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Phytochemical Composition, Antioxidant and Antitumor Activities of some Date Palm Pollen Extracts

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Abstract

The aim of the present study was to investigate the phytochemical composition and the antioxidant potential of the Date Palm pollen grains (DPP) collected from Abu-Hamad, Sharkia, Egypt. Investigation of the lipid constituents of pollen grains of Phoenix dactylifera by GLC analyses led to identification of the fatty acids mixture which consists of nine fatty acids with palmitic acid as a major and the unsaponifiable fraction that contains a series of hydrocarbons, sterols, with pentadecane as a major. Estimation of total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extract (ME) were performed by Folin-Ciocalteau reagent and aluminium chloride methods respectively. Twenty three phenolic compounds and twenty flavonoid compounds were identified and quantified by HPLC to provide the scientific basis for its biological activity. The results revealed that the methanolic extract had total phenolic content value of 62.1 ± 1.8 mg GAE/g and total flavonoid content value of 90.2 ± 2.3mg QE/g. Antioxidant activity was screened by DPPH assay. The methanolic and ethyl acetate extracts of the DPP grains showed potent antioxidant activity and high cytotoxic activities at a concentration of 25 µM against RPE-1 and MCF-7 human cell lines using MTT assay, compared to doxorubicin as a reference drug.

Keywords: Phoenix dactylifera, pollen grains, flavonoids, phenolic compounds, antioxidant, antitumor activity.

1. Introduction

The belief of many middle easterners people, that the action may the subject has been exposed to any toxic material, can be reversed bythe consumption of dates especially in the morning on empty stomach (1).

Date palm pollen grains were called “Fountain of Youth” by early Egyptians and ancient Chinese and used as a rejuvenating medicinal agent. Pollen preparations were used over the wide world as dietary supplements by increasing the total dietary intake (2).The genus *Phoenix*, belonging to the family Arecaceae, is a member of the monocotyledone plants, dioecious with male-female and flowers occurring on separate plants and consist of 17 species native to tropical Asia and Africa. Date palm (*Phoenix dactylifera* L.) was known by the Arabs as “Nakhl, Nakhil” and the tree of life (3,4).

In the Arabian Peninsula, and several countries of the Middle East and North Africa, Date palm tree fruit has become an important source of health, nutrition and economic values due to the antioxidant properties of the fruit and pollen (5-7). It considers one of the main oldest crops, since it plays an essential role in their population diet and socio-economic structure (8-11), and cultivated in arid regions in Southwest Asia and North Africa as the commercial source of dates, documented in Holy Quran and modern scientific literatures (12-14).

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In traditional medicine, the date palm pollen (DPP), is a natural herbal powder widely used for both

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male and female infertility (15). For centuries, due to low function of testicles or a disturbance of their hormonal control (16), or abnormalities in the production of sperm in testicles (17-18), the pollen of the "blessed tree" has been used for treatment and prevention of weakness of sexual activity and prostatitis.

Different parts of the genus *Phoenix*, showed antioxidant (19), anti-inflammatory, antitumor (20), antibacterial (21), antifungal, neuroprotective (22) and hepatoprotective activities (23).

Lipids, phenolic acids, flavonoids, procyanidins, saponins, triterpenes, sterols and carbohydrates and/or glycosides have been identified and isolated from Egyptian DPP (24-26).

In pollen grains, high amount of flavonoids were present and moderate amount in the shoot tip as compared with other parts of the plants (leaves and fruits tissues)(27).

Farouk *et al*., in 2015(28), stated that twenty-one compounds detected and the dimethoxytoluene isomers (19.75%), were the main aroma component of palm pollen grains of *Phoenix dactylifera* cultivated in Egypt, followed by *p*-cymen-4-ol (13.51%), caryophyllene(9.51%), and phenylethanol (8.75%).The pollen volatile oil exhibited antioxidant activity and radical scavenging with inhibition constant IC_{50} 0.89 mg/ml.

The new sulfate conjugates (quercetin 3-glucosyl sulfate, luteolin 7-glucosyl disulfate and chrysoeriol 7-glucosyl disulfate) were detectedin *P. dactylifera* fruits along with the known luteolin and chrysoeriol 7-glucosyl sulfates, chrysoeriol 7-O- *β-D*-glucoside, chrysoeriol 7-*O*-rutinoside and luteolin 7-*O*-rutinoside (29).The phenolic compounds gallic acid, catechin, caffeic acid, epicatechin, vanillic acid, coumarin, quercetin and rutin were identified and quantified in DPP extracts from Tunisian origins by HPLC (4).

