287	Naringin	2376.32	556.56
3	12.441	Rutin	3800.79	292.48
4	12.571	Hesperidin	10295.90	1062.40
5	13.467	Quercetrin	939.21	577.10
6	13.905	Isorhamnetin	17785.44	4349.10
7	14.978	Quercetin	1050.32	140.14
8	15.631	Hesperetin	1213.06	138.92
9	16.257	Kampferol	442.84	167.01
10	16.551	Apigenin	157.26	790.55

Eighteen different phenolic compounds were identified, where ethyl vanillic acid was the major phenolic acid identified in both organs, its antioxidant (Tai *et al.,* 2011) and anti-inflammatory (Jung *et al.,* 2010) activities were previously reported. Concerning the flavonoids, ten flavonoids were identified in both organs, where isorhamnetin predominates at a concentration of 17785.44 ppm and 4349.10 ppm in leaves and pollens, respectively. Isorhamnetin was reported to exhibit anti-inflammatory (Seo *et al.,* 2014), anti-obesity (Lee *et al.,* 2009), anti-oxidant (Pengfei *et al.,* 2009), and antitumor (Teng *et al.,* 2006) activities. The metabolomics fingerprints of 21 Egyptian date fruit varieties of *Phoenix dactylifera* were previously studied by Farag *et al.,* 2014. Luteolin and apigenin glycosides predominates the flavones identified while quercetin conjugates were the principle flavonols. Caffeoyl shikimic acid was the main hydroxylcinnamic conjugate.

Headspace GC/MS analysis of spathe volatiles.

Headspace volatile analysis combined with GC/MS was utilized for detection of spathe volatiles (Table 6). The percentage of total hydrocarbons, monoterpenes and sesquiterpene hydrocarbons as well as the percentage of total oxygenated compounds and oxygenated monoterpenes were calculated (Table 7).

Table 6: Identified components in spathe essential oil.

Peak no.	Identified compounds	RRI	Base peak	M+	Conc.%*
1	n-Hexanal	802.1	44	72	0.5
2	o-Xylene	870.2	91	106	0.92
3	Styrene	885	104	107	1.37
4	Heptanal	898	44	96	1.61
5	Benzaldehyde	967	77	105	0.82
6	n-heptanol	968.9	41	80	0.64
7	2-Pentylfuran	991	81	138	3.07
8	n-octanal	1005	41	100	0.97
9	3-carene	1013	93	136	1.87
10	m-Cymene	1029	119	134	1.21
11	2-Ethylhexanol	1033	57	98	4.77
12	D-limonene	1034	68	136	0.49
13	Benzene acetaldehyde	1050	91	120	0.96
14	2-octenal	1063	41	93	1.03
15	1-octanol	1075	41	95	0.75
16	p-1,3,8-Menthatriene	1090	119	134	0.32
17	Linalool	1102	91	134	0.41
18	n-nonanal	1095.6	57	124	3.44
19	(*E*,*E*)-2,6-Nonadienal	1158	41	79	0.41
20	2-nonenal	1165	43	148	0.61
21	Caprylic acid	1171	61	101	0.44
22	n-nonanol	1175	56	98	0.42
23	4-terpineol	1192	71	136	0.43
24	Estragole	1205	59	148	0.18
25	Decanal	1209	43	112	0.88
26	β-cyclocitral	1232	41	152	0.57
27	Thymoquinone	1258.8	43	194	0.29
28	Nonanoic acid	1267	60	129	0.42
29	(3E)-8-Methyl-3,7-nonadien-2-one	1274	69	109	0.33
30	Tridecane	1300	57	99	0.25
31	1-methyl naphthalene	1314	115	142	0.34
32	Isolongifolene, 9,10-dehydro-	1338	131	202	0.52
33	δ-EIemene	1345	81	204	0.3
34	α-cubebene	1360	161	204	2.87
35	8-methylene-tricyclo[3.2.1.0(2,4)]octane	1379	91	119	0.5

36	Longifolene-I2	1389	94	204	0.3
37	α-copaene	1393	119	204	18.72
38	β-cubebene	1402	161	204	2.51
39	Unknown	1410	119	202	9.23
40	α-Gurjunene	1425	118	204	0.37
41	(E)-α-Ionone	1433	121	202	0.11
42	Caryophyllene	1439	41	189	2.2
43	Unidentified sesquiterpene	1448	161	189	0.65
44	Aromadendrene	1453	119	202	1.4
45	Isocaryophyllene	1458	41	135	0.43
46	α-humulene	1476	133	136	0.94
47	Allo-aromadendrene	1481	41	189	1.82
48	γ-Muurolene	1491	41	159	1.43
49	Germacrene D	1501	161	204	2.97
50	Viridiflorene	1510	107	204	0.44
51	Elixene	1515	121	189	1.47
52	δ-cadinene	1518.9	161	204	1.95
53	Calamenene	1536	159	159	0.46
54	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	1547	119	204	0.41

