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Original Article

Nutritional and biological evaluation of *Phoenix canariensis* pollen grains

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ABSTRACT

The nutritional value of pollens of *Phoenix canariensis* Chabaud, Arecaceae, was evaluated. HPLC analysis of vitamins revealed the predominant presence of vitamin C (109.13 ppm), followed by vitamin A (53.71 ppm) and vitamin E (40.60 ppm) and the total carbohydrates (28.12%), proteins (17.10%). In addition, 16 amino acids of which nine are essential (75.07%), and seven are non-essential (24.93%) were determined. On the other hand, two steroidal saponins (dioscin and methyl protodioscin) were isolated from the pollens, their structures were elucidated by spectroscopic techniques, including ^{1}H NMR and ^{13}C NMR. Their content in the 70% ethanolic extract was quantified using reversed phase HPLC and found to be 0.013% and 19.35%, respectively. The modulatory effect of the isolated compounds on the prevention of benign prostatic hyperplasia induced in rats and their moderate curative effect on stressed testicular tissue were studied. Being a good source of carbohydrates, proteins, vitamins, and amino acids pollens of *P. canariensis* can be used as a promising source of dietary supplement. Meanwhile pollens can act as prophylactic agent against benign prostatic hyperplasia.

Introduction

Phoenix canariensis Chabaud, Arecaceae, is one of the date palm species that grows in countries around the Arabian Gulf. Genus *Phoenix* contains about nineteen species, distributed in southern Africa and Asia (Adawy et al., 2002; Jain et al., 2011). *P. canariensis* is native to the Canary Islands. It is commonly used as an ornamental palm in the United States and the Mediterranean area (Krueger, 2001). The date palm pollen of *P. canariensis* is a male reproductive dust of palm flowers used traditionally as dietary supplement (Tahvilzadeh et al., 2016).

Benign prostatic hyperplasia (BPH) is benign tumour in men, and its prevalence is age related. It is also called benign prostatic hypertrophy and nodular hyperplasia. Distinct alterations in tissue histomorphology is usually accompanying prostatic enlargement (Lee et al., 2012a).

The global interest towards herbal medicines rather than conventional treatment is increasing due to lower side effects provided by them for the treatment of chronic diseases. The date palm (*Phoenix dactylifera*) pollen grains were used by ancient Egyptians as aphrodiasic and for improving fertility in both males and females (Tahvilzadeh et al., 2016). Pollen preparations are distributed globally for dietary purposes and are used as dietary supplement by increasing the total dietary intake. They are good sources of protein, amino acids, vitamins, dietary fibre, fatty acids, enzymes, hormones and minerals (Kroyer and Hegedus, 2001). In a previous study, the authors reported that pollens of *P. canariensis* were rich in steroids (Hifnawy et al., 2016). Several studies have reported the wide activity of steroidal saponins on different cancer cells (Wang et al., 2001; Lee et al., 2012b; Chung et al., 2016).

Reviewing the available literature, only one saponin named methyl (25*S*)-protobioside was previously isolated from the leaves of *P. canariensis*, cultivated in Japan (Asami et al., 1991).

In continuation of our previous work (Hifnawy et al., 2016, 2017), further investigation of the saponin content of pollens was carried out. The therapeutic efficacy of pollens against prostatic hyperplasia has been investigated, in addition to evaluation of its nutritive value as well as quantification of the saponin content using reversed phase HPLC method.

Material, methods and apparatus

Plant material

Pollen grains of *Phoenix canariensis* Chabaud, Arecaceae, used in this study were collected in summer of the years 2013–2015 from El-Orman Botanical Garden, Egypt. Identity of the plant was confirmed by Dr. Mohamed el Gebaly (Senior Botanist) and by Mrs.