Column chromatography of hexan fraction revealed the presence of ten compounds (estradiol, estrone, clinoasterol acetate, β-sitosterol acetate, β-sitosterol caproate, β-sitosterol, cerotic acid, lignoceric acid, behenic acid and cholesterol), and (rutin, isorhamnetin, apigenin, luteolin, and naringin) were isolated from the ethyl acetate fraction of Egyptian date palm pollen (DPP)(26). The chemical investigation of the methanolic extract of date pollen revealed the presence of six phenolic compounds: caffeic acid, gallic acid, catechin, coumaric acid, chlorogenic acid, and quercetin (30).

Abbas and Ateya, 2011(26), stated that, the ethyl acetate fraction from date palm pollen possessed a strong antioxidant activity against DPPH radicals with value ($SC_{50} \geq 16.51$ µg/ ml).

The antibacterial activity of unsaponifiable fraction and saponifiable fraction of pet.ether and diethyl ether extracts of date palm had good antibacterial activity against (*Escherichia coli*, *Klebsiella* species, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus luteus*and *Staphylococcus aureus*) and high antifungal activity against *Candida albicans* except with un-saponifiable fraction of pet. ether extract (31). The methanolic extract of date palm had strong antibacterial activity and antifungal activity(30).

The objectives of this study were to investigate the lipid constituents, identify the phenolic and flavonoid compounds and quantify their total contents by RP-HPLC, and to evaluate the antioxidant, antitumor activities of date palm pollen grains (*Phoenix dactylifera*).

2. Experimental

Plant material

Date Palm pollen (DPP) of *Phoenix dactylifera* L. was collected in September (2020) from Abu-Hamad, Sharkia Governorate, Egypt. After collection, it was identified and authenticated by Prof. Dr. Mona Marzouk, Professor of plant taxonomy at the National Research Centre, Egypt. The pollen was air-dried and ground to a fine powder using a grinder. The powdered material was stored in a refrigerator at +4 º C until further use.

Extraction of lipid constituents

About 500 g of the dried powdered plant material was extracted with n-hexane. The hexane extract was filtered, dried over anhydrous sodium sulphate and evaporated *in vacuo* at 40 °C till dryness to give an oily residue. About 2 g of the oily residue were subjected to saponification process(N/2 alc. KOH), to afford the unsaponifiable matter. The liberated fatty acid mixture, after saponification was

extracted, methylated (MeOH, 4.5% HCl)(32). Samples of the isolated unsaponifiable fraction and methyl esters of fatty acids were subjected to GLC analysis.

Phenolic extraction

About 5 g ofthe defatted powdered plant material were extracted by maceration with methanol (80%; 3 x 250 mL).The combined methanol extract was evaporated *in vacuo* at 50 ºC till free from methanol and diluted with hot distilled water (250 ml). The aqueous methanolic extract was partitioned with ethyl acetate (250 ml x 3), followed by butanol (250 ml x 3). The combined solvents were dried over anhydrous sodium sulfate and evaporated till dryness. The dry residue obtained from each extract was kept for further analysis.

Determination of total phenolic and flavonoid contents

The total phenolic contents of the extract(80 % MeOH), were analyzed using a slightly modified version of the Folin-Ciocalteu's method as described by Li *et al*.,in 2008 (33). The absorbance of the samples was measured at 760 nm, and their total phenolic contents were expressed as mg of gallic acid equivalents per g of dry plant extract (mg GAE/g). Total flavonoid contents of the extract(80 % MeOH), were quantified spectrophotometrically at 430 nm using the method described by Quettier-Deleu *et al*., in 2000(34), and expressed in mg of quercetin equivalent per g of dry plant extract (mg QE/g).

