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Hydrocarbons, fatty acids and biological activity of date palm pollen (*phoenix dactylifera* L.) growing in Egypt

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Abstract*: In this study, the non-polar light petroleum ether and diethyl ether extracts of date palm (Phoenix dactylifera L.) Pollen were analyzed by Gas chromatography–mass spectroscopy (GC-MS) and found to be rich with lipids and steroids. Fatty acids include Palmitic acid 38.52% and Hydnocarpic acid 44.23% while α-Sitosterol was the most prevalent Phytosterol 14.40%. Un-saponifiable fraction and saponifiable fraction of Pet. ether and DEE extracts were screened for their in vitro antimicrobial activities against six bacterial species (Escherichia coli, Klebsiella species, Staphylococcus epidermidis, Bacillus cereus, Micrococcus luteus and Staphylococcus aureus). All fractions have good antibacterial activity against the six studied bacterial species. But it gave high antifungal activity as compared with Ketoconazole (standard) except with Un-sap. fraction of Pet. ether extract with Candida albicans and Aspergillus niger it gave antifungal activity with Un-sap. fraction of Pet. ether extract only. Finally two extracts were tested against three human cell lines HELA, MCF7 and CACO. The results showed those extracts had activity against all cell lines tested.*

Keywords: *Phoenix dactylifera L., GC-MS, fatty acid, antibacterial, antifungal and cytotoxic activity.*

I. Introduction

Phoenix dactylifera L. commonly known as the date palmis an important plant in the scorched regions of South west Asia and North Africa. The fruits which are the most commonly used part are an important source of nutrition, especially in the arid regions where due to the extreme conditions, very few plants can grow. In conversation allanguages dates are known as Sugar Palm (English), Nakhal (Arabic), Khajur (Hindi and Urdu), Karchuram (Tamil, Malayalam) and Karjura (Kannada**)** [1].

Date palm pollen is the Angiosperms-Monocotyledones; Palmaceae is a family of about 200 genera and 1,500 species. Phoenix (*Coryphoideae Phoeniceae*) is one of the genera which contain a dozen species, all native to the tropical or subtropical regions of Africa or Southern Asia; including *Phoenix dactylifera* L. [2]**.**

In recent years, it has been suggested that estrogen, may be involved in the regulating the renewal of spermatogonial stem cells [3]. Investigations have revealed that date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility. Reports have also pointed that isolation of micro elements from DPP has estrogen, sterols and other agents that may influence male fertility [4]**.** With regard to these components, snack foods have been supplemented with date pollen to improve male infertility [5]. The results were showed that the values of the proximate chemical composition of palm pollen grains were moisture (28.80%), ash (4.57%), crude fiber (1.37%), crude fat (20.74%), crude protein (31.11%) and carbohydrate (13.41%). Palm pollen grains contained a logical amount of vitamins A, E and C. They are a good source of minerals such as B, Zn, Se, Fe, Mo, Cu, Mn, Co and Ni. Leucine and lysine (3.34 and 2.95 g/100 g dry weight, respectively) were the major essential amino acid constituents. So, palm pollen grains are a good economic nutritional source can be used as human food supplements [6]. Due to the lack of the previous studiesand in continuation of our studies [7-9], we report here the chemical constituents of both extracts of light petroleum ether and diethyl ether; and also study some of the biological activities.

II. Materials And Methods

2.1. Collection of plant material:

Date Palm pollen was collected in March (2012) from Sharkyia Governorate, Egypt. Some of palm pollen (*Phoenix dactylifera* L.) was collected and kept in a refrigerator at 4°C and were identified by botany department, Faculty of Science, Zagazig University.

2.2. Material for antimicrobials:

The bacterial and fungal strains were personally obtained from the Microbiology Lab., Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were (*Escherichia coli, Klebsiella*

species, Staphylococcus epidermidis, Bacillus cereus, Micrococcus luteus and *Staphylococcus aureus*). And fungal species were (*Candida albicans* and *Aspergillus niger*) [10].

2.3. Materials for cytotoxic activity:

Human tumor cell lines: [HELA (Cervical carcinoma cell line), MCF7 (breast carcinoma cell line) and CACO (intestinal carcinoma cell line)] [11].

