Evaluation of Antioxidant, Antibacterial Potential, Nutrition Value and Acute Toxicity Study of Libyan Date Palm Pollen (DPP)

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Abstract

Pollen of date palm - DPP (*Phoenix dactylifera* L.) belongs to the Arecaceae family and grows widely in many areas including the Middle East and North Africa. *Phoenix dactylifera* L. has nutritional, medicinal, economic and ecological benefits as it could be used as a treatment of various health diseases and disorders for its antimicrobial, anti-oxidative, anti-inflammatory, anti-toxicant, and anti-cancer properties as well as its hepato-protective activities. It is also considered as an enhancer of fertility in both males and females. This research aims to assess the phytochemical screening of the Phoenix dactylifera L cultivated in Libya, test its antioxidant and antibacterial effects, and perform acute toxicity tests. The antioxidant activities of the extracts were screened using DPPH assay. The results indicated that the water-extract of palm pollen grains showed the best DPPH scavenging activity (IC5o = 0.0005). The DPPH scavenging activities recorded for palm pollen grain methanol extract were IC5o = 0.233 mg/ml and IC5o = 0.224mg/ml at 70°C and 45°C, respectively. The methanol extracts of palm pollen grain (at concentrations of 12.5mg/ml, 25mg/ml, 50mg/ml, and 100mg/ml) were tested against 5 different strains of standard bacteria (ATCC) by cup cut method. The result showed no remarkable inhibition of bacterial growth. The phytochemical screening of date palm pollen revealed the presence phytosterols, flavonoids, coumarins, tannins, phenolic compounds, amino acids, protein, a small amount of saponin, and fats. The proximate nutritional value of palm pollen grains was also analyzed. The results obtained showed that the pollen contained 18.19% moisture, 5.41% ash, 67% crude fiber, 7.32% crude fat, and 27.36% crude protein. The acute toxicity test of methanol extract showed neither mortality amongst the graded dose groups of animals nor behavioral changes at the highest dose of 5,000 mg/kg.

Keywords: Acute toxicity test, pollen, phytochemical screening, antioxidant, antimicrobial

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Introduction

Pollen of date palm - DPP (*Phoenix dactylifera L*) has been traditionally used in Egyptian and Chinese folk medicine from ancient times and is recently used in Arab countries for the treatment of various body disorders in form of herbal preparations (Al-Samarai *et al*., 2016). The important traditional use of DPP was focused on improving male and female fertility, sexual hormonal balance (Hassan, 2011), in dry and wet cough, support stomach function, restore skin youth, improves healthy nails and hair, anti-diabetic and anti-oxidant (Ben Said *et al.*, 2017). Pollen is a strong product known for male and female

fertility treatment as a food supplement due to its contents of essential components such as amino acids, fatty acids, flavonoids, saponins, and estrole (Tahvilzadeh *et al.*, 2015). Considering prostate diseases, which are age-related diseases that can mimic prostate adenocarcinoma and inflammatory prostatic cells and cause an imbalance between prostate cell growth and apoptosis (Elberry *et al.*, 2011), found that the intake DPP acts as a remedy to prostatic diseases. Besides the treatment of infertility, the palm pollen extract was also used as anti-coccidial activity with mice earlier infected with Eimeria papillata responsible for increasing apoptosis cells in intestines (Metwaly *et al.*, 2013). DPPs give better results with cytotoxic activity when tested by (Abed El-Azim *et al.*, 2015). Moreover, DPPs are effective health treatment of mouth ulcer induced by chemotherapy in carcinogenic animals. The protective effect to date palm pollen on oral mucosa is achieved by blocking oxidative free radicals. It also prevents DNA damage. Daoud *et al*., (2015) investigated the content of date palm pollen and reported the presence of polyphenols, flavonoid contents and others molecules namely gallic acid, catechin, caffeic acid epicatechin, vanillic acid, coumarin, quercetin and rutin, proteins, ascorbate, b-carotenen, a-tocopherol and lycopene, all of which are characterised with a high antioxidant activity (Daoud *et al.*, 2015). In study conducted by (Farouk *et al.*, 2015) the twenty one oils were identified in date palm pollen grains using gas chromatography and mass spectrometry. The oils were subjected to antioxidant activity measurement and the results showed strong antioxidant properties. Basuny *et al*. (2013) estimated the antimicrobial activity of pollen of olive grain and palm date against different types of bacteria. They showed the effectiveness of pollen in the inhibition of bacteria growth and its antibacterial effect due to different phenolic content. In addition, a study conducted by (Abed El-Azim *et al.*, 2015) reported the pollen's effect against two types of fungi and its ability to inhibit some strains of bacteria zones. The nutritional value refers to the quantity and quality of nutrients present in a food item. Nutrients are the food ingredients necessary to maintain optimum metabolic functions of the body. Knowing the right nutrition helps to understand our daily calorie requirements, to feed the body with minerals, vitamins and phytonutrients, to minimise obesity and risk of developing health problems as coronary heart disease, stroke, diabetes, and cancer, and to maintain vital processes such as growth, breathing, digestion, and secretion. The Pollen preparations are used worldwide for dietary purposes as diet supplement. Aim of this study is to evaluation the nutrient evaluation of date palm pollen grains. Evaluate the acute toxicity of methanolic extract of date palm pollen grains. And assessment of antioxidant activity of methanolic and aqueous extract of date palm pollen grains. Also Assessment of antibacterial activity of methanolic extracts of date palm pollen grains on different bacteria strains at different temperature degrees.

Materials and methods

2.1. Materials

2.1.1. Plant Material

Date palm pollen was collected from Tripoli in March 2017. After the collection process, the pollen was air-dried and there after the powdered material was stored in -18 ˚C until further use. The sample was authenticated by Dr. Mohamed Abo Hadra. It carry voucher number specimen (683611) from herbarium of botany department a plant taxonomist at the Department of Botany, Faculty of science, University of Tripoli, Libya.

2.1.2. Bacterial strains

The antibacterial activity of pollen extract was assessed against five bacterial species: Gram positive, methacillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus aureus* ATCC 29213; *Klebsiella pneumonia* ATCC13883, Gram-negative *Escherichia coli* ATCC 25922, *and Pseudomonas aeruginosa* ATCC 9027.

2.1.3. Experimental animals

Nineteen female rats were obtained from the pharmacology department, Faculty of Pharmacy-University of Tripoli, Libya.

2.2. Methods

2.2.1. Plant material preparation

2.2.1.1 Collection of plants

Date palm pollen was collected at the end of March of the date fields in Tripoli, Libya. This area is known as palm varieties. A total of about 310 mg of pollen was collected.

2.2.1.2 Cleaning of plants

After the collection of the plants, saving process and collection of powder must be performed to get better results.

2.2.1.3 Drying

The main purpose of drying is to remove the water content from the powder of pollen, before the plants are stored. Drying can be done by oven at 25ºc overnight, and then it should be kept in the freezer for future analysis.