Peak no.: Peak number M^+: Molecular weight. RRI: Relative retention index. *Relative concentration based on triplicate measurements using three samples. MS of unknown compound RRI: 1410, m/z (rel.int.): 91(53%)- 105(52%)- 119 (100%)- 132 (48%) - 131(35%)-145(34%) – 92 (28%)- 93 (27%). MS data of unidentified sesquiterpene RRI: 1448, m/z (rel.int.): 161 (100%)- 105 (46%)- 91 (40%)- 41 (32%) – 81 (24%)- 93 (23%)- 119 (22%) – 55 (18%).

Table 7: The calculated percentage of different classes of the spathe oil compounds.

Oil constituents	Percentage
Identified components	72.84
Unknown compounds	27.16
Monoterpene hydrocarbons	3.89
Sesquiterpenes hydrocarbons	41.51
Total hydrocarbons	48.78
Oxygenated monoterpenes	2.03
Total oxygenated compounds	23.73
Total monoterpenes	5.92
Total sesquiterpenes	41.51
Major constituent: Copaene<alpha>	18.72

A total of 52 compounds were detected. Sesquiterpene hydrocarbons constituted the most dominant class with 41.51% where α-copaene predominates (18.72%). Concerning oxygenated compounds, total oxygenated compounds were 23.73%, where 2-ethylhexanol (4.77%) was the major oxygenated compound, while oxygenated monoterpenes were found to be 2.03%,

In conclusion, the results provide the first study on volatiles of spathe from the plant while several reports were found concerning the spathe of the well-known species *Phoenix dactylifera* (Hamedi *et al.*, 2013, Jahromi *et al.*, 2014).

CONCLUSION

This study is the first report that shed light on leaves and pollens of *Phoenix canariensis* Hort. *ex Chabau* cultivated in Egypt, in addition to its spathe volatile analysis. The leaves are rich in polyphenolics, flavonoids and tannins as compared to pollens while pollens are rich in steroids. The main components identified by GLC and HPLC might suggest their incorporation in antioxidant and anti-inflammatory preparations. Further studies are required to evaluate the plant biological activities and to isolate the bioactive compounds.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

AOAC, 2000. Official Methods of Analysis of the Association of Official Analytical Chemist. 14th ed., Washington D.C.

Adawy SS, Hussein EHA, El-Khishin DA, Saker MM, El-Itriby HA. Genetic variability studies and molecular fingerprinting of some Egyptian date palm (Phoenix dactylifera L.) cultivars. II-RAPD and ISSR Profiling. Arab. J. Biotech, 2002; 5, 225-236.

Akbari M, Razavizadeh R, Mohebbi GH, Barmak A. Oil Characteristics and Fatty Acid Profile of Seeds from Three Varieties of Date Palm *Phoenix dactylifera* Cultivars in Bushehr-Iran., African Journal of Biotechnology, 2012; 11, 12088-12093.

Al-Juhaimi F, Ghafoor K, Ozcan, M M. Physical and Chemical Properties, Antioxidant Activity, Total Phenol and Mineral Profile of Seeds of Seven Different Date Fruit *Phoenix dactylifera* Linn.,Varieties. International Journal of Food Sciences and Nutrition, 2012; 63, 8489.

Al-Shahib W, Marshall R. Fatty Acid Content of The Seeds from Fourteen Varieties of Date Palm *Phoenix dactylifera* Linn. International Journal of Food Science and Technology, 2003; 38, 709-712.

Al-Zoreky NS, Al-Taher AY. Antibacterial activity of spathe from Phoenix dactylifera L. against some food-borne pathogens. Industrial crops and products, 2015; 65, 241-246.

Asami A, Hirai Y, Shoji J. Studies on the Constituents of Palmae Plants, Steroid Saponina and Flavonoids of Leaves of *Phoenix canariensis* hort. ex Chabaud, P. humilis Royle var. hanceana Becc., *P. dactylifera* L., and *Licuala spinosa* Wurmb, Chemical and Pharmaceutical Bulletin, 1991; 39, 2053-2056.

Azmat S, Ifzal R, Rasheed M, Mohammad FV, Ahmad VU. GC-MS Analysis of n-hexane Extract from Seeds and Leaves of *Phoenix dactylifera* L. Journal of the Chemical Society of Pakistan, 2010; 32, 672-676.

Ben Salah S, Emna B, Flamini G, El Arem A, Issaoui M, Dabbou S, BenYahia L, Ferchichi A, Hammami M, Achour L. Compositional Characteristics and Aromatic Profile of Date Palm Seeds from Seven Varieties Gown in Tunisia, International Journal of Food Science and Technology, 2012; 47, 1903-1908.

