

Improving the production of unsaturated fatty acid esters and flavonoids from date palm pollen and their effects as anti-breast-cancer and antiviral agents: an *in-vitro* study

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Background/aim

Pollens from different plants contain unsaturated fatty acid esters (USFAEs) and flavonoids that play a very important role as bioactive compounds. Therefore, the present study was designed to improve the production of volatile USFAEs and flavonoids from date palm pollen (DPP) in a culture of *Trichoderma koningii* and test its activities as an anti-breast-cancer and antiviral agent.

Materials and methods

The volatile esters of fermented and nonfermented date palm pollens (FDPPs) were identified using gas chromatographic–mass spectrometric (GC-MS) analysis. Antioxidant activities were determined using three different methods: the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, the ferric reducing antioxidant power assay, and the 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay. Polyphenols (phenolics and flavonoids) were also determined. Anti-breast-cancer and antiviral activities were determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results

GC-MS analysis showed an improvement in the level of USFAE in FDPP (47.99%) almost double that of the DPP results (24.11%) extract concentration. Flavonoids content of the FDPP extract (93.4 ± 6.3 mg/ml) was higher than that obtained by the DPP extract (45.4 ± 2.1 mg/ml), which was more than double the value. Antioxidant activity of the FDPP extract increased 3.16, 3.42, and 2.14 times that of the DPP extract as determined by the ABTS, ferric reducing antioxidant power (FRAP), and DPPH assays, respectively. The extract of FDPP showed strong anticancer activity against the MCF-7 cell line (IC₅₀: 9.52 µg/ml) compared with the DPP extract (IC₅₀: 96.22 µg/ml). Also, the FDPP extract had strong antiviral activity (CC₅₀: 16.5 µg/ml) compared with DPP (CC₅₀: 38.8 µg/ml). This is the first report in which the FDPP extract is used in biological studies as anti-breast-cancer and antiviral agents.

Conclusion

Fermentation of DPP by *T. koningii* improves many bioactive volatile USFAE and flavonoid contents; these have anti-breast-cancer and antiviral activity.

Keywords:

anticancer, antioxidants, antiviral, flavonoids, pollen, volatile esters

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Introduction

Cancer is a major health problem and one of the main causes of mortality worldwide. At present, breast cancer is the most common cancer in women worldwide. Currently, breast cancer (23% of all new cancer cases) is the second leading cause of death in women worldwide including Egypt as well. Despite the progress achieved in the use of anticancer drugs in the treatment of malignant diseases, they are frequently associated with unfavorable side effects. Recently, many natural products from Egyptian plants have been reported to have anticancer activity [1,2]. The therapeutic effects of dietary fatty acids on cancer cell progression have been verified by both in-vitro and in-vivo studies [3]. Saturated fatty acids are nonessential fatty acids and are harmful if excessively

ingested in food. However, polyunsaturated fatty acids (PUFAs) are designated as 'essential' for good health as their metabolic precursors cannot be synthesized in the body and must be ingested by food intake. PUFAs have important effects on the structure and physical properties of localized membrane domains. They modulate enzyme activities, carriers and membrane receptors (low-density lipoprotein receptors, insulin, antibodies neurotransmitters, drugs receptors, etc.) [4]. Some esters of fatty acids

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show antitumor activity against Ehrlich ascites tumor in mice [5]. Flavonoids are naturally occurring polyphenolic compounds with low molecular weight. These compounds are ubiquitous, found in almost all plant kingdoms. In humans, the vesicular stomatitis virus (VSV) causes an acute influenza-like illness with symptoms such as fever, muscle aches, headache, malaise, and blisters in the mouth [6]. Flavonoids were reported to have antiviral, antioxidant, antiproliferative, antitumor, anti-inflammatory, antiallergic, and anticarcinogenic properties that have been proven through in-vitro and in-vivo studies. Flavonoid consumption through plant-derived foods and from medicinal plants has beneficial health effects in humans [7–9]. Pollens are the male reproductive cells of flowers. Most noncultivated plants produce pollens. The early Egyptians and ancient Chinese used pollen as a rejuvenating medicinal agent. Date palm (*Phoenix dactylifera* L.) pollens are commonly used in the Middle East, especially in Egypt [10]. Phytochemicals screening showed that dried date palm pollens (DPPs) contain sterols, triterpenes, saponins, and glycosides [11]. A significant change in bioactive volatile and nonvolatile components of the fermentation process by *Trichoderma* spp. has been reported recently [12].