2.2.2. Extraction of plant material

The methanolic pollen extracts were subjected to phytochemical screening, antioxidant, antibacterial activity and toxicity study, were prepared by a slight modification of the methods according to (Banu and Catherin, 2015). The 90g of dried powder of Date palm pollen was divided in to two parts and extracted by using methanol (99.9%) at two temperatures; in 70 °C and 45 °C in water bath for half h then filtered. The solvent extracts were concentrated by rotary evaporator at 70, 45 °C, under reduced pressure. The extracts were weighted and stored for later use. The aqueous extract was subjected to antioxidant activity; 30g of dried powder of Date palm pollen was extracted by water

maceration for 24h, and then filtered through Filter paper, the filtered solution Freeze dryer. The dry extract and stock solutions were kept until further analysis.

$$\% \text{ Extraction yield} = \frac{\text{weight of the dry extract}}{\text{weight the sample used for extraction}} x100$$

2.2.3. Preliminary phytochemical screening

2.2.3.1. Detection of alkaloids

Few mg of each extract (Date palm pollen) was boiled in 1ml of hydrochloric acid on water bath for 5min, added two drops of mayres reagent (Potassium mercuric iodide solution), of Dragendorff's reagent (Potassium bismuth iodide solution), wagners reagent (potassium iodide and iodine) were added separately along the side of test tubes. Formation of precipitate indicate the presence of alkaloids (Pandey and Tripathi, 2014).

2.2.3.2. Detection of flavonoids

Few drops of sodium hydroxide solution 20% were added to the extract. The Formation of an intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoid (Tiwari *et al.*, 2011).

2.2.3.3. Detection of carbohydrates

2.2.3.3.1. Fehling's test

The extract were treated with 2 drops of dilute HCL in test tube, neutralized with alkali then Fehling's A & B solutions were added and heated on water bath for 10 min. The formation of a red precipitate indicates the presence of reducing sugars (Tiwari *et al.*, 2011).

2.2.3.3.2. Molisch's test

The extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and was mixed. 2 drops of concentrated sulphuric acid was added. The formation of the violet ring at the junction indicates the presence of Carbohydrates (Pandey and Tripathi, 2014).

2.2.3.4. Detection of tannins

Few mg of the extract was treated with 1% gelatin solution plus sodium chloride, the appearance of white precipitate indicates presence of tannins (Harborne, 1998).

2.2.3.5. Detection of phenols

Few mg of the extract were treated with 3 drops of ferric chloride solution. The appearance of greenish or dark blue color indicates presence phenols (Harborne, 1998).

2.2.3.6. Detection of saponins

Few mg of the extract were shaked with about 5ml of distal water for 10 minutes, foam formation indicate presence of saponin (Tiwari *et al.*, 2011).

2.2.3.7. Detection of cumarins

Two drops of the extract was dissolved in 2ml sodium hydroxide, spotted on wattmans filter paper and examine under long UV, The appearance of blue Florescence indicates the presence of cumarins.

2.2.3.8. Detection of phytosterols

2.2.3.8.1. Salkowskis test

Few mg of the extract were treated with colroform then filtrated. Few drops of concentrated sulfuric acid added to the filtrate, shaken, and allowed to stand. The formation of a golden yellow color indicates presence of triterpens (Tiwari *et al.*, 2011).

2.2.3.8.2. Libermann-Burchard's test

Few mg of the extract were treated with chloroform and filtered. The filtrates treated with few drops of acetic anhydride, boiled and cooled, conc sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols (Pandey and Tripathi, 2014).

2.2.3.9. Detection of amino acid and proteins

2.2.3.9.1. Ninhydrin test

Few mg of the extract were treated with 2 drops of ninhydrin solution. The Appearance of purple colour indicates the presence of amino acids (Harborne, 1998).

2.2.3.9.2. Xanthoproteic test

Few mg extracts were treated with few drops of concentrated Nitric acid. The appaernce of a yellow colour indicates the presence of proteins (Tiwari *et al.*, 2011).

2.2.3.10. Detection of glycosides

2.2.3.10.1. Modified Borntrager's test

A few mg of the extracts boiled with dilute sulfuric acid then filtered. The filtration cooled and added equal volumes of benzene. The benzene layer separated and treated with ammonia solution. Change the color into rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides (Tiwari *et al.*, 2011).

2.2.3.10.2. Killer killiani test

A few mg of extract treated with few ml of glacial acetic acid contaning ferric chloride, concentrated sulphuric acid was added on the wall of the tube forming a red ring at the interface and the upper layer converted to blue greenish color indicating the presence of glycosides (Ashokkumar and Ramaswamy, 2014).

2.2.3.11. Detection of Fixed oils and Fats:

2.2.3.11.1. Spot test

A few mg of extracts were pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils (Pandey and Tripathi, 2014).

2.2.3.11.2. Infra-red Spectroscopy

IR spectra of dried powder plant materials were measured by FTIR analysis. 10 mg of the dried powder was compressed in 100 mg of KBr pellet to prepare sample in capsule form discs into 80KN by using a mould and press. The thin disc of plant specimen produced was loaded in FTIR spectroscope, with a Scan range from 400 to 4000 cm with a resolution of 4 cm 1 spectrum takes about three minutes to be recorded. The reference standard was injected and recorded (Sudhira *et al.*, 2015).

2.2.4. Determination of antibacterial activity

In vitro antibacterial screening of methanolic extracts of pollen grain of date palm was carried out using cup cut diffusion method (well diffusion method). The standard bacterial strains were activated and cloned mellur hinton agar and incubating in 37 °C for 24 hours, stored in refrigerator for future experimental tests.

2.2.3.1. Cup-cut diffusion method

Antibacterial susceptibility was tested using Cup-cut diffusion method (Daoud *et al.*, 2015). The pollen extracts were dissolved in 2% (v/v) DMSO solution to get different concentration 100, 50, 25 and 12.5 mg/ml concentration (125 mg/ml DMSO), then filtered using 0.2 µ mlmillipore filter for sterilization. DMSO was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. This method was performed using freshly prepared Mueller Hinton agar with overnight culture of bacteria (107 CFU/ml). Approximately 20 ml of the agar were poured into 10 cm diameter in petri dishes and allowed to solidify. On each plate wells were made by sterile cork borer (5mm), each well was filled with 100µl of each pollen extract of different concentration. The controls were prepared by using the same solvent withoutextract, the plates were left for half an hour at room temperature for diffusion of extract into agar, then incubated at 37°C for 24 hour. The diameter of the zones of inhibition was measured to the nearest mm.

2.2.4. Determination of antioxidant activity (DPPH assay)

2.2.4.1. Quantitative DPPH assay

The free radical scavenging capacity of the Date palm pollen was determined using DPPH method. A solution was prepared in 99% methanol. The extracts of Date palm pollen were mixed with solvent to prepare the stock solution (10-250 µg/mL). Freshly prepared DPPH solution (0.001% w/v) was taken into the cuvettes, then Date palm pollen were added to every cuvette so that the final volume was 1 mL and after 30 min, the absorbance was read at 515 nm using a spectrophotometer. Ascorbic acid was used as a

reference standard and was dissolved in methanol. The control was prepared containing the same volume without any extract and reference ascorbic acid.

Percentage Scavenging of the DPPH free radical was measured by using the following equation:

$$\% \text{ scavenging Activity} = \frac{\text{Absorbance of the control- Absorbance of the test sample}}{\text{Absorbance of the control}} X100$$

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition ± standard deviation. IC_{50} value was determined from the graph obtained by using standard ascorbic acid following the formula "y=mx+c" from the slope of the graph. The IC_{50} represents the concentration where 50% inhibition of the DPPH radical is obtained. The purity and the contents of the standard ascorbic acid were confirmed using HPLC (Njoku *et al.*, 2014).