GLC analysis

The GLC analyses were carried out using the following conditions; Instrument: Varian model3700 GC. Column for unsaponifiable matters: 10 % OV-101 on chromsorb W/HP, 80/100, (2m stainless steel, 0.25mm i.d.), Column for fatty acid methyl esters:15 % DEGS on chromsorb W/AW, 80/100, 2m stainless steel, 0.25 mm i.d.), Temp. for unsaponifiable matters: column: 70 °C up to 270 °C, 4 °C/min., injector: 280 °C., Detector (FID): 290 °C. Temp. for fatty acid methyl esters: column: 70 °C upto 190 °C, 4 °C/min., injector: 240 °C., Detector :280 °C, Flow rates for both of them:N_2 and H_2: 30 ml / min., Air:300 ml /min.

HPLC analysis of phenolic compounds

Before quantization by HPLC, the sample was filtered through a 0.4 µm membrane filter into sample vial for injection. The relative concentrations of the detected flavonoids and other phenolic compounds were determined from peak areas Quantitative determination of phenolic compounds was performed using HPLC apparatus, Agilent series 1200 (Agilent, USA) equipped with autosampler injector, solvent degasser, quaternary HP pump and diode array (DAD) UV detector, or set at 280 nm for phenolic acids and 320 nm for flavonoids (35). Analysis was achieved with Eclipse DB C-18 column (5µm, 250 x 4.6 mm i.d.), temperature was maintained at 35 ºC. Separation was done using acidified water and acetonitrile (90:10 v/v); isocratic elution at a flow rate of 1 ml/min. The injection volume for all samples was 20 µl. Standard flavonoids and phenolic acids were prepared as 10 mg/50 ml solutions in methanol and serial dilutions were prepared and injected into HPLC to establish standard calibration curves. Quantification of compounds was performed based on peak area computation (external standard method). The analysis was run in triplicates and the concentrations of the identified compounds were expressed as µg/g dry weight(35,36).

In-vitro antioxidant activity

Antioxidant activity of each extract and standards (rutin and ascorbic acid) was assessed based on the radical scavenging effect of stable DPPH free radical (37). 10µl of each tested extract or standard (series of different concentrations) was added to 90 µl of a 100 µM methanolic solution of DPPH in a 96-well microtitre plate. After incubation in dark at 37°C for 30 min, the decrease in absorbance of each solution was measured at 520 nm using SpectraMax® Paradigm® Multi-Mode microplate reader. The absorbance of blank sample containing the same amount of DMSO and DPPH solution was also prepared and measured. All experiments were carried out in triplicate.

The scavenging potential was compared with a solvent control (0% radical scavenging), and the standard compound. Radical scavenging activity was calculated by the following formula:

% Reduction of absorbance = [(AB - AA) / AB] x 100, where: AB - absorbance of blank sample and AA - absorbance of tested compound (t = 30 min). The concentration of each extract required to scavenge 50 % of DPPH (IC_{50}) was determined as well (37).

MTT cytotoxicity assay

The cytotoxicity activity against RPE-1 and MCF-7 human cell lines was estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells (38). Cells were dispensed in a 96 well sterile microplate (5 x 10^4 cells/well), and incubated at 37ºC with series of different concentrations in DMSO, of each tested compound or Doxorubicin® (positive control) for 48 h in a serum free medium prior to the MTT assay. After incubation, media were carefully removed, 40 µL of MTT (2.5 mg/mL) were added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 590 nm using SpectraMax® Paradigm® Multi-Mode microplate reader. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells.

In-vitro cytotoxicity activity

Cell culture of RPE-1 (human normal Retina pigmented epithelium cells) and MCF-7 (human breast adenocarcinoma) cell lines were purchased from the American Type Culture Collection (Rockville, MD) and maintained in DMEM medium which was supplemented with 10% heat-inactivated FBS (fetal bovine serum), 100U/ml penicillin and 100U/ml streptomycin. The cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

Statistical analysis

All experiments were conducted in triplicate and repeated in three different days. All the values were represented as mean ± SD. IC_{50}s were determined by probit analysis using SPSS software program (SPSS Inc., Chicago, IL).

3. Results and Discussion

Contents of total phenolic (TPC) and total flavonoids(TFC)

TPC of the methanolic extract of date palm pollen was investigated, and the values was 62.1 ± 1.8 mg gallic acid equivalent (mg GAE/g D.W.),While that of TFC was 90.2 ± 2.3 mg quercetin equivalent (mg QE/g D.W.).