2.4. Methods:

2.4.1. Preparation of extracts:

The yellowish powder pollen was extracted exhaustively with Pet. ether and DEE. These were obtained: the non-polar (NP)–Pet. ether (60-80°C) extracts (14.2 grams) and DEE (30-45°C) extract (8.22 grams). For each extraction the powder was left 24 hours with the solvent in a Soxhlet apparatus. The extracts were collected after filtering through filter paper and concentrated on a Rota-vapor at each temperature. After that hydrolyses with alc. KOH (10%) for 6 hours (Both pet. ether and DEE extracts) over water bath under reflux. Then dilution with water followed by ether afforded the ether part (the un-saponifiable fraction) [12].

2.4.2. Liebermann–Burckhardt or acetic anhydride test:

It is used for the detection of cholesterol. The formation of a green or green-blue colour after a few minutes is positive [13]. Dissolve 1 or 2 crystals of cholesterol in dry chloroform in a dry test tube. Add several drops of acetic anhydride and then 2 drops of conc. H_2SO_4 and mix carefully. The formation of a green or green-blue colour after a few minutes is positive cholesterol.

2.4.3. GC-MS (Gas Chromatography/Mass Spectrometry) analysis:

The analytical GC-MS analyses were performed in two different equipment's: (a) Hewlett Packard 5973–6890 system, operating on EI mode and equipped with a HP 5 MS 30 m × 0.25 mm × 0.25 μ film thickness capillary columns. The carrier gas was Helium (flow rate = 1 mL/min). Temperature program: initial column temperature 60°C (for 5 min.), was raised to 280°C within 3°C/min; and held there for 15 min. The injector and detector temperatures were 220 and 280°C, respectively, (b) Finnegan trace GC ultra-system operating on EI mode and equipped with AT™ Aqua wax 30 m × 0, 32 mm × 0.25 μm film thickness capillary column. The carrier gas was Helium (flow rate = 1.5 ml /min, constant flow) and Split ratio, 1:10. Temperature program: initial column temperature 60°C (for 5 min.), then was raised to 235°C within 3°C/min and held there for 30 min (injector temperature 290°C, detector temperature 300°C). MS details (for both organs): ionization energy = 70 eV; emission = 200 μÅ; mass range = 35–650 Da; scan time = 1.25 s; scan rate (amu/s) = 500.0, scans/s = 0.7974.

All compounds were identified by comparison of their retention times ($\mathbf{R_t}$) and mass spectra with those of authentic samples and/or mainlib, Wiley 9, replib, NISTD-EMO libraries spectra and through international literature [14].

2.5. Measurement of antimicrobial Activity:

2.5.1. Preparation of the tested extracts:

The different extracts were prepared at a concentration of 100 mg/ 10 ml in Dimethyl foramide (DMF) in order to obtain a final concentration of 10 mg/ 1 ml. Ciprofloxacin was used as standard drug (50 & 100 μg/ml) and dimethyl sulfoxide (DMSO) as a control.

2.5.2. Methods of antibacterial activity:

The antibacterial activity was studied by using cup plate agar diffusion method by measuring diameter of zone of inhibition in mm. The extract was tested at the concentration of 200 ppm in 5% (DMF). The solution was poured in the cup/well of bacteria seeded agar plates. The plates were incubated at 37°C for 24 hours for E. coli where the plates of other bacterial species were incubated at 27±2°C for 24 hours.

The activity was reported by measuring the diameter for zone of inhibition in mm. Ciprofloxacin was used as standard drug for antibacterial activities. Nutrient agar was employed as culture medium and Dimethyl Sulfoxide (DMSO) were used as solvent [15].

2.5.3. Methods of antifungal activity:

Antifungal activity was screened for the newly separated samples. The cup plate method was employed to study the preliminary antifungal activity of *Candida albicans* and *Aspergillu sniger*. The extract was dissolved in 5 ml of Dimethyl Sulfoxide (1000 μg/ml) volume. Ciprofloxacin was used as standard drug (50 & 100 μg/ml) and dimethyl sulfoxide as a control. The observed zone of inhibition was measured in mm [15].

2.5.4. Methods of antitumor activity:

Potential cytotoxicity of the extract was tested using the method of Skehan *et al.*; [11]. Cells were plated in 96-multiwell plate (104 cells/well) for 24 hours. The cells were allowed to attach to the wall of the plate and then treated with the extract. Different concentrations of the extract under test (50, 100, 125, 250 and 500 µg /ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extract for 48 hours at 37°C and atmosphere of 5% CO_2. After 48 hours cells were fixed, washed and stained with Sulforhodamine B strain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured by ELISA reader. The relation between surviving fraction and extract concentration is plotted to get the survival curve of each tumor cell line [14].