2.2.5. Proximate analysis of nutritional value (AOAC method)

2.2.5.1. Moisture content

The dry Petri-dish and lid was placed in oven to dry at 105 ºC for 3h, then transfered to desiccator to cool and 3g of the sample was placed in a pre-weighed Petri dish, the sample spread to the uniformity and then placed in an oven to dry at 105 ºC for 3hours. The dish and dry sample were transferred to a desiccator to cool at room temperature reweigh the dish and it is sample, the experiments were repeated until constant weight was obtained (Soylak *et al.*, 2004).

$$\% \text{ Moisture content} = \frac{\text{weight before drying-weight after drying}}{\text{weight before drying}} x100$$

2.2.5.1. Ash content

Place crucible in muffle furnace at 550 ºC, overnight to ensure that impurities on surface crucible are burned and placed in a desiccator to cool. 5g of the sample was weighed into a crucible in a muffle furnace and heated at 550 ºC for six hours until it became gray ash. The crucible was removed from the muffle furnace using crucible tong and placed in a desiccator to cool. Re-weighed and the weight of ash was obtained by difference (Soylak *et al.*, 2004).

$$\% \text{ Ash content} = \frac{\text{weight of Ash}}{\text{weight of sample}} x100$$

2.2.4.5.2. Fat content

The bottle was washed and dried in oven at 105 ºC overnight and then placed in a desiccator to cool. Place the bottle in the apparatus, 3g of the dried sample were taken and weighed accurately into labeled thimbles. The extraction thimble was plugged with a cotton and transfered to soxhelt apparatus. The bottle was filled with 200 ml of petroleum ether and boiled at 40-60 ºC. The soxhlet apparatus was allowed to reflux for one hours. The thimble was removed carefully, and the petroleum ether on top of the container was collected and drained into another container for reuse. The flask was removed and boiled for an hour at 105 ºC. Finally transferred from the oven into a desiccator to cool before weighing (Soylak *et al.*, 2004).

$$\% \text{ Fat content} = \frac{\text{weight of fat}}{\text{weight of sample}} \text{ x100}$$

2.2.4.5.3. Fiber content (Weende's method)

2g of the sample was weighed into a 250 ml conical flask and 200 ml of 1.25% $H2S0_4$ was added and the mixture was boiled under reflux for 30 minutes. The solution was filtered with whatman filter paper; the residue was rinsed thoroughly with hot water until it was no more acidic when tested using pH paper. The residue was transferred into a 250 ml beaker and 200 ml of 1.25% NaOH was added and boiled for 30 minutes in a digestion apparatus after which it was filtered and rinsed with distilled water until the filtrate was neutral when tested with pH paper. The residue transferred into a crucible and placed in electric oven at 100 ºC for eight hours to dry. Then removed and placed in a desiccator to cool before weighing. After weighing, the sample cooled in a desiccator and reweighed.

$$\textbf{Fiber} = \frac{\text{wt of crucible ashing+content before} \times \text{wt crucible after ashing}}{\text{wt of sample}} \times 100$$

2.2.4.5.4. Protein content (Kjeldahl method)

The total nitrogen was determined and multiplied by a conversion factor of 6.25 to obtain the protein content. Weighed 1g of the sample added tablet catalyst tablet (10g of $NaSO_4$, 1g of CuSO) into a Kjeldahl digestion flask, with 12ml of $H2SO_4$, and heated at digestion stand at 420 ºC for 1h until chemical decomposition complete and changed to blue green color, let digestion flask to cool. Adding 20 ml deionized water. Then 25 ml of 40% of NaOH and placed in the Kjeldahl distillation apparatus. The mixture was distilled until a total of 50ml distillate was collected into 250ml conical flask containing boric acid and titrated with 0.1N HCL. The end point of the titration was observed when the color of the distillate changed to the initial color of the mixture of boric acid and screen methyl red indicator which was light pink (Soylak *et al.*, 2004).

$$\% \text{ Protein} = \frac{\text{Volume of standard acid X 0.1N X 14.007 M.Wt x 6.25}}{\text{Wt of sample (mg)}} \text{ x100}$$

2.2.6. Elemental analysis

2.2.6.1. Preparation of sample by wet digestion

0.5g of pollen sample was weighed and placed in 25ml of volumetric flask, and 5ml of concentrated HNO_3 was added. The volumetric flask was covered then placed on hot plate and heated for 2h at 120c until brown fumes. After cooling 1ml of H_2O_2 was added and heated to become clear. The volumes were completed by 25ml of ultra-pure water and filtered with 0.45 µ mlmillipore membrane filter (Jothy *et al.*, 2011).

2.2.6.2. Mineral content

The minerals were determined by using inductively coupled plasma-optical emission spectrometry (ICP-OES). Ten minerals (Ba, Cd, Cu, As, Cr, Fe, Pb, Mn, Ni and Zn) were quantified, the calibration standards were prepared from multi elemental standard after dilution.

2.2.7. Acute toxicity study

2.2.7.1. Target animals

Healthy Wister rats weighing between120-200gm obtained from local animals house of the pharmacology department, faculty of pharmacy, University of Tripoli, Libya. Those rats were distributed into three groups, three animals in each group *i.e.*, treated groups had given dose 500 mg/kg as low dose whereas another group had received 5000 mg/kg in high dose; the control group received distilled water only. The animals are randomly selected ones and marked on the tail for individual identification. All rats must be maintained on a 12-h dark place and located at room temperature approximately 23 °C with constant humidity before experiment. The animals were maintained into laboratory conditions for a week before starting the experiment. The animals must be fasting 12 h prior to the treatment. The body weight of the rats were determined after the fasting period, and the dose calculated to body weight as the volume of the extracts solution given to the mice is 10 mL/kg body weight.

2.2.7.2. Procedure for acute oral toxicity study

The acute toxicity study was conducted in accordance procedure of the Organization for Economic Co-operation and Development (OECD) (Shaheen *et al.*, 1986). It was conducted by using a total of nine male rats. The animals were divided into 3groups of 3mice each. The First group was given 500 mg/kg, the second was given 5000 mg/kg body weight of sterile the extract, to possibly establish the range of doses producing any toxic effect. In addition, the third group of rats was set up as the control group, the animals were given only sterile distilled water. The animals were observed in detail for any indications of toxic effect within the first six hours after the treatment period, and

daily further for a period of 14 days. The surviving animals were weighed and monitored daily for visual behavioral changes as well as mortality.

2.2.8. Determination of pollen viability (staining test)

The viability of pollen grains was determined by spread pollen on slide and staining by using one to two drops of 1% acetocarmine solution, Pollen viability was examined under a light microscope, if the pollen grains are able to staining with red color that it is mean viable whereas colourless grains were considered non-viable (Serrano *et al.*, 2010).

2.2.9. Scanning Electron Microscopy (SEM)

Pollen grains of date male palm sample was weighed and prepared to remove fat by soxhlet extraction before the injection in SEM. The dried material was mounted directly on stubs using double-sided conductive tapes, coated with 40 - 60 nm of gold (a thin layer) and then observed in the microscope (Tabbiruka *et al.*, 2014).