The present study was designed to improve the production of unsaturated fatty acid ester (USFAE) and flavonoids from DPP in a culture of *Trichoderma koningii* as well as to examine DPP and fermented date palm pollen (FDPP) extracts as anti-breast-cancer and antiviral agents (VSV).

Materials and methods

Raw materials and chemicals

Date palm pollen

Egyptian dry DPP (*P. dactylifera* L.) was obtained from the Department of Medicinal and Aromatic Plants, Ministry of Agriculture (Cairo, Egypt).

Microorganisms and culture conditions

T. koningii was obtained from Microbial Chemistry Lab., National Research Center (Dokki, Cairo, Egypt) and maintained on potato dextrose agar slants at 30°C for 72 h. The spore suspensions were prepared by adding 10 ml of sterilized water to slant cultures and the surface was gently rubbed with a sterilized wire loop. The fermentation was carried out in 250 ml Erlenmeyer flasks containing 5 g of DPP moistened to 50% (v/w) with distilled water. One milliliter of spore suspension (10⁶ spores) was used as an inoculum. The cultures were incubated at 30°C for 3 days by solid-state fermentation.

Date palm pollen extraction

Fatty acid alkyl esters were obtained by the trans-esterification of triglycerides (in most cases, vegetable oils and animal fats) with methanol or ethanol [13]. Volatile (esters) and nonvolatile (phenolic and flavonoid) compounds were extracted from DPP grain (DPP and FDPP) samples with ethanol. Ethanol is a better extracting solvent compared with methanol [14]. The extraction was performed on the basis of the modification reported by Rajeswari *et al.* [15]. Five grams of each of DPP and FDPP was weighed into a 250 ml conical flask and extracted using 150 ml 95% ethanol. The flask was shaken every hour for the first 6 h, then kept aside and shaken again after 24 h. The solvent layer was separated from the solid residue by centrifuging at 2000g for 10 min. The clear supernatant was transferred to a clean rounded flask and evaporated using a vacuum evaporator at less than 50°C. The extract was concentrated to 2 ml and stored at –20°C until further use.

Gas chromatographic–mass spectrometric analysis

About 2 µl of each extract was used. The analysis was carried out using a coupled gas chromatography (Hewlett-Packard Model 5890; Hewlett-Packard, Perkin Elmer Co., USA)/mass spectrometry (Hewlett-Packard-MS 5970; Hewlett-Packard). The ionization voltage was 70 eV and the mass range was *m/z* 39–400 amu. The oven temperature was maintained initially at 50°C for 5 min and then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas at a flow rate of 1.1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology). Quantitative determination was carried out on the basis of peak area integration. Interpretation on mass spectrum gas chromatographic–mass spectrometric (GC–MS) was carried out using the database of the NIST library.

Determination of phenolic content

The phenolic content was determined according to the Folin–Ciocalteu procedure. It was determined by means of a calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalent per milliliter of sample [16].

Determination of flavonoid content

The total flavonoid content was determined as reported by Thaipong *et al.* [16]. It was expressed as milligram of catechin equivalent per milliliter of sample [16].

Determination of antioxidant activity

DPPH assays

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay was used as reported by Thaipong *et al.* [17]. The antioxidant activity was determined by a calibration curve prepared with ascorbic acid and expressed as milligram of ascorbic acid equivalent per milliliters of sample [17].

ABTS assay

For the 2,2-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assay, the procedure followed the method of Arnao *et al.* [18]. Results are expressed in millimoles per liter Trolox equivalents per milliliter extract. Additional dilution was needed if the ABTS value measured was beyond the linear range of the standard curve [18].

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power assay was performed according to the Benzie and Strain's [19] method. Results are expressed in millimoles per liter Trolox equivalents per milliliter extract. Additional dilution was needed if the ferric reducing antioxidant power value measured was beyond the linear range of the standard curve [19].

