Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 05 | Issue : 04 | Oct.-Dec.| 2015
Pages: 945-949

Chemical Composition and antioxidant activity of Date Palm pollen grains (*Phoenix dactylifera L. Palmae)* essential oil for Siwe Cultivar Cultivated in Egypt

[1]A. Farouk, [2]A. Metwaly and [3]M. Mohsen

[1]Flavor and Aromatic Chem. Dept., National Research Center, Dokki, Giza, Egypt.
[2]Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.
[3]Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt.

ABSTRACT

Volatile constituents of Date palm pollen grains essential oil cultivated in Egypt, were hydrodistilled and identified using gas chromatography – mass spectrometry. Twenty - one compounds detected accounting for 74.2% of the total oil, whereas dimethoxytoluene isomers (19.75%), p-cymen-4-ol (13.51%), caryophyllene (9.51%) and phenylethanol (8.75%) constitute the major aroma components. Antioxidant activity and radical scavenging were investigated using diphenpierylhydrazyl DPPH and β-Carotene-linoleic bleaching assays. The obtained results revealed an activity in pollen volatile oil with inhibition constant 50 IC_{50} 0.89 mg/ml. This is in agreement with the total phenolic content, which measured for the oil and identified 57.9 mg GA/g according to Folin-Ciocalteu reagent.

Key words: **Date** Palm pollen grains, essential oil, *Phoenix dactylifera L.*, DPPH, total phenolic**.**

Introduction

Date Palm (*Phoenix dactylifera L.* Palmae) is native to the Middle East region over centuries ago, whereas, date represents an essential meal especially in Arab countries (Copley *et al*. 2001; Miller *et al.,* 2003). Several medicinal applications have been reported for different components of date palm, for example, date palm pollens which are used as folk medicine remedy for curing male fertility and to promote fertility in women (Bahmanpour*et al*., 2006; Hassan, 2008). The Inflorescence of the male date tree is composed of spathe that surrounds many buds containing pollen (Hassan., 2011) many other therapeutic properties for palm pollen extracts have been studied as antioxidant and antimicrobial activities using different solvents (Amany*et al*., 2013 ; Abd El-Azim., *et al*., 2015). Therefore, chemical compositions have been given more attention in order to find out the main constituents responsible for such bioactivities.

Hassan (2011), showed the chemical composition of palm pollen grains as moisture (28.8%), ash (4.57%), crude fiber (1.37%), crude fat (20.74%), crude protein (31.11%), carbohydrate (13.41%), vitamins (A,E and C), minerals and amino acids. While Bishr and Desouky (2012), made a comparative study for the nutritional value of four different Egyptian palm pollen, based on their vitamins, minerals and amino acids contents. Again, chemical analysis for the palm pollen as well as antioxidant and antimicrobial activities of their ethanolic extract were performed by Arafat *et al*. (2013). In addition to vitamins and amino acids complete profile, they described fatty acids composition and the total phenolic and flavonoids contents of the pollen ethanolic extract. Flavonoids were studied extensively before by Abbas and Ateya (2011), where estradiol, esteriol, estrone and others identified in pollen for the first time.

Using different Polar solvents, Abd El Azim *et al*. (2015), separated six phenolic and flavonoid compounds and studied the antibacterial and antifungal activities of such extracts. Jahromi *et al*. (2014), were the first extracted the hydrodistilled essential oil of spathes from 10 varieties cultivated in Iran, with different identified chemical classes e.g. terpenes, aldehydes, and methoxylated aromatics. However, nothing could be traced in the literature regarding the volatile constituents of palm pollen as well as their bioactivities. Therefore, the aim of the present study is to identify the main constituents of these volatiles and study the antioxidants activity based on their total phenolic content.

Material and Methods

Plant materials and chemicals

Date Palm pollen Grain (semi-dried) obtained from New Valley Governorate. Diethyl ether and Methanol purchased from (Fisher chemicals). Sodium bicarbonates , linoleic acid ≥ 99% , Tween 40 , β-Carotene ≥ 97%

Corresponding Author: A. Farouk, Flavor and Aromatic Chem. Dept., National Research Centre, Dokki, Giza, Egypt.
E-mail: amrfarouk01@gmail.com

, Folin-Denis Reagent for total phenolic , DPPH (2,2-Diphenyl -1-picrylhydrazyl) and Gallic acid (97.5-102.5%) were obtained from Sigma Aldrich Chemical Co . (St Louis, MO, USA).