2.2.10. Thermo- gravimetric Analysis (TGA)

2.2.10.1. Thermo-gravimetric and Differential Thermal Analysis

TGA method was used to determine the weight loss percentage. 3 mg of pollen grains sample was weighed and put in the sample cell in the TGA –DTA machine which was then operated and the thermogram was obtained by heating gradually from ambient temperature at a scanning rate of 25 ºC /5 min up to 100°C and switch the gas to N^2 at 20 ml/ min. Different components present in the sample were decomposed at different temperatures and result percent of weight loss was recorded (Sharif *et al.*, 2010).

Results and discussions

3.1 Extraction yields

The extraction of finely dried date palm pollen from 45.12g using methanol yielded 30.47% at 70 °C and 45.41g at 45°C yielded 27.75% of DPP crude extracts. The yield color at 70°C was a strong yellow, while it was a pale yellow at 45°C (Table 1). Methanol is a highly active solvent which facilitates the extraction of more bioactive polyphenols, tannins, saponins, flavon, terpenoids and anthocyanin compounds compared with other solvents. It is characterized by its strong ability to penetrate the cell wall of plant material and extract the intracellular components (Harborne, 1998). The difference between the extract yields and the colors from the same plant materials in the present study could be due to the temperature levels used. The increase in coloring substances and yields weight follows the increase in temperature until 70°C. Temperatures higher than 70°C may cause the degradation of the color of plant materials, as reported by (Sharif *et al.*, 2010).

Table (1): Extraction yield and colors of DPP grains.

Methanol solvent	Extraction yield %	Color
At temperature 70 °C	30.47	Dark yellow
At temperature 45 °C	27.75	Pale yellow

3.2. Preliminary phytochemical screening of *P dactylifera* pollen

The medicinal properties of plants are due to the presence of several active components which are exclusively accumulated in different part of the plant. These active components protect against many disease as they are characterized by their antibacterial and antioxidant properties, among others. The phytochemical screening of the palm pollen extracts revealed the presence of phytosterols, flavonoids, coumarins, tannins, phenolic compounds, amino acids, and protein along with the presence of small amounts of saponin and fats. The screening also revealed the absence of alkaloids and anthra quinone glycosides. The ratios are reported in (Table 2). A study by (Al-Samarai *et al.*, 2016) on the phytochemical screening of ethanol extracts of Iraq palm pollen showed the presence of alkaloids, carbohydrate, protein, amino acides with trace amounts. The pollen was reported to be rich in flavonoids, phytosterol. No coumarins, quinones, other glycosides and saponin were found. Another study by (Al-Samarai *et al.*, 2016) reported that methanol extracts of palm pollen grain contained many types of flavonoids, consistent with (Abed El-Azim *et al.*, 2015) who found that the palm pollen grain contains high quantities of lipids and steroids. DPP is considering an excellent food and drug resource among natural products. The phytosterols in DPP is a potential male fertility enhancer as it is a gonadal stimulant and can improve lipid metabolism. Polyphenolic compounds, which have growth promoter properties and a strong antimicrobial activity, can assist in curing inflammatory diseases and have powerful antioxidant and anticancer effects. Some flavonoids, such as quercerin and rutin, have hepatoprotective activities. Besides, the bioactive fat and lipids contents in palm pollen increase the immune response activity (Kostova *et al.*, 2011). Coumarins have the ability to act as anticancer, antiviral, excellent antioxidant, anticoagulant agents. Therefore, it is possible to use palm pollen in natural pharmaceutical formulations (Rohini and Srikumar, 2014), as well as an anti-inflammatory treatment of chronic infections (Bentrad *et al.*, 2017).

Table (2): Phytochemical screening for two extracts of *P dactylifera* pollen.

Phytochemical tests	Reagents	Methanol 70	Methanol 45
Phytosterol	Salkowski	+++ve	++ve
	Libermann burchard	+ve	+ve
Flavonoids	Alkaline reagent	+++ve	+++ve
tannins& phenolic compounds	Ferric chloride	+++ve	+++ve
Alkaloids	Dragendorff	-ve	-ve
	Mayer	-ve	-ve
	Wagner	-ve	-ve
Glycosides	Killer killiani	++ve	+ve

	Modified Brontrager s	-ve	-ve
Saponin	Foam test	+ve	+ve
Cumarins	Sodium hydroxide	+++ve	++ve
Amino acides and protein	Xanthoprteic	+++ve	+++ve
Oil and fats	Spot test	+ve	+ve
Carbohydrate	Fehling	+++ve	++ve
	Molishe s	+++ve	+++ve

High (+++), medium (++), low (+), none (-).

3.3. FTIR Spectroscopy analysis

The infrared spectra of date pollen were recorded by (FTIR) and run under Infrared region of 400–4000 cm−1 range. The present study was undertaken with a view to identify the functional groups present and purity of sample. The compatibility of Libyan DPP sample with standard are 97.1% as show in the Figure (2). FTIR spectrum of DPP were obtained and depicted in Figure (1) which confirmed the presence of functional group. The intense bands occurring at 3437.15 cm-1 , 3414 cm-1, 2924.09 cm-1,1546.91 ,1639.49 cm-1,and 1056.99 cm-1 corresponding to N-H/O-H (carboxillic acid) / O-H str / aromatic ring/ C=O/ C-O str / stretching. This confirms the presence of functional groups in date palm pollen (DPP), such as carboxylic acids, amines, amides, phenols, and others. Strong absorption band observed around 3437.15 cm-1 indicates the presence of amines and amides, strong absorption band observed near 2854.65 cm-1 shows C-H symmetric stretching of methylene group, strong absorption band observed near 1639 cm-1 were due to the presence of C=O stretching. Finally the sample was in a good agreement with standard.