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Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture. Voucher specimens (numbered 5.10.2015), were deposited in the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Experimental

General experimental procedures

^{1}H NMR 400 MHz and ^{13}C NMR 100 MHz were measured on Bruker spectrometer. The chemical shifts being represented as ppm with tetramethylsilane as an internal standard. Melting point (uncorrected) were determined using Yamagimoto micro-melting point apparatus MP 500 D (Japan). Agilent 1100 apparatus was used for HPLC analysis of vitamins. Amino acid determination was done using automatic amino acid analyzer (AAA 400), INGOS Ltd. Quantification of saponins was done using reversed phase HPLC (Waters 2690 Alliance, USA) equipped with a Waters 996 photodiode array detector. Diaion HP-20 (synthetic polyaromatic adsorbent with pore volume 1.3 ml/g, surface area 500 m^2/g, pore radius □200), (Sigma Aldrich, Germany). Column chromatography (CC) was performed using silica gel (40–63 μm, Merck Co., Darmstadt, Germany). Pre-coated silica gel 60 F_{254} (0.25 mm thickness; Merck Co., Darmstadt, Germany) was used for TLC. Spots were detected using *p*-anisaldehyde-sulphuric acid spray reagent, Libermann-Burchard reagent (Huang et al., 1961).

Isolation and identification of saponin content

The butanol fraction 22.5 g obtained by fractionation of 155 g 70% ethanol extract prepared from 1 kg powder of pollens of *P. canariensis* was found to be rich in saponins (Tiwari et al., 2011); thus, it was subjected to fractionation with the aim of isolation of its major constituents.

Butanol fraction of pollens (15 g) previously prepared were applied on diaion HP-20 column (80 g, 80 cm × 5 cm). Elution started with 1500 ml distilled water followed by 25% methanol then 50% methanol in water increments till 100% pure methanol (Hifnawy et al., 2017). Fractions were collected (250 ml each) and monitored by TLC using solvent system (S) [ethyl acetate:methanol:water:formic acid (100 ml:16 ml:12 ml:1 drop)]. The spots were visualized under UV light and after spraying with *p*-anisaldehyde and heating at 110 °C for 5 min, similar fractions were pooled together to yield two collective fractions. The collective fraction eluted with 100% methanol (310 mg) was subjected to further chromatographic separation on silica gel H column (60 cm × 2 cm) using gradient elution (DCM-MeOH) mixtures and monitored by TLC in solvent system (S), yielding two saponins eluted by [DCM:MeOH (80:20)]; compound **1** (16 mg, white powder, R_f 0.37 in solvent system (S)) and compound **2** (45 mg, white powder, R_f 0.29 in solvent system (S)).

Nutritional value of pollens

HPLC analysis of vitamins. HPLC analysis of vitamins was carried under the following conditions: the column Hypersil-BDS-C_{18} (4.6 mm × 250 mm), the mobile phase used was methanol at 1 ml/min flow rate. The injection volume was 5 μl. Vitamins A, E and C were detected using UV detector at wavelength 325 nm, 292 nm and 254 nm, respectively (Nöll, 1996; Pyka and Sliwiok, 2001; Romeu-Nadal et al., 2006).

Determination of protein content. Micro-Kjeldahl method was used to determine the total protein content in pollens (AOAC, 1995).

Determination of amino acids. The amino acids content in pollens was determined spectrophotometrically after acid hydrolysis (Block et al., 1958; Okoronkwo et al., 2017).

Determination of total carbohydrates. Total carbohydrates were determined colorimetrically (DuBois et al., 1956; Wahba et al., 2017).

Quantification of the saponin content using RP-HPLC

Quantification of saponins content was carried out on the 70% ethanol extract as it was found efficient in extraction of major constituents. It was done using reversed phase HPLC equipped with photodiode array detector. 10 μl injection volume of the 70% ethanolic extract was injected in each run at 25 °C. The separation of sample solution was performed on a C18 (150 mm × 4.6 mm, 3 μm). The solvent flow rate was 1 ml/min. Gradient elution system composed of acetonitrile as solvent A and 0.2% phosphoric acid in water as solvent B was applied. Detection was carried out at 210 nm. Serial dilutions of dioscin (**1**, 99% purity) and methyl protodioscin (**2**, 99% purity) [20, 50, 100, 150, 200, 250 μg/ml] were prepared from stock solution having 1 mg/ml concentration. Results of dioscin and methyl prodioscin in *P. canariensis* were expressed as the mean of three determinations.