Assessment of the lipid constituents

Unsaponifiable fraction

The results obtained in Table(1) showed that the GLC analysis of the unsaponifiable fraction consists mainly of a mixture of a series of n-alkanes from C-10 to C-25, that all representing 65.87 % of total unsaponifiable material, in addition to campasterol (1.50 %), stigmasterol(4.32 %) and β-sitosterol(8.96 %). Identification of these compounds were carried out by comparison of their retention time with the available reference compounds.

Abed El-Azim *et al.*, in 2015(31), reported that the GC-MS of the unsaponifiable fraction of pet. ether extract of DPP contain a series of saturated, unsaturated hydrocarbons and steroids, in which α-sitosterol was the major phytosterol(14.40 %).

Fatty acid fraction

GLC analysis of the fatty acid methyl esters revealed the presence of 9 fatty acids(99.95 %), the major component is palmitic acid ($C_{16(0)}$), which comprises a relative percent (50.48 %), followed by linoleic acid $C_{18(2)}$ (13.69 %), and lauric acid $C_{12(0)}$ (8.55 %). The results obtained in Table (2) for the fatty acids pattern of *P. dactylifera* pollen had 69.79 % saturated fatty acids, 5.34 % monounsaturated fatty acids and 24.82 % polyunsaturated fatty acids. The metabolic precursors of polyunsaturated fatty acids cannot be synthesized in the body and must be taken by food. The PUFAs play an important role in modulating enzyme activities, carriers and membrane receptors of insulin, antibodies neurotransmitters, drugs receptors, etc., and display antitumor activity against Ehrlich ascites tumor in mice (39). Abed El-Azim *et al.*, in 2015(31), reported that the GC-MS of pet. ether extract of date palm pollen revealed the presence of 7 fatty acids.

The major is hydnocarpic acid 44.23 %, followed by palmitic acid 38.52 %. The GC/MS analysis of date palm pollen contains a high amount of linoleic acid (13.69 %), linolenic acid (5.64 %) and oleic acid (5.34 %). Dupont and White, in 1990(40), reported that linoleic acid is an important essential fatty acid required for growth, physiological functions and maintenance. The presence of high level of linoleic acid in the plant part could be beneficial as dietary supplements and help in weight management, in the prevention of cancers, asthma, high blood pressure, cardiovascular diseases, gastro intestinal and immune systems dysfunction.

The highest value of omega-9 oleic acid is considered one of the healthier sources of fat in the diet which helps in the production of antioxidants, minimize cholesterol level, high blood pressure and chest pain (41).

Ghanem *et al*., in 2015(39), stated that the GC-MS analysis of the ethanolic extract of DPP, consist of unsaturated fatty acids(USFAs), and six saturated fatty acid esters. The results obtained that the monounsaturated fatty acids palmitoleic and oleic acids, comprised 2.12 % and 2.73 % of total volatiles. The major saturated fatty acids ester of DPP was hexadecanoic acid-ethyl ester (35.32%) of the total volatiles.

The predominant USFA of DPP was ethyl-9-hexadecenoate, 9,12,15-octadecatrienoic acid-ethyl ester, and ethyl oleate, as monounsaturated fatty acid, comprised 8.42 % and 1.35 % of the total volatiles of DPP. The 9,12,15-octadecatrienoic acid-ethyl ester, as PUFAs, comprised 9.68 % of the total volatiles of DPP.

Table 1. GLC data of unsaponifiable fraction of *Phoenix dactylifera* pollen grains.

Peak No.	Rt (min.)	Relative %	Compounds
1	5.53	4.50	Decane, n-C_{10}
2	6.81	7.92	Undecane, n-C_{11}
3	8.36	0.65	Tridecane , n-C_{13}
4	12.03	28.23	Pentadecane , n-C_{15}
5	13.14	3.06	Hexadecane , n-C_{16}
6	14.67	5.05	Heptadecane, n-C_{17}
7	16.62	0.59	Octadecane, n-C_{18}
8	17.42	6.51	Nonadecane, n-C_{19}
9	19.85	1.73	Uncosane, n-C_{21}
10	23.70	4.63	Tetracosane, n-C_{24}
11	25.60	3.00	Pentacosane, n-C_{25}
12	32.00	1.50	Campasterol
13	34.20	4.32	Stigmasterol
14	36.70	8.96	β -sitosterol

Table 2. GLC analysis of FAME fraction of *Phoenix dactylifera* L. pollen grains.