Claus EP, Tyler V. Pharmacognosy 5th Ed., Lea Febiger, Philadelphia, 1967; 271-273.

Daksha A, Jaywant P, Bhagyashree C, Subodh P. Estimation of sterols content in edible oil and ghee samples. Electronic Journal of Environment, Agriculture and Food Chemistry, 2010; 9, 1593-1597.

Earp CF, Akingbala JO, Ring SH, Rooney LW. Evaluation of several methods to determine tannins in sorghums with varying kernel characteristics. J. Cereal Chemistry, 1981; 58, 234-238.

Elgindi MR, Singab AN, El-Taher EMM, Kassem MES. A comprehensive review of Phoenix (Arecaceae). Research journal of pharmaceutical, biological and chemical sciences, 2015; 6, 966-974.

Evans WC, 2002. Trease and Evans Pharmacognosy. 15th Ed., 50, WB Saunders Elsevier Company Ltd.

Farag MA, Wessjohann LA. Volatiles Profiling in Medicinal Licorice Roots Using Steam Distillation and Solid-Phase Microextraction (SPME) Coupled to Chemometrics. Journal of Food Science, 2012; 77, 1179-1184.

Farag MA, Mohsen M, Heinke R, Wessjohann LA. Metabolomic fingerprints of 21 date palm fruit varieties from Egypt using UPLC/PDA/ESI-qTOF-MS and GC-MS analyzed by chemometrics. Journal of food research international, 2014; 64, 218-226.

Fieser LF, Fieser M. 1959. Steroids. Reinhold Publishing Co., New York, 727-743.

Finar, IL. 1973. Organic chemistry, sixth ed., Longman group Ltd., England.

Garcia B, Alberto M, Eliseo S, Amparo T. Triterpenes, Waxes and Tricin in *Phoenix canariensis* hort ex. Cabaud, Journal of Natural Products, 1981; 44, 111-113.

Geissman, TA. The Chemistry of Flavonoid Compounds, Macmillam Company, New York, 1962; 318-320.

Gilman E, Watson D. 2006. *Phoenix canariensis* Canary Island Date Palm.Bulletin ENH-598. Institute of Food and Agricultural Sciences, University of Florida.

Goupy P, Hugues M, Boivin P, Amiot M. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. Journal Sci. Food Agriculture, 1999; 79, 1625-1634.

Hamedi A, Mohagheghzadeh A, Rivaz S. Preliminary Pharmacognosy Evaluation and Volatile Constituent Analysis of Spathe of *Phoenix dactylifera* L., Pharmacognosy Journal, 2013; 5, 83-86.

Hammami S, Jarraya H, Ben Salem S, Hamdi B, Ben Salah A, Devi P, Nefzi A, Mighri Z. Isolation and Structure Elucidation of Novel Alkaloid from *Phoenix canariensis* hort ex. Cabaud. Palm Tree, Journal de la Societe Chimique de Tunisie, 2010; 12: 105-108.

Jahromi MF, Moein M R, Etemadfard H, Johromi Z. Variation of Spathe Essential Oil Composition of Ten Iranian Date Varieties (*Phoenix dactylifera* L.). International Journal of Plant, Animal and Environmental Sciences, 2014; 4: 51-56.

Johnson DV. 1998. Tropical palms, FAO Forestry report, non-wood Forests Products No.10. FAO, Rome.

Jung HJ, Song YS, Kim K, Lim CJ, Park EH. Assessment of the anti-angiogenic, anti-inflammatory and antinociceptive properties of ethyl vanillin. Archives of pharmacal research, 2010; 33, 309-316.

Kchaou, W., Abbès, F., Blecker, C., Attia, H.,Besbes, S. Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (Phoenix dactylifera L.). Industrial crops and products, 2013; 45: 262-269.

Krueger R. 2001. Date palm germplasm overview and utilization in the USA. Proceedings international conference on date palms.

Lee J, Jung E, Lee J, Kim S, Huh S, Kim Y, Kim Y, Byun SY, Kim YS, Park D. Isorhamnetin Represses Adipogenesis in 3T3-L1 Cells. Obesity, 2009; 17, 226-232.

Liolios CC, Sotiroudis GT, Chinou I. Fatty Acids, Sterols, Phenols and Antioxidant Activity of *Phoenix theophrasti* Fruits, Growing in Crete, Greece, Plant Foods for Human Nutrition, 2009; 64, 52-61.

Mansouri A, Embarek G, Kokkalou E, Kefalas P. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera* L.), Food Chemistry, 2005; 89,411-420.