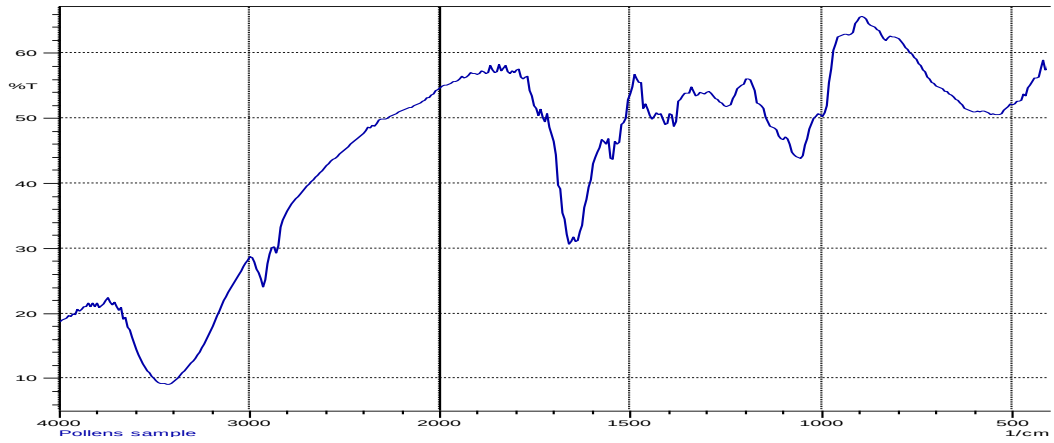

Fig. (1): FTIR spectrum of date palm pollen sample**.**

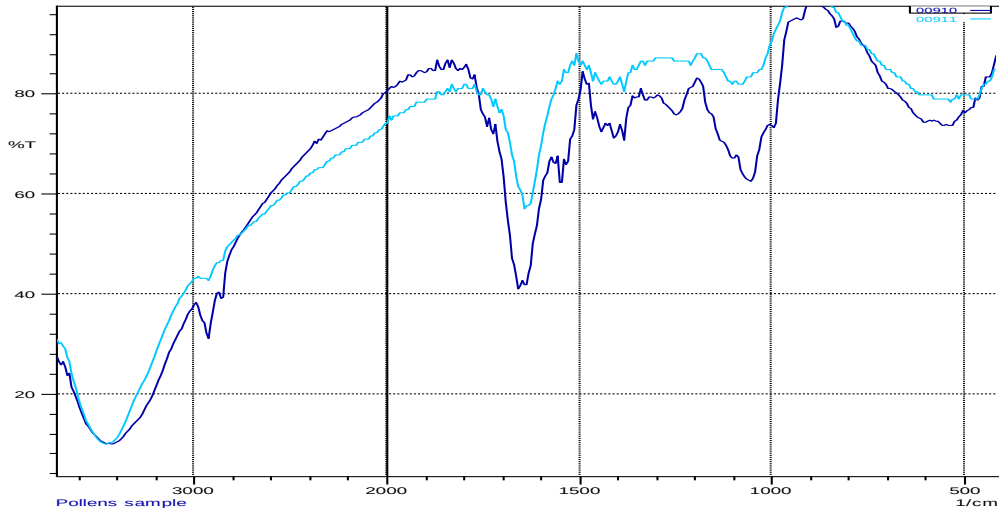

Fig. (2): FTIR spectrum of standard and sample of date palm pollen

3.4. Quantitative antioxidant activity palm pollen extracts

3.4.1. DPPH free radical scavenging activity method

The DPPH radical scavenging assay is a simple, quick, and sensitive method used for the screening and evaluation of the antioxidants activity and free radical scavenging ability of plant extracts. The extracts of date palm pollen were quantitatively investigated for antioxidant activity and the values are compared to the ascorbic acid used as reference standard. The results are presented in Tables (3) and (4), in Figures (3) and 4) where the activity was expressed by IC_{50} values. The values was refer to sample concentration required to scavenge DPPH radical by (50%), the lower IC_{50} reflect to greater antioxidant activity of the property (Daoud *et al.*, 2015).

Table (3): In vitro DPPH antioxidant activity of methanolic extract of DPP grains

Extracts	IC_{50} ($m_{g/ml}$)
Methanol extract at 70 ºC	0.233
Methanol extract at 45 ºC	0.224
Ascorbic acid references	0.05

Table (4): In vitro DPPH antioxidant activity of the aqueous extract of DPP grains.

Extracts	IC_{50} (mg/ml)
Aqueous extract	0.00075
Ascorbic acid references	0.05

Data are displayed with mean values

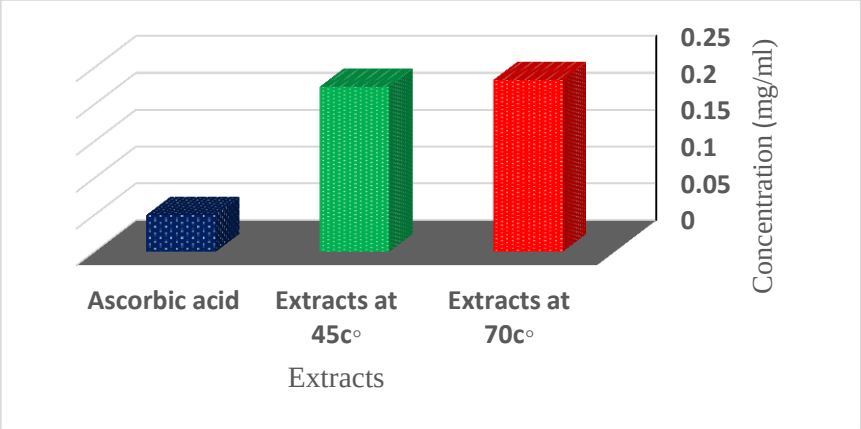

Fig. (3): DPPH radical scavenging capacity (IC_{50}) of DPP methanolic extracts and standard.

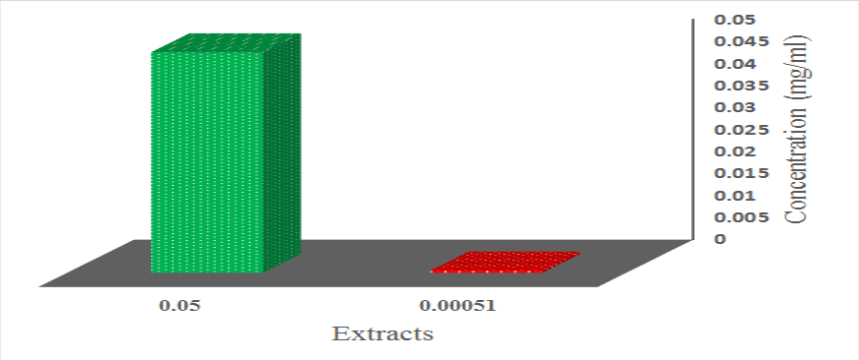

Fig. (4): DPPH radical scavenging capacity (IC_{50}) of DPP aqueous extract and standard.

The finding revealed that methanol extract at 70 °C has IC_{50} value (0.233 mg/ml) and another methanol extract at 45 °C has IC_{50} value (0.224mg/ml) less than IC_{50} value ascorbic acid (0.05mg/ml). These findings are similar to data obtained by (Daoud *et al.*, 2015) on the DPPH scavenging activity of IC_{50} value of Tunisian date palm pollen ethanol extract (0.144 mg/ml). The results show few differences in IC_{50} between the two. The change in the IC_{50}, it may because the use of heat in extraction in the present work produces little alteration in antioxidant activity of date palm pollen compounds. In the thermal stability work the results was showed loss of weight might be related to loss of moisture and volatile components, these volatile compounds in other study by (Farouk *et al.*, 2015) were possess IC_{50} 0.89mg/ml. The excellent result was shown in the aqueous DPP extract which has IC_{50} (0.0007mg/ml) potent with 100 times of Ascorbic acid. This activity may be attributed to extra concentration of active compounds were extracted with more polar solvent as water.