Histopathological study of the pollen grains activity on benign prostatic hyperplasia and on stressed testicular tissue in rats

Material: 10% neutral-buffered formal saline for 72 h at least, ascending grades of alcohol (70%, 80%, 90%, and finally absolute alcohol), xylene, soft paraffin wax, hard paraffin, haematoxylin (Merck, Darmstadt, Germany) and eosin (H&E) (Drury and Wallington, 1980).

Experimental animals

Thirty adolescent male Wister rats, six weeks old and weighing approximately 150 g, were divided into five groups (six rats, each) (Jeyaraj et al., 2000; Lepor, 2004).

The first group of six rats was treated with placebo subcutaneous 0.2 ml olive oil (vehicle) injection every other day for 28 days (negative control group). The second group of six rats was treated hormonally with 0.2 ml subcutaneous injections containing 9 mg/kg body weight dihydrotestosterone (DHT) and 0.9 mg/kg body weight estradiol valerate every other day for 28 days (positive control group). The third, fourth and fifth groups, each of six rats were treated hormonally in the same way as positive control in addition to oral administration of *P. canariensis* pollens [70% ethanolic extract, butanol fraction and saponin fraction (mixture of **1** and **2** 1:1)], respectively in a dose of 100 mg/kg every other day for 28 days. At the end of 28 days, the rats were sacrificed then the prostate gland and the testicles for each rat were removed, separately.

All animals specimens were immediately dissected after death and fixed in 10% neutral-buffered formal saline for at least 72 h. All the specimens were washed for half an hour with in tap water and then dehydrated using ascending grades of alcohol (70–80–90% and finally absolute alcohol), cleared in xylene, impregnated in soft paraffin wax at 55 °C and then embedded in hard parrafin. Serial sections of 6 μm thick were cut and stained with haematoxylin (Merck, Darmstadt, Germany) and eosin (H&E) Drury,Wallington 1980 for histopathological investigation. Images were captured and corrected for contrast and brightness using Adobe Photoshop version 8.0, but not otherwise manipulated.

The procedure used was in accordance with the guidelines of EU Directive 2010/63/EU for animal experiments and were approved by the Medical Research Ethics Committee of the National Research Centre, Giza, Egypt, registration number (6/014).

At the end of the experiment, the rats were sacrificed under sodium pentobarbitone anaesthesia according to guidelines for euthanasia in the Guide for the Care and Use of Laboratory Animals (Council, 2010).

Table 1
Amino acids content in pollens of *Phoenix canariensis*.

Peak number	Amino acid	Percentage of amino acid in gm
1	Aspartic acid	5.75
2	Threonine[a]	1.24
3	Serine	3.10
4	Glutamic acid	4.33
5	Proline	0.03
6	Glycine	5.35
7	Alanine	5.10
8	Valine[a]	2.70
9	Methionine[a]	0.19
10	Isoleucine[a]	1.69
11	Leucine[a]	2.56
12	Tyrosine	1.27
13	Phenylalanine[a]	2.44
14	Histidine[a]	1.86
15	Lysine[a]	55.67
16	Arginine[a]	6.72
% of essential amino acids		75.07
% of non-essential amino acids		24.93

[a] Essential amino acids.

Results

Nutritional value of pollens

Vitamins A, E and C were found to be 53.71, 40.60 and 109.14 ppm, respectively. The total carbohydrates percentage was found to be 28.12% and proteins were 17.10% dry weight. The amino acids content was presented in Table 1.

Quantification of saponins in the 70% ethanolic extract

The concentration of methyl protodioscin and dioscin in the 70% ethanolic extract of pollens was 19.35% and 0.013%, respectively. Standard calibration curve of methyl protodioscin and dioscin are represented in Figs. 1 and 2.