Essential Oil Extractions

Palm pollen Grain essential oil under study were obtained by steam –distillation for 3h using a Clevenger – type apparatus according to the method of Giray *et al.*(2008).The obtained essential oil was closed under nitrogen gas and stored in airtight glass vials covered with aluminum foil at – 20ºC.

Gase Chromatography- Mass Spectrometry analysis (GC-MS)

The obtained essential oil of (palm pollen grain) analyzed using GC-MS apparatus. Separation was performed on Trace GC Ultra Chromatography (Thermo Scientific, USA), equipped with ISQ-Mass (Thermo Scientific, USA) and 60m x 0.25mm x 0.25mm TG-5MS capillary column (Thermo Scientific, USA). The column separation programmed from 50ºC with hold time 3min and temperatures increase at rate 4ºC/min to 140ºC with hold time 5min, then at rate 6ºC /min to 260ºC with 5min. isothermal hold. The injector temp was 180ºC, Ion source temp 200ºC and the transition line temperature was 250ºC. The carrier gas was helium with constant flow rate 1.0 ml min-1. The mass spectrometer had a scan range from m/z 40 to m/z 450. Ionization energy was set at 70 eV. The identification of compound based on the comparison the MS computer library (NIST library version 2005) and the relative percentage of the oil constituents was calculated from GC peak areas. A linear retention was calculated for each compound using the retentiontimes of a homologous series of C6 – C26 n-alkanes (Adams, 1995).

The antioxidant activity measurements

DPPH radical scavenging assay

The potential antioxidant activity of Date palm pollen grain essential oil were assessed according to Hatano *et al*. (1998), 1ml of each solution of different concentrations (0.01- 1 mg/ml) of the extracts was added to 3 ml of 0.004% methanolic 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical solution. The mixture was shaken vigorously and left to stand at room temperature for 30min; the absorbance of the resulting solution was measured at 517nm using UV spectrophotometer (Evolution 300 Thermo UV -VIS) which all tests was run in three replicates and averaged. Then the % inhibition was calculated by the following equation:

$$\% \text{ radical} = (AB - AS) / AB \times 100$$

Where AB = absorbance of blank, AS = absorbance of scavenging activity sample From calibration curves, obtained at different concentrations of the extracts, the IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

β-Carotene bleaching assay

Antioxidant activity of the aqueous solution was determined by a β -carotene / linoleic acid system as described by Taga *et al*. (1984).Approximately 10 mg of β-carotene was dissolved in chloroform (10mL). The carotene–chloroform solution, (0.2 mL) was pipetted into a boiling flask containing 20mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator at 40 °C for 5 min, and distilled water (50 mL) was added to the residue slowly with vigorous agitation, to form an emulsion. A portion of the emulsion (5 mL) was added to a tube containing the sample solution (0.2 mL) and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 200 µL of water instead. The antioxidant activity was expressed as % inhibition percentage with reference to the control after a 60 min incubation using the following equation:

$$AA = 100 (DRC - DRS)/ DRC$$

Where AA = antioxidant activity; DRC = degradation rate of the control = [ln(a/b)/60]; DRS = degradation rate in presence of the sample= [ln(a/b)/60]; a = absorbance at time 0; b = absorbance at 60 min.

Determination of the total phenolic contents

Total phenolic content of the essential oils obtained from Date Palm pollen Grain was determined using Folin–Ciocalteu reagent according to a modified method of (Singleton *et al*., 1999) using gallic acid as the standard. The oil solution (0.5 mL) was mixed with 2.5 mL of 10%Folin–Ciocalteu reagent, 2.5 mL of a 7 % aqueous solution of $NaHCO_3$ Then, the mixture was vigorously vortexed. The reaction mixtures were thereafter incubated in a thermostat at 45ºC for 45 min before the absorbance at 765 nm was measured. The concentration of essential oils was set to 1 mg mL-1. The same procedure was also applied to standard solutions of gallic acid with concentration (0.01-0.1 mg/ml) and The calibration equation for gallic acid was:

$$y = 0.0233x + 0.057 \ (R^2 = 0.9957)$$

Where y is the absorbance and x is the concentration of gallic acid in mg mL^{-1}.