3.5. Antibacterial activities of the DPP grains against bacterial strains

Table (5) shows the anti-bacterial activity of date palm pollen extracts by cup cut method against five bacteria strains. The potency of each strain was evaluated based on the diameters of the inhibition zones surrounding the wells; if there is no inhibition, it assumed that there is no antibacterial activity. As in Table (5), negative results were obtained against all types of gram positive and gram negative bacteria for two extracts

and clear zones of inhibition were produced by positive control. The results of this experiment were different from the prior literature findings, which implies that the extract of palm pollen grain may exhibit antibacterial activity depending on the concentration of the extract and the target bacterial strain as in the (Banu and Catherin, 2015) who reported that palm pollen grain ethanol extracts resulted in inhibition zones with diameters of 2.5mm, 3.5mm, 3mm, 7mm against *Klebsiella Pneumaniae* at concentrations 60%, 70%, 80%, and 90% respectively, zones with diameters of 2.5mm and 1.5mm with concentrations of 60% and 70% of *Pseudomonas aeruginosa*, and negative results against *Staphylococcus aureus*. Another study of the ethanol extracts of palm pollen grains from two Tunisian cultivars by (Daoud *et al.*, 2015) reported zones with diameters of 12mm and 14mm against *Staphylococcus aureus*, 10mm and 10.5mm diameter zones against *E. coli*, and negative effects to one extract, while the other extract resulted in a 8mm diameter inhibition of zone against *Klebsiella Pneumaniae*. However, prior literature on palm pollen extracts reported a low to moderate antimicrobial activity especially against human pathogenic and fungi. An inhibition zone with a diameter of 10 mm suggests that the antibacterial activity is low, while a diameter between 10 and 15 mm suggests that the antibacterial activity is moderate (Bentrad *et al.*, 2017).

Table (5): Zone of inhibition of date palm pollen in two extracts

Extracts	**Methanol extract at 70 ºC**						**Methanol extract at 45 ºC**					
Bacterial strains	**Inhibition of zone (mm)**						**Inhibition of zone (mm)**					
Concentration of extract	**100**	50	25	12.5	DMSO*	Cip	100	50	25	12.5	DMSO*	Cip
Staphylococcus aureus	-	-	-	-	-	35	-	-	-	-	-	25
Klebsiella. pneumoniae	-	-	-	-	-	40	-	-	-	-	-	32
Pseudomonas aeruginosa	-	-	-	-	-	30	-	-	-	-	-	26
E. coil	-	-	-	-	-	34	-	-	-	-	-	23
MRSA*	-	-	-	-	-	35	-	-	-	-	-	25

*= *Methicillin-resistant Staphylococcus aureus*
Data are displayed with mean values ± SD (n=3)
The refrence standard of bacteria (ciprofloxacin 5µg)
(-) =absent
DMSO*= 2%

3.5. Determination of the nutritional value of the *P dactylifera* pollen

Previous chemical analyses showed pollen grains are different from other pollens collected from different places as the principles of chemical analysis focus on moisture, ash, crude fiber, crude fat, crude protein, carbohydrates, vitamins, and minerals. In this study, the pollen grains were examined for their contents of moisture, ash, crude fiber, crude fat, crude protein and minerals and the obtained data are reported in Table (6). The moisture content of *P dactylifera pollen* is calculated at 18.19%. This value was lower than the values of 28.80% and 29.00% obtained by (Hassan, 2011) and (Banu and Catherin, 2015), respectively, and higher than the value of 8.041% obtained by (Al-Samarai *et al.*, 2016). The decrease in moisture content leads to the decrease in the growth of microorganisms and helps in a safe long period storage. The protein content in the *P dactylifera pollen* was calculated at 27.36%, a value lower than 39.80% obtained

by (Banu and Catherin, 2015). The value of crude protein content was within the values (19.45-31.1%) obtained by (Hassan, 2011) proteins are essential for human health and are needed to maintain the vital function of body cells and growth. They are also required by pregnant and breastfeeding mothers. The protein quantity in the pollen grain are mainly more than the 19% found in shrimp as reported by (Stein, 2014), and more than the 22.90% in chicken breasts reported by (Petracci *et al.*, 2014). The total ash content in palm pollen grains is 5.41%, nearly compatible with the 5.58%, estimated by (Al-Samarai *et al.*, 2016). Furthermore, (Banu and Catherin, 2015) reported a higher content of 6.20%, while (Hassan, 2011) found only 4.57%. The total ash refers to amount of minerals beneficial to human health along with some toxic metals. Fats are an excellent source of energy. Palm pollen grains contain 7.32% of the crude fat, consistent with the value of 7.67% reported by (Al-Samarai *et al.*, 2016). Other studies reported different results: 20.7 4% reported by (Hassan, 2011) and 31.50% by (Basuny *et al.*, 2013). The value obtained for fiber content was 0.68 %. This value was within the range estimated by (Al-Samarai *et al.*, 2016; Banu and Catherin, 2015; Hassan, 2011). The variation in type and content may be owing to many factors such as the differences in the collection time and seasons, maturation age, location of cultivates, and conditions of handling and storage after collection (Hassan, 2011). The pollen grains are valuable natural food sources rich in protein and minerals, and contain well- balanced essential amino acids, vitamins and over 100 kinds of enzymes and cofactors (Hassan, 2011). Nutritionally, the pollen are high in protein, low in saturated fat and calories, and have a neutral flavor, which make pollen a natural potential additive in pharmaceuticals formulations, also in salads, cakes, and soups.

Table (6): Proximate nutritive values of DPP.

Parameter (%)	Mean ± S. D.
Moisture	18.19 ±0.003
Crude fiber	0.67 ±0.001
Total Ash	5.41 ±0.001
Crude fat	7.32 ±0.007
Crude protein	27.36 ±0.003

3.6. Elemental analysis

The date palm pollen possessed a variety of metals which play a crucial role in the development of biological functions and growth of the human body such as Mn, Fe, Zn, Cr, Cu, Mo, and Ni. The results of the elemental analysis obtained by AAS techniques are shown in Table (6) in ppm of dry weight of the palm pollen grains with a precision of about ± 99 %. The predominant mineral was zinc (36.8), followed by iron (27.5), manganese (24.1), cupper (4.73), nickel (1.20), and chromium (0.29). This study's findings vary in the concentration of the quantified elements compared with other studies. In the studies by Al-Samarai *et al.* (2016) and Hassan (2011) zinc contents were 2.810 and 2.799, iron contents 2.410 and 8.500, manganese 2.840 and 19.600, cupper 3.196 and 3.658, and nickel 3.024 and 1.698, respectively. Some plants may be contaminated with

nonessential metals for human body like Hg, Pb, As, and others. The presence of these elements is dangerous as they have toxic effects and widely present in plants (Leal *et al.*, 2013). Date palm pollen is free from heavy metals which are usually below the detection limit. The ratio of arsenic, cadmium, and lead as observed in Table 6 with values below 0.02, 0.002, and 0.03, respectively. Metals may accumulate from soil, water, and the variation of the flora and growth state: the soil and geographic origin of pollen can cause a large difference in the composition and concentration of minerals in the pollen**.** These elements are essential for life and the deficiency of vital elements in the human body can cause many diseases. The presence of Mg may decrease coma, neurological disturbances and diabetes mellitus. The body also requires sufficient amounts of zinc to produce testosterone, maintain a functional male reproductive system, prevent of hair loss, and overcome free radicals. Iron also helps in protecting the body from anemia, regulating hemoglobin blood levels, while a copper deficiency causes brain disease in infants, anemia in adults. Chromium helps patients with diabetic mellitus to regulate blood sugar, supports muscle and burns fat in the human body, supports healthy bones by inhibiting the loss of calcium and increasing the rate of milk production by pregnant and lactation mothers (Zafar *et al.*, 2010).