Compound isolated as white powder 16 mg, soluble in methanol, R_f value 0.37 in system (S), m.p. 243–246 °C, giving green colour with *p*-anisaldehyde spray reagent. ^{1}H NMR: δ (CD_3OD, 400 MHz) δ_H: 5.2914 (1H, d, J = 4.88 Hz, 2 Rha., H-1), 5.1068 (1H, d, J = 1.16 Hz, 4 Rha, H-1), 4.4766 (1H, m, H-6), 4.4120 (1H, d, J = 7.8 Hz, Glu.H-1), 1.1511 (3H, bs, H-21), 0.9503 (3H, s, H-19), 0.8728 (3H, d, H-27), 0.7122 (3H, s, H-18). ^{13}C NMR: (CD_3OD, 100 MHz) δ_c: 140.48 (C-5), 121.24 (C-6), 109.20 (C-22), 101.60 (Rh II C-1), 100.91 (Rh I C-1), 99.03 (Glu C-1), 80.81 (C-16), 78.1 (Glu C-4), 77.87 (C-3), 77.60 (Glu C-3), 77.40 (Glu C-2), 76.5 (Glu C-5), 72.52 (Rh I C-4), 72.32 (Rh II C-4), 71.04 (Rh II C-2), 71.01 (Rh II C-3), 70.96 (Rh I C-3), 70.78 (Rh I C-2), 68.38 (Rh I C-5), 66.45 (C-26), 62.32 (C-17), 56.38 (C-14), 50.30 (C-9), 41.50 (C-20), 40.01 (C-13), 39.52 (C-12), 37.15 (C-4), 36.63 (C-1), 36.50 (C-10), 31.77 (C-7), 31.39 (C-15), 31.32 (C-8), 31.01 (C-2), 30.03 (C-25), 29.34 (C-24), 26.30 (C-23), 20.57 (C-11), 18.42 (C-19), 18.30 (Rh I C-6), 16.56 (Rh II C-6), 16.45 (C-18), 16.07 (C-27), 15.35 (C-21). Compound **1** is identified as dioscin.

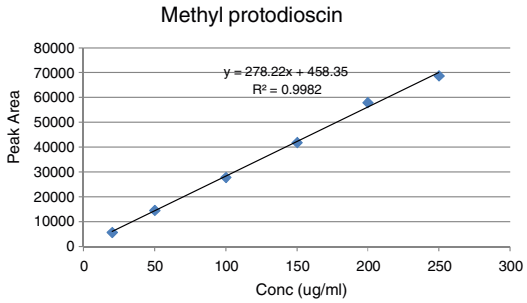


Fig. 1. Standard calibration curve of methyl protodioscin (**1**).

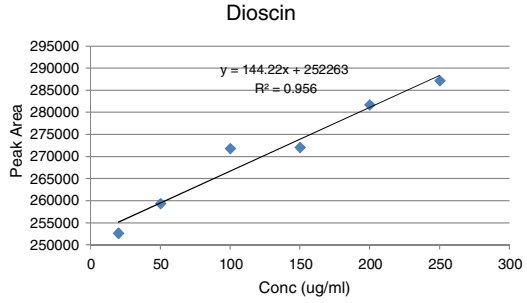
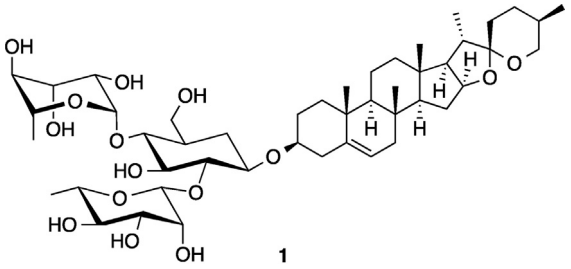
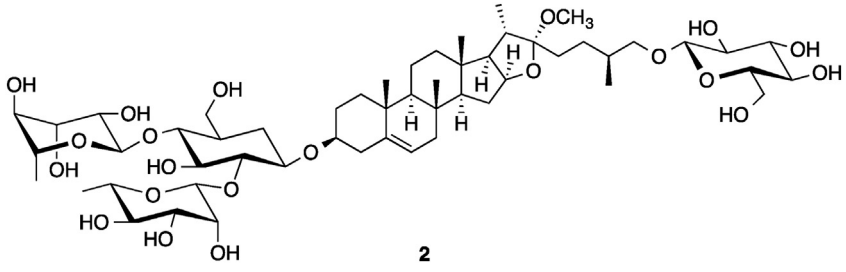


Fig. 2. Standard calibration curve of dioscin (**2**).