Peak No.	Rt(min.)	Relative %	Compounds
1	11.00	8.55	Lauric acid n-$C_{12(0)}$
2	13.98	1.19	Myristic acid n-$C_{14(0)}$
3	15.55	50.48	Palmitic acid, n-$C_{16(0)}$
4	18.87	3.58	Stearic acid, n-$C_{18(0)}$
5	19.41	5.34	Oleic acid, n-$C_{18(1)}$
6	20.45	13.69	Linoleic acid, n-$C_{18(2)}$
7	22.06	5.64	Linolenic acid, n-$C_{18(3)}$
8	24.51	5.49	Arachidonic acid, n-$C_{20(4)}$
9	27.07	5.99	Lignoceric acid, n-$C_{24(0)}$

Quantitative determination of phenolic compounds

The present study was performed in order to estimate qualitatively and quantitatively the flavonoid and other phenolic contents of *P. dactylifera* pollen grains (80 % MeOH) cultivated in Egypt by high performance liquid chromatography (HPLC), using the available standards was carried out.

The results in Table 3 (Figure 1), showed that the main flavonoids were apigenin 6-*C*- glucose 8-*C*-rhamnose followed by luteolin 6-*C*- arabinose 8-*C*-glucose. The highest amount recorded using HPLC analysis of flavonoids in pollen grains extract was apigenin 6-*C*- glucose 8-*C*- rhamnose (1932.94 µg/g DW). The lowest amount recorded in pollen grains extract was kaempferol (8.09µg/g).

The main phenolic compounds in table 4 were pyrogallol, e-vanillic and ellagicacids (3888.82, 2029.97 and 1209.30 µg/g DW) respectively. The lowest amount recorded in pollen grains extract was reversterol(1.38 µg/g). Eight phenolic compounds were identified and quantified by HPLC analysis of DPP extracts from Tunisian origins (gallic acid, catechin, caffeic acid, epicatechin, vanillic acid, coumarin, quercitin and rutin) (4). Abed El-Azim *et al*., in 2015(30), stated that the methanolic extract of Egyptian date pollen revealed the presence of six phenolic compounds: caffeic acid, gallic acid, catechin, coumaric acid, chlorogenic acid, and quercetin. Abbas and Ateya, in 2011(26), isolated and identified five flavonoid components (rutin, isorhamnetin, apigenin, luteolin, and naringin) from the ethyl acetate fraction of Egyptian date palm pollen (DPP).Eight phenolic acids were recorded in *P. dactylifera* dried fruits (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, and ferulic acid) by high performance liquid chromatography (24).

Table 3. Quantification of some flavonoid compounds identified in pollen of *Phoenix dactylifera* using HPLC.

No.	Rt (min.)	Flavonoid compounds	Test results of flavonoids (µg/100 g D.W)
1	9.35	Luteo.6-arabinose 8-glucose	782.76
2	10.55	Luteo.6- glucose 8- arabinose	39.28
3	11.56	Apig. 6-arabinose 8-glactose	31.86
4	11.90	Apig.6- rhamnose 8- glucose	57.81
5	12.07	Apig. 6- glucose 8- rhamnose	1932.94
6	12.19	Luteolin	652.91
7	12.32	Luteo. 7- glucose	94.39
8	12.40	Naringenin	501.75
9	12.46	Rutin	419.81
10	12.63	Hesperidin	332.85
11	13.06	Apig. 7-O-neohespiroside	685.89
12	13.25	Apig. 7- glucose	13.93
13	13.40	Quercitrin	238.39
14	13.97	Quercetin	10.12
15	15.12	Kaemp. 3(2-p-coumaroyl) glucose	219.56
16	15.61	Hespertin	42.38
17	16.38	Kaempferol	8.09
18	16.55	Rhamentin	15.39
19	16.75	Apigenin	11.09
20	16.87	Acacetin	123.03