Table (7): Mineral composition of DPP grains

Mineral	Concentration (ppm)
Zn	36.8
Ba	0.54
Cu	4.73
Fe	27.5
Mn	24.1
Ni	1.20
Cr	0.29

Table (8): Heavy metals

Metals	Concentration (ppm)
As	<.02
Cd	<.002
Pb	<.03

3.6.1. Acute toxicity study

The acute toxicity effect of the of methanolic extract of date palm pollen at 70 °C on male rats on the appearance and the general behavioral pattern are shown in Table (7). No toxic symptoms or mortality were observed in any rats, which lived up to 14 days post extract administration at dose level 500mg/kg and 5000mg/kg body weight. The behavioral patterns of animals were observed first 6h and followed by 18h in both vehicle treated (water) and extract-treated (extract) groups and the animals were normal and didn’t shows any difficulty in breathing, or loss appetite, muscular tremors, or abdominal

cramps, sweating and general weakness. There are no previous reports on any toxic effect to palm pollen grain. Thus the LD_{50} of the of date palm pollen being greater than 5000mg/kg body weight. It thought to be safe as food or for medicinal purpose (Shaheen *et al.*, 1986).

Table (9): Acute toxicity effect of methanolic extract of DPP at difference doses.

Dose	500mg/kg				5000mg/kg			
Observation	**6h**	**18-h**	**daily**	**control**	**6-h**	**18-h**	**Daily**	**Control**
Skin	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N
Breathing	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N
Coma	N	N	N	N	N	N	N	N
Tremor	N	N	N	N	N	N	N	N
Mortality	None	None	None	None	None	None	None	None

N=normal
None =not observed.
Data are displayed with mean values ± SD (n=3)

3.7. Thermo-Gravimetric Analysis (TGA)

Thermo-Gravimetric Analysis (TGA) method was applied to investigate and understand the thermal stability of the natural products, pharmaceuticals and others, so some of phytochemicals compound are very sensitive to heat and can easily decompose and degrade. Therefore, the thermal constancy properties of any medicinal plants play a vital role in pharmacological stability of compounds when manufactured as drug form during processing, formulation, packaging, and storage. The estimation of thermal treatments also are frequently used in the industry of food materials whose antioxidant efficiencies can be affected by heat (Farhoosha and Nyström, 2017). The thermal stability of date palm pollen was assessed using thermogravimetric analysis before extraction. TGA curve was plotted between temperature and weight of the sample Figure (5). These results show that little change of weight at 22– 100 ◦C were observed and exhibit 15.8% of weight loss which might be associated with the loss of water and volatile molecules which is consistence with (Nadiye-Tabbiruka *et al.*, 2014). There is twenty one volatile compounds was detected in DDP according to (Farouk *et al.*, 2015). DDP shows minimum initial temperature decomposition which might be related to its stability but further HPLC studies are required to confirm the stability.

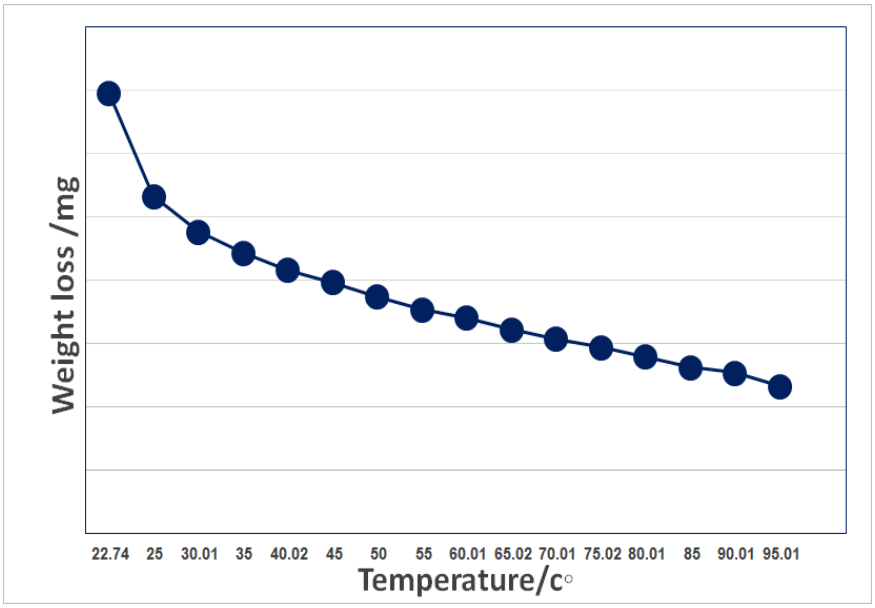

Fig. (5): TGA of weight loss curves of the thermal degradation of date palm pollen

3.8. Pollen grains viability

Viability of pollen grains of the males are high in most of the pollen grains under investigation, the viability were showed most pollen grain colored with red color.

3.9. Description of palm pollen grains under Scanning Electron Microscopy (SEM)

The mature pollen grain consists of a double wall: a thin inner layer called the intine and a tough outer layer known as the exine, which features various sculptural patterns. These characteristics of the markings are often valuable for identifying genera and species. Additionally, other morphological characteristics of pollen grains, such as symmetry, shape, surface texture, size, apertures, and polarity, have been categorized as unique pollen units (Soliman and Al-Obeed, 2013). The figure (6) describes the pollen grains of males of *phoenix dactylifera* L. and their shape is monad, symmetric mono sulcate, elliptical-oblate shaped, the aperture of palm pollen has one deep line in the polar surface ,usually opened with rounded tips slightly shorter than polar view long axis positioned centrally in polar face of pollen grain (simple aperture). The surface is reticulate with irregular semi-circular pores and appear as roughness spongy form, The size of DPP pollen grains is between 20 to 75 microns has, a diameter of about 21 -27 microns.