1

Compound **2** isolated as white powder 23 mg, soluble in methanol, R_f 0.29 in system (S). m.p. 185–189 °C, giving green colour with *p*-anisaldehyde spray reagent.^{1}H NMR: δ (CD_3OD, 400 MHz) δ_H: 5.4037 (1H, br, 2Rh, H-1), 5.1950 (1H, br, 4Rh, H-1), 4.5260 (1H, d, J = 7.8 Hz, Glu I H1), 4.4242 (1H, d, J = 7.8 Hz,Glu II H-1), 1.2567 (3H, bs, H-21), 1.0943 (3H, s, H-19), 0.87 (3H, bs, H-27) 0.8102 (3H, s, H-18). ^{13}C NMR: (CD_3OD, 100 MHz) 140.46 (C-5), 121.27 (C-6), 110.45 (C-22), 101.78 (Rh II C-1), 101.20 (Rh I C-1), 101.10 (Glu II C-1) 99.06 (Glu I C-1), 80.81 (C-16), 78.19 (Glu I C-4), 78.01 (C-3), 77.94 (Glu I C-3), 75.28 (Glu I C-2), 76.5 (Glu I C-5), 72.52 (Rh I C-4), 72.32 (Rh II C-4), 71.04 (Rh II C-2), 71.51 (Rh II C-3), 70.96 (Rh I C-3), 70.78 (Rh I C-2), 68.43 (Rh I C-5), 67.67 (C-26), 62.24 (C-17), 56.37 (C14), 50.29 (C-9), 41.50 (C-20), 40.00 (C-13), 39.51 (C-12), 37.13 (C-4), 36.63 (C-1), 36.50 (C-10), 31.77 (C-7), 31.39 (C-15), 31.32 (C-8), 31.01 (C-2), 30.00 (C-25), 29.33 (C-24), 26.30 (C-23), 20.57 (C-11), 18.43 (C-19), 18.30 (Rh I C-6), 17.16 (Rh II C-6), 16.58 (C-18), 16.07 (C-27), 15.35 (C-21). Compound **2** is identified as methyl protodioscin.



2

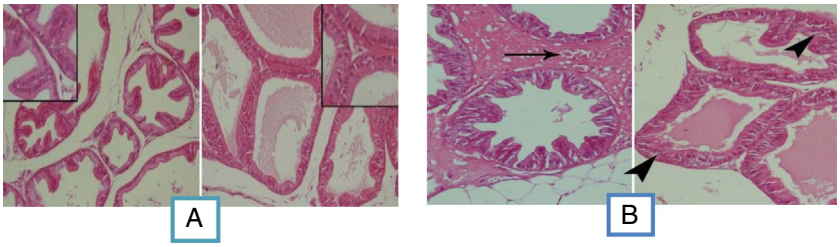


Fig. 3. A photomicrograph of two sections of prostate gland from (A) normal control rat showing acini lined by one layer of columnar cells that sometimes makes folds protruding in the lumen of the acini (H&E, ×10 and ×20); (B) positive control rat shown in the left side stromal hyperplasia with dilated blood vessels (arrow). On the right side epithelial hyperplasia is observed, where there is crowding of the epithelial cells with increase of their height (arrow head) (H&E, ×10).