Anticancer activity

Cell cultures and treatments

The human breast cancer cell line (MCF-7) was obtained from the American Type Culture Collection (Rockville, Maryland, USA). Cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% MEM nonessential amino acid solution, and 1% penicillin streptomycin solution (10 000 U of penicillin and 10 mg of streptomycin in 0.9% NaCl) in a humidified atmosphere of 5% CO₂ and 95% air at 35°C. The passage number range for both cell lines was maintained between 20 and 25. The cells were cultured in 75 cm² cell culture flasks. For experimental purposes, cells were cultured in 96-well plates (0.2 ml of cell solution/well). The optimum cell concentration as determined by the growth profile of the cell line was 2×10^5 cells/ml (cells were allowed to attach for 24 h before treatment with tested extracts). The stock solution was filtered with Minisart filters Merck (Darmstadt, Germany) (0.22 µm). The cells were exposed to 11 dilutions (0.16, 0.32, 0.64, 1.28, 2.56, 5.12, 10.24, 20.48, 40.96, 81.92, and 163.84 µg/ml) of test extracts. Cell monolayers were washed with PBS and the additional serially diluted materials were dispensed to the precultured plates to determine the toxicity of test materials [20].

MTT assay

The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay is based on the protocol described for the first time by Mossmann [21]. The assay was optimized for the cell lines used in the experiments. Briefly, for the purposes of the experiments at the end of the incubation period, cells were incubated for 4 h with 0.8 mg/ml of MTT and dissolved in serum-free medium. Washing with PBS (1 ml) was followed by the addition of DMSO (1 ml) and gentle shaking for 10 min so that complete dissolution was achieved. Aliquots (200 µl) of the resulting solutions were transferred to 96-well plates and absorbance was recorded at 560 nm using the microplate spectrophotometer system (Spectra Max190; Molecular Devices). The concentration required for 50% inhibition of cell viability (IC₅₀) was calculated [21].

Antiviral assay

Cells and viruses

Vero and BHK-21 cells were grown in Eagle's minimal essential medium (MEM; GIBCO) containing 5% inactivated calf serum and 50 g/ml gentamicin. Maintenance medium (pH 7.5) consisted of MEM supplemented with 1.5% inactivated calf serum and gentamicin. The VSV was propagated on Vero cells. Virus stocks were plaque assayed on Vero cells [22].

Cytotoxicity assay

Vero cells grown in 96-well tissue culture plates were incubated with 12 concentrations (0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 102.4, 204.8, and 409.6 µg/ml) of each extract for 24 h at 37°C. Cell viability was measured using the MTT procedure. The cytotoxicity of each extract was expressed as the 50% cytotoxic concentration (CC₅₀), which is the concentration required to reduce cell viability to 50% of the control [22].

Statistical analysis

The results are reported as mean ± SD for at least three experiments. Statistical differences were analyzed using the one-way ANOVA test.

Results

Gas chromatographic–mass spectrometric analysis of date palm pollen and fermented date palm pollen

Extract of DPP and FDPP were analyzed by gas chromatography coupled with mass spectrometry. Twenty-three volatile compounds were identified. GC–MS analysis showed that the extract of DPP included four unsaturated fatty acids, six saturated

fatty acid esters, six USFAEs, and a small number of benzene derivatives (Table 1).

Palmitoleic and oleic acids, as monounsaturated fatty acids, comprised 2.12 and 2.73% of total volatiles. The saturated fatty acids ester (SFAE) identified in DPP included dodecanoic acid-ethyl ester, tetradecanoic acid-ethyl ester, hexadecanoic acid-methyl ester, hexadecanoic acid-ethyl ester, octadecanoic acid-ethyl ester, and docosanoic acid-ethyl ester. In general, the predominant SFAE of DPP was hexadecanoic acid-ethyl ester (35.32%) of the total volatiles. The USFAEs identified in DPP included ethyl-9-hexadecenoate, 9,12,15-octadeca trienoic acid-ethyl ester, ethyl oleate, linoleic acid-ethyl ester, 11-eicosenoic acid-methyl ester, and 9-hexadecenoic acid-9-octadecenyl ester. In general, the predominant USFAE of DPP were ethyl-9-hexadecenoate, 9,12,15-octadeca trienoic acid-ethyl ester, and ethyl oleate. Ethyl-9-hexadecenoate and ethyl oleate, as monounsaturated fatty acids, comprised 8.42 and 1.35% of the total volatiles of DPP. 9,12,15-Octadeca trienoic acid-ethyl ester, as PUFAs, comprised 9.68% of the total volatiles of DPP. However, GC-MS analysis showed that the extract of FDPP included two unsaturated fatty acids, seven saturated fatty acid esters, six USFAEs, and a small number of benzene derivatives (Table 1).