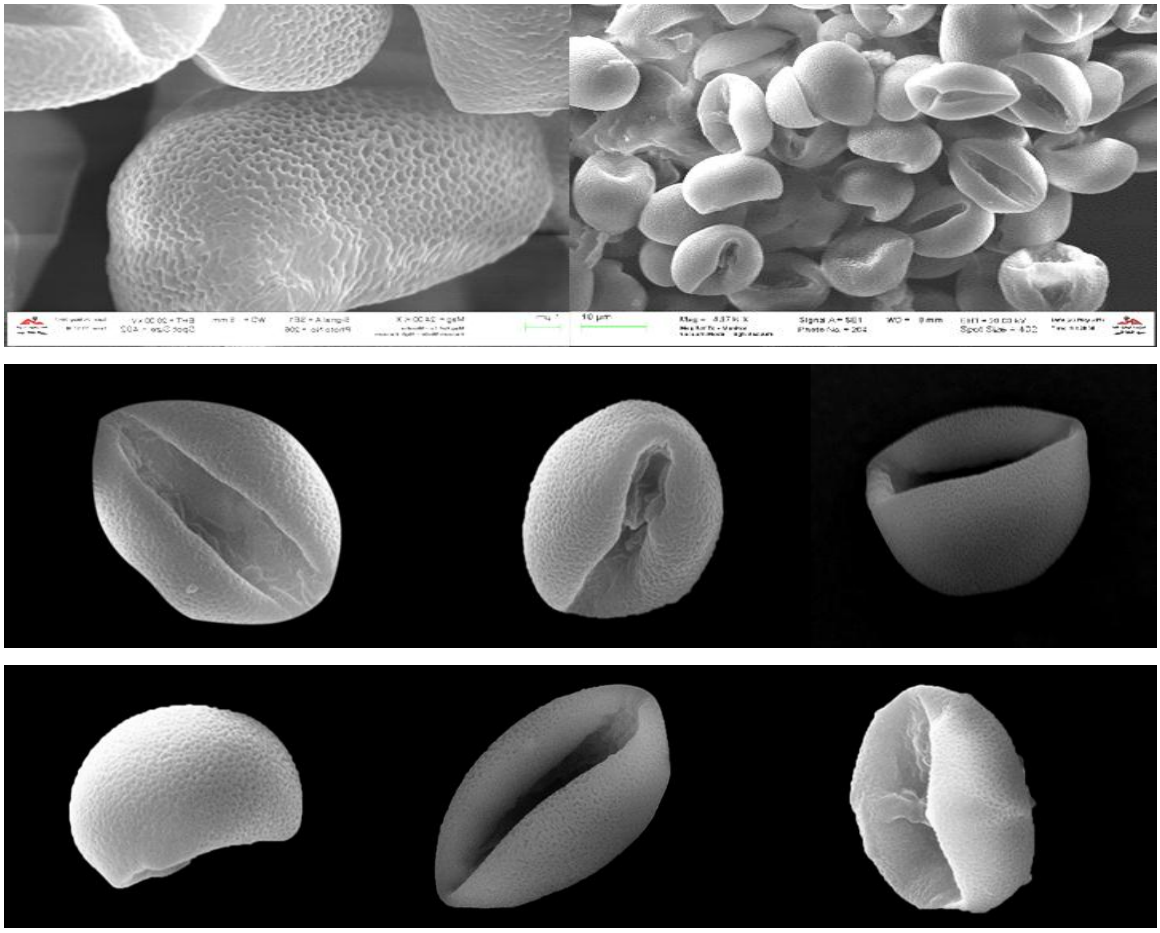

Fig. (6): DPP under scan electrical microscope.

Conclusion

To conclude, the study revealed that the preliminary phytochemical analysis indicates that DPP contains phytosterols, flavonoids, coumarins, tannins, phenolic compounds, amino acids, and proteins, along with small amounts of saponins and fats. Notably, the aqueous extract of date palm pollen exhibited higher antioxidant activity, likely due to the presence of polyphenolic compounds. Conversely, the methanolic extracts showed a lower IC50, potentially linked to the heat used in the extraction process. Thermogravimetric analysis indicated a 15% weight loss in DPP, primarily due to water loss and evaporation of volatile compounds, which were previously reported to have an IC50 of 0.89 mg/ml. Additionally, DPP is rich in nutritious components such as minerals, proteins, fiber, and fatty acids, making it a valuable economic resource that could be incorporated into dietary supplements and body care products, as well as formulated into natural pharmaceuticals. Furthermore, the methanolic extract at 70ºC was found to be safe, with an LD50 greater than 5000 mg/kg body weight and no apparent in vivo toxicity in animal models. However, the DPP extracts did not demonstrate antibacterial activity against the bacterial strains tested. The significance of DPP is also noted in the Holy Quran and supported by previous research and traditional medicine literature, which highlight its potential for promoting overall health and rejuvenation. Therefore, encouraging the cultivation of date palm trees in Libyan farms and promoting their consumption is essential, especially given

the confirmed safety of their use. Future research should focus on the identification and isolation of steroidal active compounds in DPP, particularly in relation to fertility issues. Date palm pollen represents an efficient, safe, and low-cost resource rich in various compounds, including potential plant steroidal compounds, gonadotrophic hormones, carotenoids, bioflavonoids, triterpenoids, and antioxidants. Studies should also investigate the anticancer, antidiabetic, and antihypertensive properties of DPP extracts. Date palm pollen can be included in daily diets due to its nutritional benefits; it can be consumed with honey, cooked into meals, or added to bread or cakes. Additionally, DPP could be developed into pharmaceutical preparations for topical use in treating or preventing various skin conditions and addressing skin aging.

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تقييم القدرة المضادة للأكسدة والمضادة للبكتيريا والقيمة الغذائية ودراسة السمية الحادة لحبوب لقاح نخيل التمر الليبي

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الملخص العربي

تُعتبر حبوب لقاح نخيل التمر DPP - (*Phoenix dactylifera* L.) جزءًا من عائلة (Arecaceae)، وتنمو بشكل واسع في العديد من المناطق، بما في ذلك الشرق الأوسط وشمال إفريقيا. يمتاز نخيل التمر بفوائد غذائية وطبية واقتصادية وبيئية، حيث يمكن استخدامه لعلاج العديد من الأمراض والاضطرابات الصحية بفضل خصائصه المضادة للميكروبات، والمضادة للأكسدة، والمضادة للالتهابات، والمضادة للسموم، والمضادة للسرطان، بالإضافة إلى أن له أنشطة وقائية للكبد. كما يُعتبر محفزًا للخصوبة لدى كل من الذكور والإناث. تهدف هذه الدراسة إلى تقييم الفحص الفيتوكيميائي لنخيل التمر المزروع في ليبيا، واختبار تأثيراته المضادة للأكسدة والمضادة للبكتيريا، وإجراء اختبارات السمية الحادة. تم فحص أنشطة مضادات الأكسدة للمستخلصات باستخدام اختبار DPPH. أظهرت النتائج أن المستخلص المائي من حبوب لقاح النخيل أظهر أفضل نشاط في تجريف DPPH (IC50 = 0.0005). كانت أنشطة تجريف DPPH المسجلة لمستخلص الميثانول من حبوب لقاح النخيل IC50 = 0.233 ملجم/مل و IC50 = 0.224ملجم/مل عند ٧٠ درجة مئوية و٤٥ درجة مئوية على التوالي. تم اختبار مستخلصات الميثانول من حبوب لقاح النخيل (بتركيزات ١٢.٥ ملجم/مل، ٢٥ ملجم/مل، ٥٠ ملجم/مل، و١٠٠ ملجم/مل) ضد خمسة سلالات مختلفة من البكتيريا القياسية (ATCC) باستخدام طريقة القطع. أظهرت النتائج عدم وجود تثبيط ملحوظ لنمو البكتيريا. كشف الفحص الفيتوكيميائي لحبوب لقاح نخيل التمر عن وجود فيتواستيرولات، وفلافونويدات، وكومارينات، وتانينات، ومركبات فينولية، وأحماض أمينية، وبروتين، وكمية صغيرة من الصابونين، ودهون. كما تم تحليل القيمة الغذائية التقريبية لحبوب لقاح النخيل. أظهرت النتائج أن اللقاح يحتوي على ١٨.١٩% رطوبة، و٥.٤١% رماد، و٦٧% ألياف خام، و٧.٣٢% دهون خام، و٢٧.٣٦% بروتين خام. أظهر اختبار السمية الحادة لمستخلص الميثانول عدم وجود وفيات بين مجموعات الحيوانات ذات الجرعات المختلفة، ولا تغييرات سلوكية عند أعلى جرعة قدرها ٥٠٠٠ ملجم/كجم.

الكلمات الدالة: اختبار السمية الحادة، حبوب لقاح نخيل التمر، مضادات الأكسدة، مضادات الميكروبات.