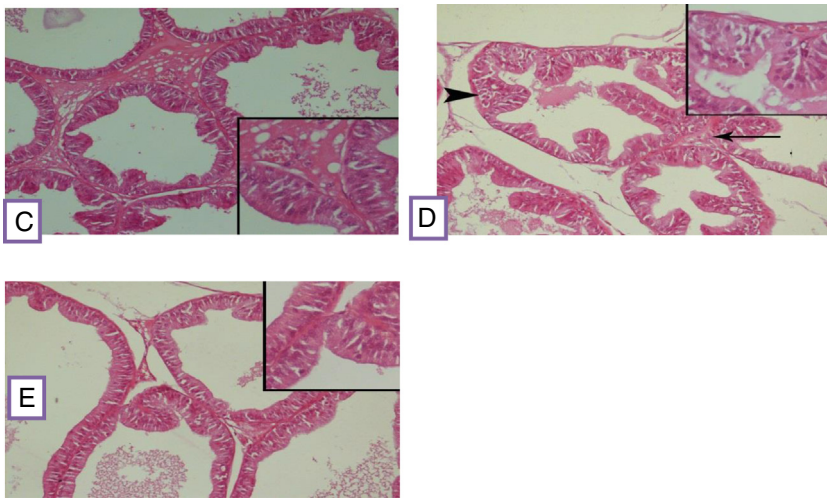


Fig. 4. A photomicrograph of a section of prostate gland from a rat treated with (C) 70% ethanolic extract of pollen grains shows mild decrease in both stromal and epithelial hyperplasia; (D) the butanol fraction showed marked decrease in stromal hyperplasia (arrow) while the epithelial hyperplasia is still noticed (arrowhead and in the higher magnification part); (E) the two isolated saponins appears close to normal (H&E, ×10 and ×20).

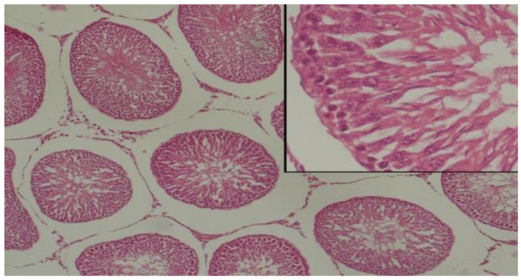


Fig. 5. A photomicrograph of a section of testis from a normal control rat shows seminiferous tubules with loose connective tissue in between. The upper right part of the figure shows the different layers of cells in the seminiferous tubules at a higher magnification (H&E, ×10 and ×20).

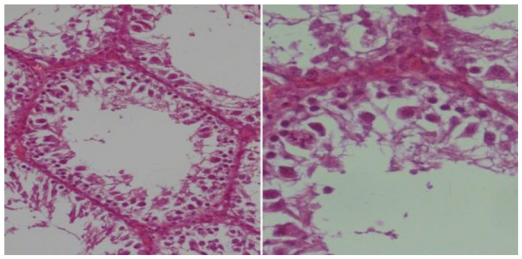


Fig. 6. A photomicrograph of two sections of testis from a positive control rat shows depletion of most of the seminiferous tubules of cells on the left side. The right side is a higher magnification of a part of the previous section shows presence of only spermatogonium cell and primary spermatocyte cell layers. Some of the primary spermatocytes appear abnormal (H&E, ×10 and ×20).

Histopathological study of P. canariensis*, pollens activity on benign prostatic hyperplasia and on stressed testicular tissue, in rats*

Histopathological changes produced after treatment with 70% ethanolic extract, butanol fraction and saponin fraction compared to normal and positive control are shown in Figs. 3–7. Treatment with saponin fraction showed effect close to normal on prostate gland and testicular tissue shown in Figs. 4 and 7, respectively as compared to normal and positive control shown in Figs. 3, 5 and 6.

Discussion

Column chromatographic fractionation of butanol fraction of pollens led to isolation of two saponins identified as dioscin (**1**) and methyl protodioscin (**2**). Their positive Salkowski and Libermann-Büchard tests indicating their steroidal nature and their positive results with Molish's test indicate their glycosidic nature. The ^{1}H NMR spectral data of compound **1** revealed the presence of four methyl protons at δ_H 0.71 (H-18), 0.87 (H-27), 0.95 (H-19) and 1.15 (H-21), respectively. Characteristic anomeric proton of glucose δ_H 4.41 (1H, d, Glu.H-1), where the coupling constant J = 7.8 Hz indicating β-orientation. The olefinic protons resonated as a broad singlet at δ_H 4.47 indicated the presence of a (C=C) in the ring system. Characteristic anomeric proton of rhamnose 5.29 (1H, br, 2Rha., H-1), 5.10 (1H, br, 4Rha, H-1). Number of multiplet signals either at or between 1.14 and 2.09 ppm characteristic for different methylene and methane protons in the structure. The ^{13}C NMR spectral data of compound **1** revealed the presence of 45 carbon atoms with positive Libermann test suggested that steroidal saponin moiety