III. Results And Discussion

After sequential extraction of the plant with soxhlet using two of the organic solvents are Pet. ether and DEE. The preliminary phytochemical study revealed those extracts of date palm pollen hydrocarbons and fatty acids. All fractions obtained were subjected to GC/MS to identify the chemical constituents of it.

3.1. The Un–saponifiable fraction of the light-petroleum extract of date palm pollen:

The Un-saponifiable fraction gave a positive (deep green color) Liebermann test. This means that cholesterol is present in the Pet. ether extract. The Un-saponifiable fraction was identified by GC/MS as shown in figure (1); to contain a series of saturated long chain hydrocarbons as shown as: 2-Ethyl hexanol, n-Nonane, n-Dodecane, 2,4-Dimethyl hexane, 2-Methyl octane, N-Pentadecane, N-Decane, N-Tridecane, 2,6-Dimethyl heptadecane, 6-Methyl octadecane, Tetradecane and contain a series of unsaturated and long chain hydrocarbons as shown as; (9Z)-1,9-Hexadecadiene, (4E)-10-Methyl-4-undecene, 1-Undecene, 1-Undecyne and contain a series of steroids (3α)-Cholest-5-en-3-ol, (3α,4α)-4-Methyl-cholesta-8,24-dien-3-ol, (23S)-Ethyl cholest-5-en-3α-ol, (24S)-24-Methyl cholest-7-ene-3α,5α,6α-triol, α-Sitosterol. This series of hydrocarbons also have not been isolated before from this plant or from any species of this family.

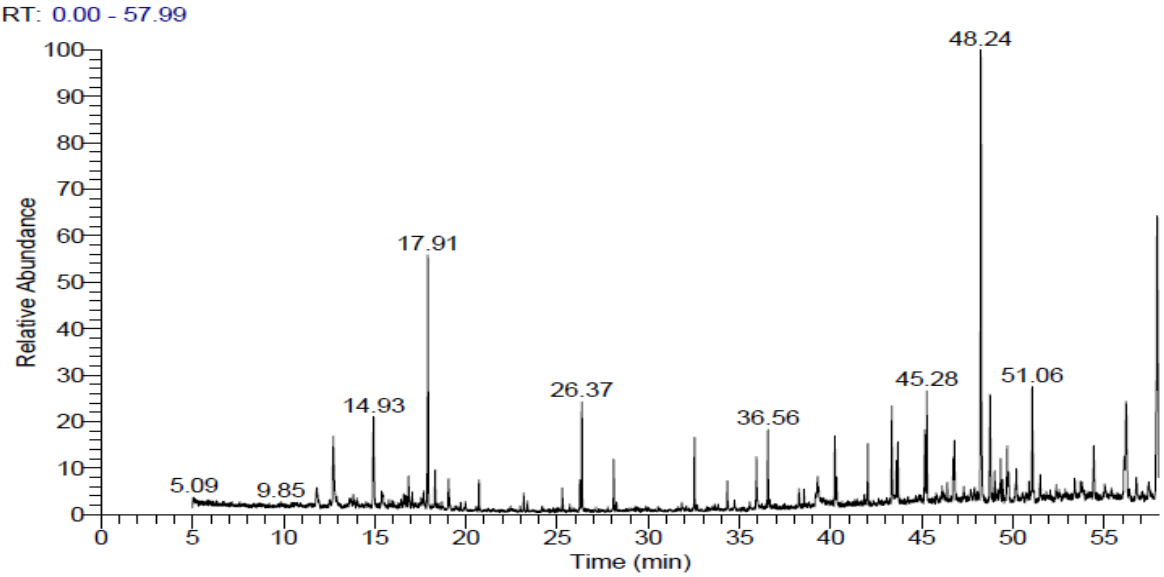

Figure 1: Gas chromatogram /Mass Spectroscopy of un-saponifiable of the light petroleum extract of the date palm (*Phoenix dactylifera* L.).

3.2. The saponifiable fraction of the light Pet. ether extract of date palm pollen:

The saponifiable fraction (potassium salt of fatty acid) was acidified by dilute HCl till acidic medium then extracted with ether and methylated with diazomethane followed by analyzed GC/Ms. This extract was found as shown in figure (2); to contain : Palmityl ester (C16:0), Hydnocarpyl ester (C16:1), Oleyl ester (C18:1), 11-Cyclopentaneundecanyl ester (C16:1), (S)-(-)-Citronellyl ester (C10:1), 9-Octadecen-12-ynyl ester (C19:3), Oxayl ester (C4:0). This series of fatty acid also have not been isolated before from this plant or from any species of this family.