Results and Discussion

GC-MS analysis of the essential oil isolated from Egyptian *Phoenix dactylifera L.*palmae, showed the identification of 21 Compound accounting for (74.2%) of the total amount Figure (1) and Table (1). The major compounds which identified were dimethoxytoluene isomers (19.45%), P-cymene-4-ol (13.51%), caryophyllene (9.51%), phenyl ethyl alcohol (8.75%) and caryophyllene oxide (3.71%). In addition to terpenes and aromatic compounds detected, fatty acids e.g. oleic acid (1.83%) and linoleic acid (1.24%) were the predominant identified in this class, which is in accordance to (Mohamadi *et al*., 2014). Fatty acids profile in date pollen grains have been studied extensively before, while volatile constituents only detected in spathe among the three main components of a inflorescence. With respect to the findings of the present study, spathes essential oil have a higher amounts of dimethoxytoluene isomers (84%), cayophyllene (5.5%) and caryophyllene oxide (2.4%) (Demirci *et al*., 2013). Dimethoxytoluene dramatically decreased in pollen grains while terpenes remains with other newly identified compounds e.g. phenyl ethanol, methoxy vinyl phenol and isopropyl benzaldehyde.

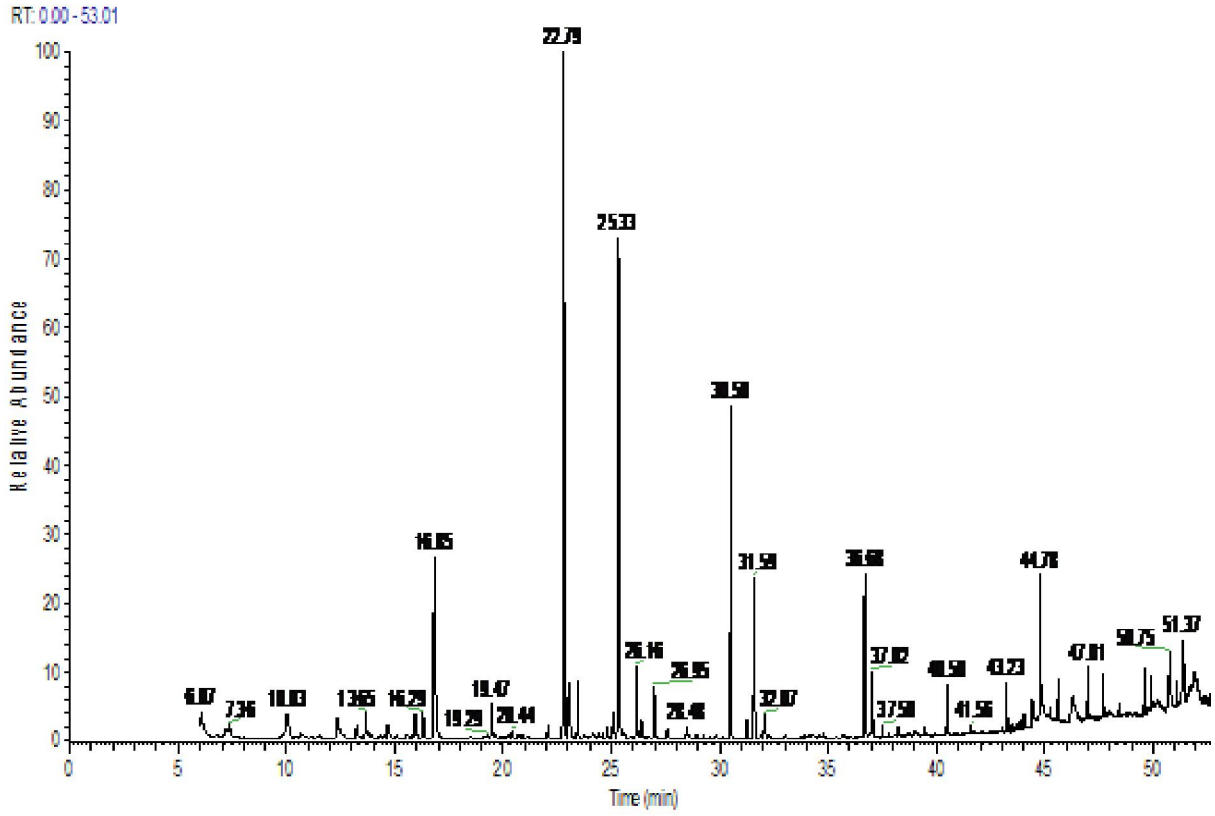
Table 1: Identification of compounds for the essential oil isolated from Egyptian (*Phoenixdactylifera* L.)