Mattila P, Astola J, Kumpulainen J. Determination of flavonoids in plant material by HPLC with Diode-Array and Electro-Array Detections. Journal of Agri. Food Chem., 2000; 48, 5834-5841.

Melo CM, Morais TC, Tomé AR, Brito GAC, Chaves MH, Rao VS, Santos FA. Anti-inflammatory effect of α, β-amyrin, a triterpene from Protium heptaphyllum, on cerulein-induced acute pancreatitis in mice. Inflammation Research, 2011; 60, 673-681.

Mohtasheem M, Waseemuddin A, Iqbal A, Husan B, Ahmad VU, Ali MS. Biological activity and phytochemistry of *Phoenix*species. Hamdard Medicus, 2005;48.

Nehdi IA, Omri S, Khalil MI, Al-Resayes SI. Characteristics and Chemical Composition of Date Palm, Phoenix canariensis hort. ex Chabaud, Seed and Seed Oil. Industrial Corp and Products, 2010; 32,360-365.

Nehdi IA, Zarrouk H, Al-Resayes SI. Changes in Chemical Composition of *Phoenix canariensis* hort. ex Chabaud Palm Seed Oil During the Ripening Process. Scientia Horticulturae, 2011; 129, 724-729.

Ogungbenle HN. Chemical and Fatty Acid Composition of Date Palm Fruit *Phoenix dactylifera* Linn., Flour. Bangladesh Journal of Scientific and Industrial Research, 2011; 46, 255-258.

Peach K, Tracey MV. Modern Methoden Der Pflazen Analyse. Tgottingen, Heidelberg, 1955; 3, 450.

Pengfei L, Tiansheng D, Xianglin , Jianguo W. Antioxidant properties of isolated isorhamnetin from the sea buckthorn marc. Plant foods for human nutrition, 2009; 64, 141-145.

Pujari RR, Vyawahare NS,Thakurdesai PA. Neuroprotective and antioxidant role of Phoenix dactylifera in permanent bilateral common carotid occlusion in rats. Journal of Acute Disease, 2014; 3, 104-114.

Rahmani AH, Aly SM, Ali H, Babiker AY, Srikar S. Therapeutic effects of date fruits (Phoenix dactylifera) in the prevention of diseases via modulation of anti-inflammatory, anti-oxidant and anti-tumour activity. International Journal of Clinical and Experimental Medicine, 2014; 7.

Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. Pharmacological research, 2008; 57, 451-455.

Saafi E, Trigui M, Thabet R, Hamammi M, Achour L. Common Date Palm in Tunisia: Chemical Composition of Pulp and Pits, International Journal of Science and Technology, 2008; 43, 2033-2037.

Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The story of beta-sitosterol-a review. European Journal of Medicinal Plants, 2014; 4, 590.

Seo K, Yang JH, Kim SC, Ku SK, Ki SH, Shin SM. The antioxidant effects of isorhamnetin contribute to inhibit COX-2 expression in response to inflammation: a potential role of HO-1. Inflammation 2014; 37, 712-722.

Singab AN, El-Taher EMM, Elgindi MR, Kassem MES. Phoenix roebelenii O'Brien DNA profiling, bioactive constituents, antioxidant and hepatoprotective activities. Asian Pacific Journal of Tropical Disease, 2015; 5, 552-558.

Singleton VL, Orthofer R, Lamuela RRM. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods Enzymol., 1999; 299,152-178.

Tai A, Sawano T, Yazama F. Antioxidant Properties of Ethyl Vanillin in vitro and in vivo. Bioscience, biotechnology, and biochemistry, 2011; 75, 2346-2350.

Teng BS, Lu YH, Wang ZT, Tao XY, Wei DZ. In vitro anti-tumor activity of isorhamnetin isolated from Hippophae rhamnoides L. against BEL-7402 cells. Pharmacological research, 2006; 54, 186-194.

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia, 2011; 1, 98-106.

Vogel Al. 1967. A Text book of practical organic chemistry. Longmans and green Co. Ltd., London.

Wagner H, Baldt S, Zganski EM, 1983. Droger Analyse. Springer Verlag, Berlin, 6.

Zaid A, Arias-Jimenez EJ. 2002. Date Palm Cultivation. FAO Plant production and protection paper 156, Rev.1. FAO, Rome.

Zhao G, Etherton TD, Martin KR, Heuvel JPV, Gillies PJ, West SG, Kris-Etherton PM. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochemical and biophysical research communications, 2005; 336, 909-917.

Zohary D, Hopf M. 2000. Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe, the Nile Valley Oxford University Press, Oxon, UK.

How to cite this article:

Hifnawy MS, Mahrous AMK, Ashour RMS. Phytochemical investigation of *Phoenix canariensis* Hort. ex Chabaud leaves and pollen grains. J App Pharm Sci, 2016; 6 (12): 103-109.