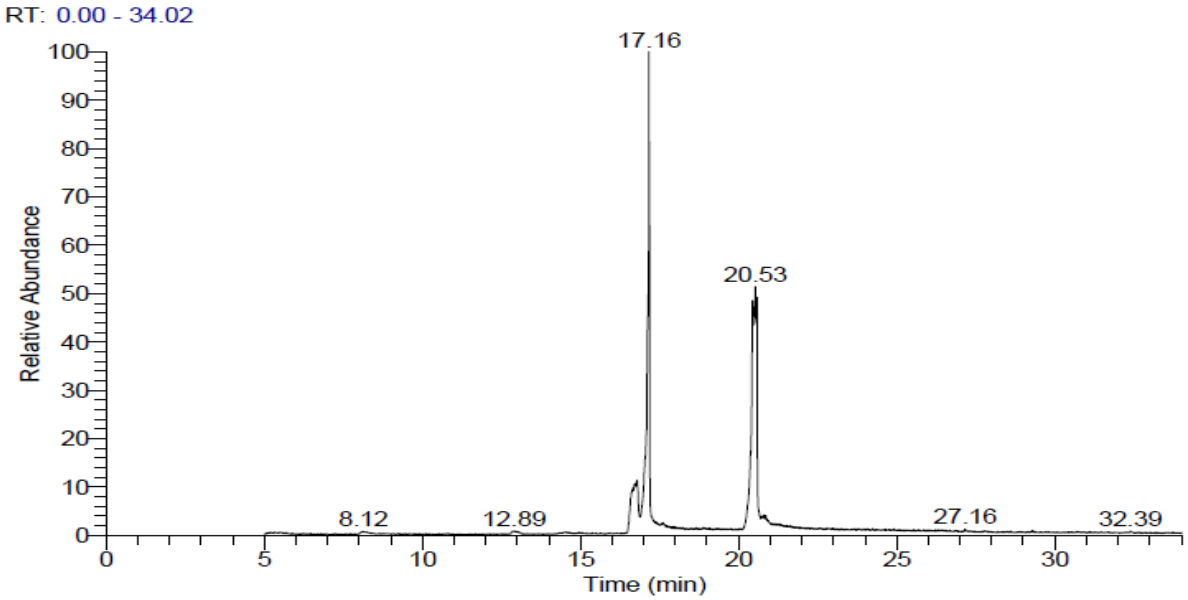

Figure 2: Gas chromatogram /Mass Spectroscopy of saponifiable of the light petroleum extract of the date palm (*Phoenix dactylifera* L.).

3.3. The Un-saponifiable fraction of the DEE extract of date palm pollen:

Also the Un-saponifiable fraction was identified by GC/MS as shown in figure (3); to contain a series of saturated long chain hydrocarbons as shown as: 1-Propyne, Hentriacontane, Heptanal, 1,7-Heptanediol, 2-Tri-fluoro acetoxy dodecane, N-Docosane, (E)-4-Oxo-2-heptenal, 2,6,10-Trimethyl tetradecane, N-Nonacosane, 3-Ethyl-5-(2'-ethyl butyl)-octadecane, n-Hexadecane, 2,6-Dimethyl heptadecane, N-Octanal. And the neutral portion was shown by GC/MS to contain a series of important compounds (5α)-Pregnane-3,20-α-diol, 5-Bromo-2-{[(6-methyl-2-pyridinyl) amino]methyl}1-hisoindole-1,3-(2H)-dione, 1,6:3,4-Dianhydro-2-deoxy-α-d-lyxo-hexopyranose, 5-Methoxy-1-aza-6-oxabicyclo(3.1.0) hexane, [2,2'-Bianthracene]-9,9',10,10'-tetrone,1,1',8,8'-tetrahydroxy-3,3'-bis(hydroxyl methyl), Diethyl [1]-Benzoselenopheno [3,2β] [1]- Benzoseleno phene-2,7-dicarboxylate and 1,3,5-Tris [(4-(S)-Phenyl-2-oxazolinyl) methyl]-2,4,6-trimethyl benzene. This series of hydrocarbons also have not been isolated before from this plant or from any species of this family.