9,12-Octadecadienoic and oleic acids, as monounsaturated fatty acids, comprised 1.03 and 1.37% of the total volatiles of the FDPP extract. The SFAE identified by the GC-MS in FDPP extract included tetradecanoic acid-ethyl ester, pentadecanoic acid-ethyl ester, pentadecanoic acid-14-methyl-methylester, hexadecanoic acid-ethyl ester, heptadecanoic acid-ethyl ester, octadecanoic acid-ethyl ester, and docosanoic acid-ethyl ester. In general, the predominant SFAE of FDPP was hexadecanoic acid-ethyl ester, comprising 36.58% of the total volatiles. The USFAE identified in FDPP included ethyl-9-hexadecenoate, 16-octadecenoic acid-methyl ester, linoleic acid-ethyl ester, 9,12,15-octadecatri-enoic acid-ethyl ester, ethyl oleate, and 6,9,12,15-docosatetraenoic acid-methyl ester. In general, the predominant USFAE of FDPP extract were ethyl-9-hexadecenoate, linoleic acid-ethyl ester, and 9,12,15-octadeca trienoic acid-ethyl ester. Ethyl-9-hexadecenoate and ethyl oleate, as monounsaturated fatty acid esters, comprised 9.83 and 2.00% of the total volatiles of the FDPP extract. Linoleic acid-ethyl ester and 9,12,15-octadeca trienoic acid-ethyl ester, as polyunsaturated fatty acid esters, comprised 21.06 and 13.93% of the total volatiles of the FDPP extract. Results showed that fermented palm pollen extract of *T. koningii* increased the level of linoleic acid-ethyl ester 4.8 times that in DPP extract;

Table 1 Gas chromatographic-mass spectrometric analysis of date palm pollen and fermented date palm pollen by *Trichoderma koningii*

Type of compounds	Identified compounds	R_t	Area%		MW	Molecular formula
			DPP	FDPP		
Saturated fatty acid esters	Dodecanoic acid-ethyl ester	35.27	0.27	—	228	$C_{14}H_{28}O_2$
	Tetradecanoic acid-ethyl ester	40.73	0.69	0.57	256	$C_{16}H_{32}O_2$
	Pentadecanoic acid-ethyl ester	43.26	—	0.59	270	$C_{17}H_{34}O_2$
	Pentadecanoic acid-14-methyl-methylester	44.02	—	0.29	270	$C_{17}H_{34}O_2$
	Hexadecanoic acid-methyl ester	44.08	0.19	—	270	$C_{17}H_{34}O_2$
	Hexadecanoic acid-ethyl ester	45.88	35.32	36.58	284	$C_{18}H_{36}O_2$
	Heptadecanoic acid-ethyl ester	48.03	—	0.83	298	$C_{19}H_{38}O_2$
	Octadecanoic acid-ethyl ester	50.29	3.02	3.42	312	$C_{20}H_{40}O_2$
	Docosanoic acid-ethyl ester	58.28	0.49	1.16	368	$C_{24}H_{48}O_2$
Unsaturated fatty acid esters	Ethyl-9-hexadecenoate	45.28	8.42	9.83	282	$C_{18}H_{34}O_2$
	16-Octadecenoic acid-methyl ester	48.21	—	0.87	296	$C_{19}H_{36}O_2$
	9,12,15-Octadeca trienoic acid, ethyl ester	49.61	9.68	13.93	306	$C_{20}H_{34}O_2$
	Linoleic acid-ethyl ester	49.77	4.39	21.06	308	$C_{20}H_{36}O_2$
	Ethyl oleate	49.96	1.35	2.00	310	$C_{20}H_{38}O_2$
	11-Eicosenoic acid-methyl ester	50.91	0.16	—	324	$C_{21}H_{40}O_2$
	6,9,12,15-Docosatetraenoic acid-methyl ester	53.04	—	0.30	346	$C_{23}H_{38}O_2$
	9-Hexadecenoic acid-9-octadecenyl ester	56.44	0.11	—	504	$C_{34}H_{64}O_2$
	14-Pentadecenoic acid	42.51	0.21	—	240	$C_{15}H_{28}O_2$
Unsaturated fatty acid	Palmitoleic acid	44.81	2.12	—	254	$C_{16}H_{30}O_2$
	9,12-Octadecadienoic acid	49.30	0.85	1.03	280	$C_{18}H_{32}O_2$
	Oleic acid	49.49	2.73	1.37	282	$C_{18}H_{34}O_2$
	Butylated hydroxyl toluene	33.03	0.78	2.11	220	$C_{15}H_{24}O$
Benzene derivatives	1-(4'-Methoxy phenyl)pyrene	49.69	13.26	—	308	$C_{23}H_{16}O$