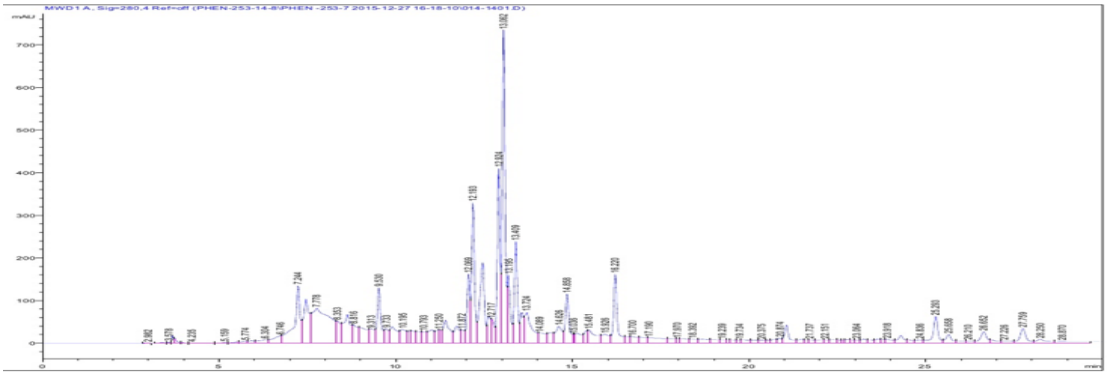

Figure 1. HPLC chromatogram of flavonoids in pollen of *Phoenix dactylifera* using HPLC.

Table 4. Quantification of some phenolic compounds identified in pollen of *Phoenix dactylifera* using HPLC**.**

No.	R_t(min.)	Phenolic compounds	Test results of Phenolic compounds (µg/100 g D.W)
1	7.16	Gallic	33.88
2	7.27	Pyrogallol	3888.82
3	7.89	4- Amino- benzoic	14.83
4	8.52	Protocatechuic	19.09
5	8.58	Catechein	214.63
6	9.14	Chlorogenic	47.99
7	9.52	Catechol	240.65
8	9.69	Epicatachin	15.84
9	9.78	Caffiene	22.68
10	10.03	P.oH.benzoic	49.48
11	10.25	Caffeic	7.49
12	10.38	Vanillic	19.67
13	11.76	P.coumaric	28.66
14	11.98	Ferulic	13.20
15	12.84	Reversterol	1.38
16	13.04	Ellagic	1209.30
17	13.19	e-vanillic	2029.97
18	13.52	α-Coumaric	17.55
19	13.66	Benzoic	405.24
20	14.00	3,4,5 methoxycinnamic	28.56
21	14.31	Coumarin	5.41
22	14.50	Salycilic	229.18
23	15.49	Cinnamic	9.10

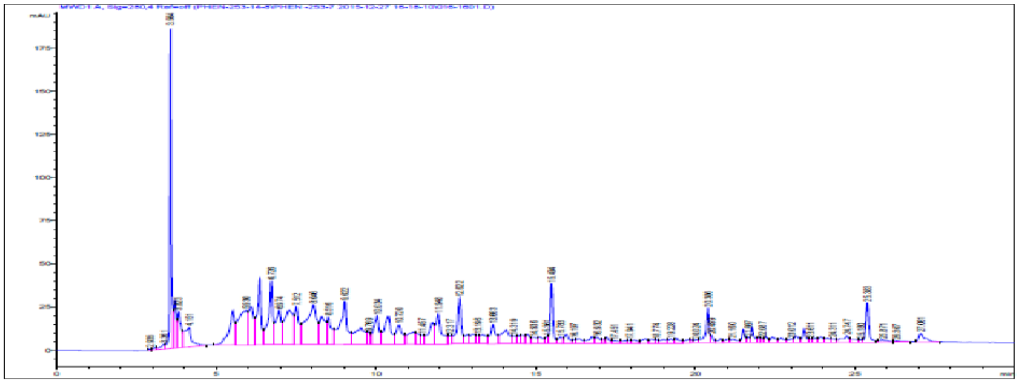

Figure 2. HPLC chromatogram of phenolic compounds in pollen of *Phoenix dactylifera* using HPLC.

Table 5. The antioxidant IC_{50} values of four extracts using DPPH assay.