S/N	Compound	KI	% Area Palm pollen (Egypt)
1.	3-Octene	799	1.29
2.	o - Cymeme	1022	1.19
3.	Benzyl Alcohol	1032	0.58
4.	Phenyl acetaldehyde	1043	1.05
5.	Gamma-Terpinene	1062	0.24
6.	Linalool	1098	1.12
7.	Phenyl Ethyl Alcohol	1110	8.75
8.	Cresol	1190	0.33
9.	3,4 DimethoxyToulene	1230	18.16
10.	P- Isopropyl Benzaldhyde	1257	1.89
11.	2,4 DimethoxyToulene	1266	1.59
12.	P- Cymene- 4-ol	1287	13.51
13.	2-Methoxy -4-vinylPhenol	1317	2.00
14.	2,3,5- Trimethoxy Toluene	1404	0.55
15.	Caryophyllene	1418	9.51
16.	Humulene	1440	0.82
17.	CaryophylleneOxid	1581	3.71
18.	GeranylTiglate	1700	0.37
19.	Palmitic acid	1984	4.47
20.	Oleic acid	2144	1.83
21.	Linoleic acid	2152	1.24
22.	Total		74.2%

The antioxidant activity of date pollen essential oil tested by DPPH radical scavenging and β-carotene – linoleic bleaching assays. Recorded activities are presented in Table (2), where lower IC_{50} values indicate higher activity. The essential oil under investigation, exhibited antioxidant activity IC_{50} 0.89 mg/ml, in agreement with Arafat *et al*. (2013), which showed a higher activity for the ethanolic extract. Again, the inhibiting effect for linoleic acid oxidation and the subsequent bleaching of β-carotene was 62.2% which assured the DPPH radical scavenging assay result (58.8%). Total phenolic constituents of the palm pollen essential oil were determined by experimental methods involving Folin-Ciocalteau test. The total phenolic concentration was calculated as Gallic acid equivalents. The result indicated a higher phenolic content 57.9 mg GA/g however; it is less than detected in pollen ethanolic extract reported by Arafat *et al*. (2013). The above result is in accordance with the antioxidant activity investigated for pollen essential oil, as well as with the references reported the responsibility of plant phenolic compounds including flavonoids, for potent antioxidant, antimutagenic and anticarcinogenic activity (Middleton and Kandaswami, 1994) and (Rice-Evans *et al.,* 1997).

Table 2: Antioxidant activity of Egyptian (Palm Pollen Grain) essential oil

Material	IC_{50} / mg ml $^{-1}$ (DPPH)[a]	β-Carotene / bleaching, %	Total phenolic content [a] mg GA/g	(DPPH)[a] , %
		for 1mg ml $^{-1}$	for 1mg ml $^{-1}$	1mg ml -1
Palm Pollen grain Egypt	0.89	62.2	57.9	58.8



Fi. 1: GC-MS chromatogram for Egyptian Phoenix dactylifera L essential oil

References

Abbas, F.A., A. Ateya, 2011. Estradiol, Esteriol, Estrone and Novel Flavonoids from Date Palm Pollen. Australian Journal of Basic and Applied Sciences. 5: 606-614.