DPP, date palm pollen; FDPP, fermented date palm pollen; MW, molecular weight.

it was 4.39% of the total volatile esters of DPP and increased to 21.06% in FDPP. In general, the level of total USFAEs in FDPP (47.99%) is almost twice that in DPP (24.11%) as shown in Fig. 1.

Polyphenols (phenolics and flavonoids)

The results indicated that the flavonoid content of the FDPP extract was 93.4 ± 6.3 mg/ml. This value was higher than the value of 45.4 ± 2.1 mg/ml obtained by the DPP extract. It was observed that the levels of phenolic compounds of DPP and FDPP were 38.1 ± 1.0 and 39.3 ± 1.4 mg/ml (Table 2).

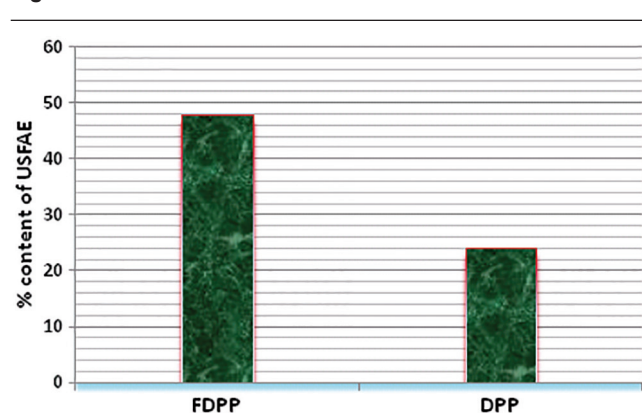
Antioxidant activity

Table 3 shows that the means of the antioxidant activity levels in FDPP were 1.664 ± 0.03 and 1.656 ± 0.02 mmol/l Trolox equivalent/ml extract as determined by ABTS and FRAB assays and 0.240 ± 0.01 mg ascorbic acid equivalent/ml extract by DPPH assay, whereas the means of the antioxidant activity levels in DPP were 0.525 ± 0.02 and 0.484 ± 0.01 mmol/l Trolox equivalent/ml extract by ABTS and FRAB assays and 0.112 ± 0.00 mg ascorbic acid equivalent/ml extract by the DPPH assay.

Anticancer activity

The data obtained in Fig. 2 show the cytotoxic effect of DPP and FDPP extracts against the MCF-7 breast cancer cell line. The cytotoxicity of each extract was expressed as the 50% cytotoxic concentration (IC_{50}), which is the concentration required to reduce cell viability to 50% of the control. Both DPP and FDPP extracts showed powerful activity as anti-breast-cancer agents. The FDPP extract showed strong anticancer activity against MCF-7 cells (IC_{50} : $9.52 \mu\text{g/ml}$) compared with the DPP extract (IC_{50} : $96.22 \mu\text{g/ml}$) (Fig. 2).

Figure 1



Total percentage of unsaturated fatty acid esters (USFAE) in date palm pollen (DPP) and fermented date palm pollen (FDPP).