Extracts	IC_{50} (µg/ml) ± SD
Methanol	32± 0.21
Butanol	42.6± 0.28
Ethyl acetate	28.9± 0.12
Hexane	72.7± 0.32
Rutin (µM)	21.7± 3.9
Vit C (µM)	39.3± 4.5

In-vitro antioxidant activity

In this study, the antioxidant activity for four extracts has been investigated as well, using DPPH assay. The results revealed that, the four investigated extracts showed a dose dependent activity. Their corresponding IC_{50}s are shown in Table 5. From these results we conclude that, all the methanolic and ethyl acetate extracts showed potent antioxidant activity compared to rutin and ascorbic acid. These results are agree with Abbas and Ateya, in 2011(26), who stated that, the ethyl acetate fraction from date palm pollen possessed a strong antioxidant activity against DPPH radicals with a value ($SC_{50} \geq 16.51$ µg/ml).

These results are in accordance with these reported by Daoud *et al*., 2015(4), who stated that, the results of the antioxidant activity of one of the Tunisian date palm pollen (Tozeur cultivar) showed that the best DPPH scavenging activity was recorded for DPP-T acetone extract (IC_{50} =46.56 ±0.28 µg/ml), and showed weak antioxidant activity for DPP-T ethanol extract (IC_{50} = 144.86• ± 0.54), with no activity being recorded for hexane and chloroform extracts. The results obtained in this study are in agreement with Ghanem *et al*., in 2015(39), who stated that, some antioxidants that play an important role in reactive oxygen species scavenging contained in palm pollen extracts which are considered rich sources of nutritive substances, polyphenols, and bioactive compounds.

Cytotoxic activity

Four different extracts (methanol, butanol, ethyl acetate and hexan extracts) were examined *in vitro* for their cytotoxic activities against RPE-1 and MCF-7 human cell lines using MTT assay. The percentage of the intact cells was measured and compared to the control (Figure 3). The obtained results showed that all extracts showed high cytotoxic activities at a concentration of 25 µM against both cell types. After the screening of the cytotoxic activities at different concentrations of each extract the IC_{50} values were calculated as indicated in Table 6.

Table 6. The cytotoxicity IC_{50} values of the four extracts using MTT assay against MCF-7 and RPE-1 human cell types.

Extracts	MCF-7	RPE-1
	IC_{50} (µg/ml)	
Methanol	22.1	21.6
Butanol	24.4	22.5
Ethyl acetate	22.9	21.6
Hexan	21.4	20.1
Doxorubicin	19.1	16.8

The results of our study exhibited high anti-tumor activity against the MCF-7 cell line compared with the results of DPP ethanol extract obtained by Ghanem *et al*., 2015 (IC_{50}=96.22 µg/ml) (39).The hexane extract exhibited the highest cytotoxic activity against RPE-1 and MCF-7 human cell lines using MTT assay (IC_{50} = 20.1µg / ml, 21.4 µg / ml respectively). These results are agree with Aydin, 2005(42), who concluded that the beneficial health effects of oils containing oleic and linoleic acids to reduce body fat and in providing many physiological benefits for humans and animals such as reducing the incidence and severity of cancer, boosting the immune system, modulate stearyl CoA desaturase. Linolenic acid, reduces cardiovascular disease and increases prostate cancer risks, and there is a close relation between large consumption and their positive effects on human health (43).

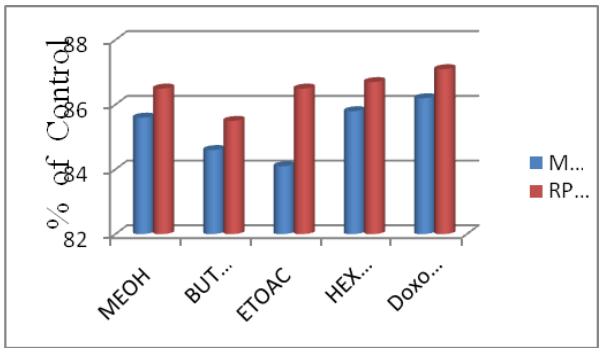

Figure 3. Cytotoxic activity of Date Palm pollen four extracts [(methanol (M), butanol (B), ethyl acetate (E) and hexane (H)] against MCF-7 and RPE-1 human cells, using MTT assay at concentration of 25 ppm.

4. Conclusion

Date pollen extracts have a promising potential to be used in nutraceutical, pharmaceutical, and medicinal products, and as a supplement for antioxidants. Date pollen extracts are rich sources for nutritive substances, polyphenols, and bioactive compounds which exhibited cytotoxicity activities

against RPE-1 and MCF-7 human cell lines compared to control.

Conflicts of interest

The authors declare no conflicts of interests.

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