Abed El-Azim, M.H., F.A. Yassin, S.A. Khalil, A.M. El-Mesalamy, 2015. Hydrocarbons, fatty acids and biological activity of date palm pollen (*Phoenix dactylifera* L.) growing in Egypt. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS). 10: 46-51.

Abed El-Azim, M.H., A.M. El-Mesalamy, F.A. Yassin, S.A.Khalil, 2015. Identification Phenolic and Biological Activities of Methanolic Extract of Date Palm Pollen (*Phoenix dactylifera*). J Microb Biochem Technol. 7:1.

Adams, R.P., 1995. Identification of essential oil component Gase chromatography / Mass Spectroscopy, Allured publishing corp., USA.

Arafat, K. H., A. M. Mohamad and S. Elsharabasy, 2013. Influence of environmental conditions, salinity and root exudates on incidence and disease severity of Lasiodiplodia theobromae that caused root rot of date palm offshoots and biocontrolling. J. Biol. Chem. Environ. Sci., 8(1), 73-91.

Bahmanpour, S., T. Talaei, Z.Vojdani, 2006. Effect of *Phoenix dactylifera* pollen on sperm parameters and reproductive system of adult male rats. Iran J Medicinal Sci. 31: 208-12.

Bishr, M., S.Y. Desoukey, 2012. Comparative Study of the Nutritional Value of Four Types of Egyptian Palm Pollens. Journal of Pharmacy and Nutrition Sciences. 2:50-56.

Copley, M.S., P.J. Rose, A. Clapham, D.N. Edwards, M.C.Horton, 2001. Detection of palm fruit lipids in archaeological pottery from Qasr Ibrim, Egyptian Nubia. Proc Biol Sci. 268: 593-597.

Demirci, B., M. Tsikoli, U.R. Bernier, N.M. Agramonte, S.I. Alqasoumi, M.A.Al-Yahya, A.J.Al-Rehaily, H.S.Yusufoglu, F. Demirci, K.H. Başer, I.A. Khan, N. Tabanca, 2013. *Phoenix dactylifera* L. spa the essential oil: chemical composition and repellent activity against the yellow fever mosquito. Acta Trop. 128: 557-560.

Giray, E.S., S. Kirici, D. A. Kaya, M.Turk, Z. Sumez, M. Inan, 2008. Comparing the effect of sub critical water extraction of Lavandulastoechas. Talanta .70: 930-935.

Hassan, H.M., 2008. Biochemical characterization of palm pollen extracts as a biotransmitter in different systems. Ph.D., Thesis, Cairo University Giza, Egypt.

Hassan, H.M.M., 2011. Chemical Composition and Nutritional Value of Palm Pollen Grains. Global Journal of Biotechnology & Biochemistry. 6: 01-07.

Hatano, T., H. Kagawa, T. Yasuhara, T.Okuda, 1988. Two new flavonoids and other constituents in licorice root their relative astringency and radical scavenging effects. Chem. Pharm Bull. 36:1090-2097

Jahromi, M.A.F., M.R.Moein, H. Etemadfard, Z. Jahromi, 2014. Variation of the essential oil composition of ten Iranian date varieties (*Phoenix DactylIfera* L.) International Journal of Plant, Animal and Environmental Sciences. 4: 51.

Middleton, E. J. and C. Kandaswami, 1994. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In: Harborne, J.B. (Ed.), The Flavonoids, pp. 619–652, Chapman & Hall, London.

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. Eur J Clin Nutr 57: 427-430.

Mohamadi, M., T. Shamspur, A. Mostafavi, 2014. Comparison of the essential oil content and composition of the spathe, buds and pollen of *Phoenix dactylifera*. Nat Prod Res. 28: 205-7.

Rice-Evans, C.A., N.J. Miller, G. Paganga, 1997. Antioxidant properties of phenolic compounds.s. Trends Plant Science, 2:152–159.

Singleton, V.L., R. Orthofer, R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-178.

Taga, M.S., E.E.Miller, D.E.Pratt, 1984. Chia Seeds as source of Natural lipid Antioxidants. J. Am. Oil Chem. Soc. 61:928-931.