Antivirus activity

The data obtained in Fig. 3 show the cytotoxic effect of both DPP and FDPP extracts against VSV. Figure 3 represents the CC_{50} values, which are expressed in $\mu\text{g/ml}$. The FDPP extract showed strong antiviral activity (CC_{50} : $16.5 \mu\text{g/ml}$) compared with the DPP extract (CC_{50} : $38.8 \mu\text{g/ml}$).

Discussion

In recent years, the use of microbial lipids, which can be obtained by fermenting non-food feedstocks,

Table 2 Phenolic and flavonoid content of date palm pollen and fermented date palm pollen

Extracts	Phenolic ^c		Flavonoids ^d	
	mg/ml extract	mg/g pollen	mg/ml extract	mg/g pollen
DPP	38.1 ± 1.0^a	53.4 ± 1.9^a	45.4 ± 2.1^a	63.5 ± 4.1^a
FDPP	39.3 ± 1.4^a	55.1 ± 2.0^a	93.4 ± 6.3^b	130.7 ± 10.2^b

All data are presented as mean $\pm$ SD; DPP, date palm pollen; FDPP, fermented date palm pollen; ^cResults expressed as milligram of gallic acid equivalent per milliliters of extract and per gram dry sample; ^dResults expressed as milligram of catechin equivalent per milliliter of extract and per gram dry sample; Values in the same column with different superscripts are significant at $P < 0.05$.

Table 3 Antioxidant activity of date palm pollen and fermented date palm pollen extracts by ABTS, FRAB, and DPPH assays

Extracts	ABTS	FRAB	DPPH
	(mmol/l TE/ml)	(mmol/l TE/ml)	(mg AAE/ml)
DPP	0.525 ± 0.02^a	0.484 ± 0.01^a	0.112 ± 0.001^a
FDPP	1.664 ± 0.03^b	1.656 ± 0.02^b	0.240 ± 0.01^b

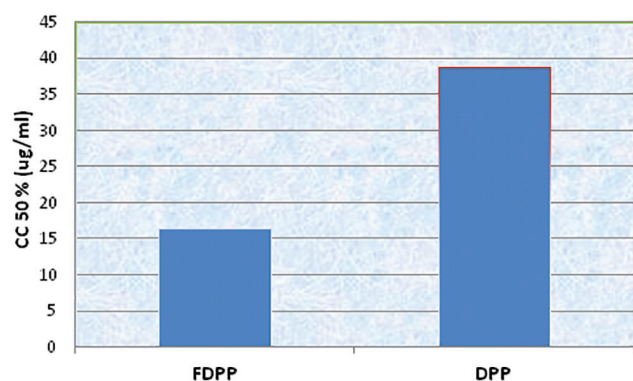
All data are presented as mean $\pm$ SD; AAE, ascorbic acid equivalent; ABTS, 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid); DPP, date palm pollen; DPPH, 2,2'-diphenyl-1-picrylhydrazyl; FDPP, fermented date palm pollen; TE, Trolox equivalent; Values in the same column with the different superscripts are significantly at ($P < 0.05$).

Figure 2



Cytotoxicity (IC_{50}) of date palm pollen (DPP) and fermented date palm pollen (FDPP) extracts against MCF-7 breast cancer cell lines.

Figure 3



Cytotoxicity (CC₅₀) of date palm pollen (DPP) and fermented date palm pollen (FDPP) extracts against vesicular stomatitis virus.

has attracted increasing attention [13]. A number of oleaginous microorganisms such as microalgae [23], yeasts [24], and fungi [25] have been tested for their capability for lipid accumulation. In addition to variations in lipid content, pollen also varies in the relative proportions of fatty acids as well as in their diversity [26]. Our results were agreement with those of Boatose *et al.* [26], who reported that oleic and palmitoleic acids were identified as unsaturated fatty acids in palm pollen, whereas Hazem [27] reported that the predominant fatty acids of palm pollen grains were palmitic, linoleic, and myristic acid. Al-Shahib and Marshall [28] showed that the major fatty acid composition in date pollen extracts may differ within the varieties and different climatic conditions of growing. Monounsaturated fatty acids and PUFAs contribute toward the characteristics of the aroma [29].

Our GC–MS results showed that palmitoleic and oleic acids, the main monounsaturated fatty acids, in the DPP extract showed a good flavor as reported by many authors [30–32].