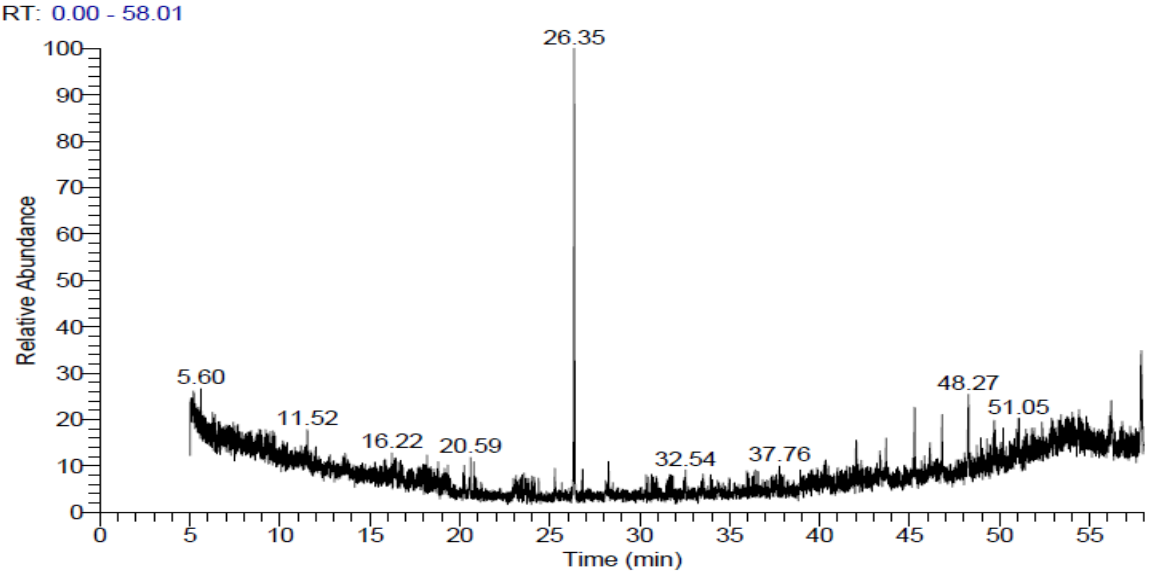

Figure 3: Gas chromatogram /Mass Spectroscopy of un-saponifiable of the diethyl ether extract of the date palm (*Phoenix dactylifera* L.).

3.4. The saponifiable fraction of the DEE extract of date palm pollen:

The saponifiable fraction (potassium salt of fatty acid) was then acidified with dilute HCl till acidic medium then extracted with ether, and methylated with diazomethane and analyzed by GC/MS, shows the presence of Heptanyl ester (C7:0), 3-Ethyl heptanyl ester (C9:0), Nonanyl ester (C9:0), Capryl ester (C10:0), Undecanyl ester (C11:0), Lauryl ester (C12:0), Palmityl ester (C16:0), 11-Cyclopentyl undecanyl ester (C16:1), Oleyl ester (C18:1), Stearolyl ester (C18:2), 11,14-Octadecadiynoyl ester (C19:2), 10-Undecynyl ester (C11:2), Hydnocarpyl ester (C16:3), Linolelaidyl ester (C19:2), Oxayl ester (C4:0). This series of fatty acid also have not been isolated before from this plant or from any species of this family, as shown in figure (4).