Volatile esters are responsible for the fruity nature of fermented extracts and thus constitute a vital group of aromatic compounds. Many fermentation parameters are known to affect volatile ester production, especially ethyl esters, where ethyl esters were the predominant esters in DPP and FDPP extracts, as shown in our results. Analysis of the expression of the ethyl ester biosynthesis genes *EEB1* and *EHT1* after the addition of medium-chain fatty acid precursors suggested that the expression level is not the limiting factor for ethyl ester production, as opposed to acetate ester production. Together with the previous demonstration that the addition of medium-chain fatty acids, which are the substrates for ethyl ester formation, to the fermentation medium causes an increase in the formation of the corresponding ethyl esters, this result further supports

the hypothesis that precursor availability plays an important role in ethyl ester production [33].

Aroma-active esters are formed intracellularly by fermenting yeast cells. As they are lipid soluble, ethyl esters can diffuse through the cellular membrane into the fermenting medium. Unlike acetate ester excretion, which is rapid and complete, the transfer of ethyl esters to the fermenting medium decreases markedly with increasing chain length. The rate of ethyl ester formation is dependent on three factors: the concentrations of the two cosubstrates (the acyl coenzyme A component and ethanol) and the activity of the enzymes involved in their synthesis and hydrolysis. Hence, all parameters that influence substrate concentrations or enzyme activity may affect ethyl ester production [34]. In strain selection, specific attention to the levels of expression of other genes (e.g. fatty acid synthesis genes) appears to be more relevant than the levels of expression of the *EEB1* and *EHT1* genes for the final ethyl ester levels [35].

Many phytochemical studies of DPP report results that are in agreement with our results, which indicated the presence of many flavonoids compounds in DPP extracts [36–38]. This experiment was designed to improve the production of flavonoids from DPP by culture of *T. koningii*. The present results showed an improvement in the flavonoid content in FDPP (93.4 ± 6.3 mg/ml); it was more than twice that of the DPP results (45.4 ± 2.1 mg/ml). Considerable effort has been directed toward elucidating the flavonoid biosynthetic pathway from a molecular genetics point of view by identifying and analyzing genes or cDNAs for flavonoid biosynthetic enzymes and regulatory factors. Moreover, analysis of enzyme structures and functions involved in flavonoid biosynthesis, generation of transgenic plants, development of protein engineering tools, and heterologous production of flavonoids in microbial systems has led to considerable progress in the characterization of the flavonoid biosynthesis pathway. This pathway produces a large number of secondary metabolites, such as lignins, stilbenes, phenolic acids, and other polyphenols [36].

Pollen may be considered an effective natural and functional dietary food supplement because of its remarkable content of polyphenol substances and significant radical scavenging capacity, in addition to their nutritional–physiological implications and their health-promoting effect [39]. Antioxidants are chemicals that interact and deactivate the free radicals, therefore preventing them from causing harm [40]. Studies indicated that the aqueous extracts of dates have potent antioxidant activity [41]. The antioxidant activity is attributed to the wide range of phenolic

compounds in dates including p-coumaric, ferulic, sinapic acids, flavonoids, and procyanidins [42].

Abdulaziz [43] indicated that the Egyptian dates were rich in phenolic compounds and flavonoids with high antioxidant activity. The excellent antioxidant activity can be attributed not only to the presence of the polyphenols but also that of volatile constituents. These components could transform free radicals such as DPPH into nonradical DPPH-H [44,45].

This experiment was designed to evaluate the role of DPP as an antioxidant *in vitro* using three different methods: ABTS, FRAB, and DPPH. Data indicated that the FDPP extract showed stronger antioxidant activity than the DPP extract. In general, data showed that the fermentation process of date palm pollen (FDPP) by *T. koningii* increased the antioxidant activity levels 3.16, 3.42, and 2.14 times that of nonfermented (row) DPP as determined by ABTS, FRAB, and DPPH assays, respectively. This may be attributed to the high levels of unsaturated fatty acids and polyphenol content as well as the presence of butylated hydroxyl toluene, a strong natural antioxidant, which represents 2.11% of the total volatiles in FDPP extract (Table 1).

Our results are in agreement with those of many previous studies on date palm pollen. Rahmani *et al.* [46] reported that palm pollen extracts contained some antioxidants that play an important role in reactive oxygen species scavenging. Al-Farsi and Lee [47] reported that date extracts have the potential to be used as a supplement for antioxidants in nutraceutical, pharmaceutical, and medicinal products. Date extracts are rich sources for nutritive substances, polyphenols, and bioactive compounds. Date extracts have been shown to impair the cytotoxicity of azoxymethane-induced cancer in colonic tissue in rats [48].

It is important to explore the extraction of health-promoting bioactive compounds, such as antimicrobial, antioxidant, anti-inflammation, and anticancer activities.

Pollens from different plants were reported to have antibacterial [49], antifungal [50], antioxidant [51], and anti-inflammatory activity [52]. To our knowledge, no data have been published on FDPP and its activity against breast cancer. We designed the current work to study whether DPP and FDPP extracts show cytotoxicity in MCF-7 (human breast cancer cell lines). In general, the results indicated that both DPP and FDPP extracts have anti-breast-cancer activity. The results indicated a significant difference between DPP and FDPP extracts ($P < 0.05$). Increasing the dose decreased the cell viability significantly. This bioactivity may be attributed to the presence of polyphenolic compounds (phenolics

and flavonoids). Flavonoids are known for their immunomodulatory and anti-inflammatory activities and inhibition of proinflammatory cytokine production and their receptors [53]. They also inhibit histamine release from different tissues [54] and downregulate transcription factor NF- κ B/I κ B involved in immune and inflammatory responses [55]. Metwaly *et al.* [56] reported that palm pollen has an anti-inflammatory effect. Different studies have reported that USFAEs can modulate the activity of the components of intracellular signaling [57–59].

Particular attention has been paid to the role of Ca^{2+} entry through store-operated channels [60]. Thus, store-operated Ca^{2+} entry (SOCE) has been involved in cell signaling that occurs in nonexcitable cells to induce different cell processes including gene regulation and cell growth [61]. It has been observed recently that the addition of PUFA to cells cultured *in vitro* modifies the extent of SOCE [62]. Thus, oleic acid can inhibit SOCE in Ehrlich tumor cells, possibly because they intercalate into the plasma membrane and directly affect the activity of the channels involved [62].

In addition, a SOCE-inhibitory effect induced by oleic acid in a colon adenocarcinoma cell line has been reported recently [63]. Studies on the activity of other esters by Kato *et al.* [64] showed that esters of fatty acids and the propylene glycol ester of myristic acid were the most effective against the tumor. You *et al.* [65] showed that USFAEs of 4'-methyl-deoxypodophyllotoxin increased in-vivo antitumor activity. David [66] showed that the protection of olive oil against breast cancer may be because of oleic acid components.

The following study aims to investigate the antiviral potential of DPP and FDPP extracts against the VSV. Because of the alarming emergence of resistance to antiviral drugs, there is a need to identify new naturally occurring antiviral molecules [6].

We tested the FDPP extract for antiviral properties. The MTT assay is probably one of the most widely used colorimetric indicators of cell viability, and it assesses mitochondrial cellular function on the basis of the enzymatic reduction of the tetrazolium salt by the mitochondrial dehydrogenases in viable cells [21].

In this study, the cytotoxicity of the DPP and FDPP extracts was evaluated by this assay. Our results showed that FDPP is rich in polyphenols and alkyl esters. Tillekeratne *et al.* [67] have reported that alkyl esters have antiviral activity. Some studies have shown the effectiveness of plant extracts as in-vitro inactivators of several enteric viruses; this was attributed to phenolic compounds. Several commercially available phenolic

compounds of plant origin were effective; these natural antiviral agents seem to depend on the stability of these complexes, such as tannins and related compounds, during passage through the digestion system [68]. This is the first report in which fermented palm pollen extracts have been used in biological studies as anti-breast-cancer and antiviral agents.

In conclusion, fermentation of Egyptian DPP by *T. koningii* improves many bioactive volatile USFAEs and flavonoid contents. FDPP extract has strong antioxidant activity and anti-breast-cancer activity. It has also antiviral activity. Further investigations are needed to be applied as additives, in food and pharmaceutical industries.

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Conflicts of interest

There are no conflicts of interest.

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