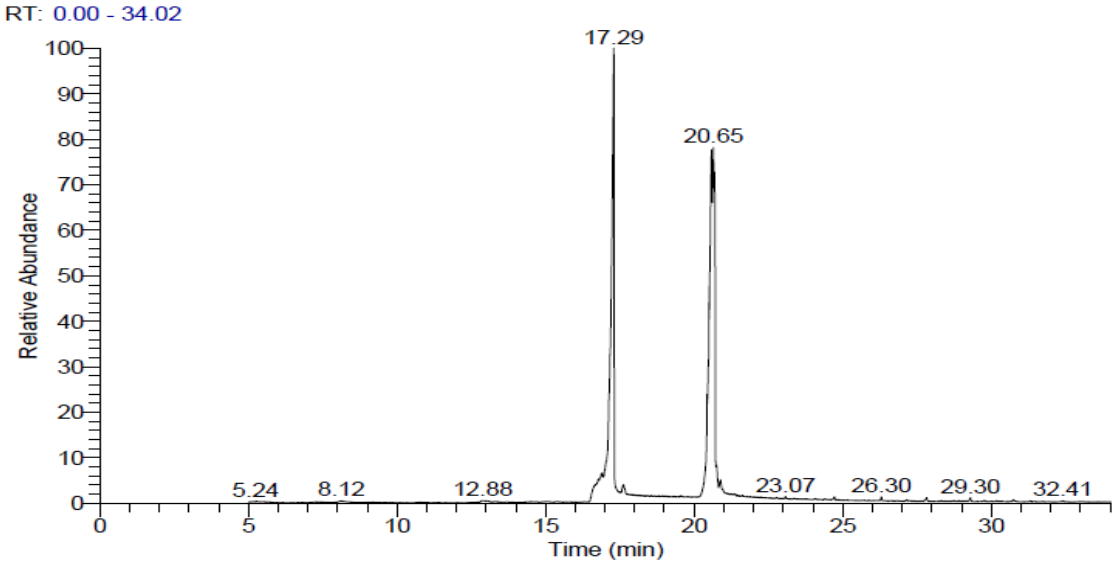

Figure 4: Gas chromatogram/Mass Spectroscopy of saponifiable of the DEE extract of the date palm (*Phoenix dactylifera* L.).

3.5. Results of biological investigation of date palm pollen:

3.5.1. Results of Antimicrobial activity:

The four fractions of (Pet. ether and DEE ether extract) showed different antimicrobial activities. All the extraction from dates palm pollen (*Phoenix dactylifera* L.) [Un-saponifiable fraction and saponifiable fraction of Pet. ether and DEE ether extracts] were screened for their in vitro antimicrobial activities against six bacterial species (*Escherichia coli*, *Klebsiella* species, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*) were measured by inhibition the zone in mm.

3.5.1.1. Results of Antibacterial activity:

It has a good inhibition zone value with all species which was near to the antibiotic value; this means that Un-saponifiable fraction and saponifiable fraction of Pet. ether and DEE ether extracts have good antibacterial activity against the six studied bacterial species, data in Table (1).

Table 1: Antibacterial activities of the extracts of dates palm pollen (*Phoenix dactylifera* L.)

Drugs	*Escherichia coli*	*Klebsiella Species*	*Staphylococcus epidermidis*	*Bacillus cereus*	*Micrococcus luteus*	*Staphylococcus aureus*
Ciprofloxacin	20	20	22	20	24	22
Un-sap. fraction of Pet. ether extract	15	18	15	18	15	20
Sap. fraction of Pet. ether extract	15	18	15	17	15	15
Un-sap fraction of DEE extract	13	-	20	15	-	12
Sap. fraction of DEE extract	15	18	-	15	20	15

3.5.1.2. Results of Antifungal activity:

The two extracts (four fractions) showed different antifungal activities against the tested fungal strains. All fractions gave high antifungal activity as compared with Ketoconazole (standard) except with Un-sap.

fraction of pet. Ether extract with *Candida albicans*, but with *Aspergillus niger* it gave antifungal activity with Un-sap. fraction of pet. ether extract only, data in Table (2).

Table 2: Antifungal Activities of the extracts of dates palm pollen (*Phoenix dactylifera* L.)

Drugs	*Candida albicans*	*Aspergillus niger*
Ketoconazole	8.25	7.25
Un-sap. fraction of Pet. ether extract	-	7.15
Sap. fraction of Pet. ether extract	7.25	-
Un-sap fraction of DEE extract	8.15	-
Sap. fraction of DEE extract	8.00	-

3.5.2. Results of Anti-tumor activity:-

The Pet. ether and DDE extracts of date palm pollen were tested against three human cell lines [HELA (Cervical carcinoma cell line), MCF7 (breast carcinoma cell line) and CACO (intestinal carcinoma cell line)]. The results showed those extracts had activity against all cell lines tested. The results of IC_{50} were listed in the following Tables (3) and (4).

Table 3: IC_{50} value of the different cell lines of the light petroleum ether extract of date palm pollen (*Phoenix dactylifera* L.)

Name of cell line	IC_{50} value
HELA	18.5 µg / ml
MCF7	18.5 µg / ml
CACO	13.03 µg / ml

Table 4: IC_{50} value of the different cell lines of the DEE extract of date palm pollen (*Phoenix dactylifera* L).

Name of cell line	IC_{50} value
HELA	18.5 µg / ml
MCF7	11.5 µg / ml
CACO	14.8 µg / ml

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