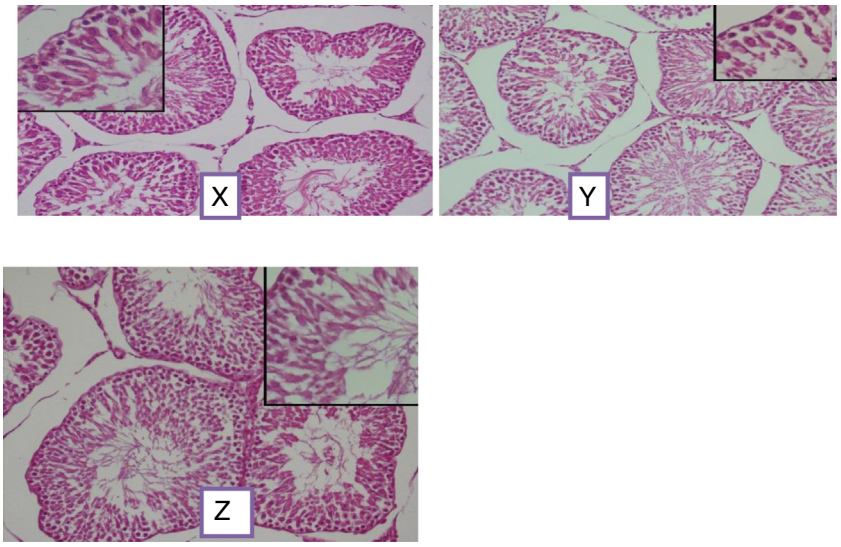


Fig. 7. A photomicrograph of a section of testis from a rat treated with (X) 70% ethanolic extract showing most of the seminiferous tubules appear normal, although some of them show stopping of cell layers at the level of spermatids with no evidence of presence of mature sperms; (Y) butanol fraction shows nearly the same results of the group treated with 70% ethanolic extract; (Z) the two isolated saponins shows normalization of the testicular tissue.

attached to sugars. The signals at δ_C 140.48 (C-5), 121.24 (C-6) and absence of signals at 129–138 indicated steroidal nucleus. Three anomeric carbon signals at δ_C 101.60, 100.91, 99.03, and two C-6 carbon signals of rhamnose at δ_C 18.30, 16.56 indicated the sugar moiety of this compound was elucidated to be one glucose and two rhamnose. A doublet signal appears at δ_C 62.32 assigned to be C-17 of the compound. From ^{1}H NMR spectrum, ^{13}C NMR spectrum, comparing these data with published data of saponins (Idaka et al., 1991), and by direct comparison with authentic sample (Sigma Aldrich, Germany); (co-chromatography, melting point and mixed melting point), the compound **1** is identified as dioscin.

Compound **2** showed four methyl protons at δ_H 0.81 (H-18), 0.87 (H-27), 1.09 (H-19) and 1.25 (H-21), respectively. In addition to two anomeric protons characteristic for glucose δ_H 4.52 (1H, d, J = 7.8 Hz, Glu I H-1), 4.42 (1H, d, J = 7.8 Hz, Glu II H-1), where the coupling constant J = 7.8 Hz indicating β-orientation. The olefinic protons resonated as a broad singlet at δ_H 4.47 indicative of the presence of a (C=C) in the ring system. Characteristic two anomeric protons of rhamnose δ_H 5.40 (1H, br, 2Rha., H-1), δ_H 5.19 (1H, br, 4Rha, H-1). Number of multiplet signals either at or between 1.14 and 2.09 ppm assigned to different methylene and methane protons in the structure. The ^{13}C NMR showed characteristic signals at δ_C 140.46 (C-5), 121.27 (C-6) and absence of signals at 129–138 indicated steroidal nucleus in addition to four anomeric carbon signals at δ_C 101.78, 101.20, 101.10, 99.06, and two C_6 carbon signals of rhamnose at δ_C 18.30, 17.16. The sugar moiety of this compound was elucidated to be two glucoses and two rhamnoses. From ^{1}H NMR spectrum, ^{13}C NMR spectrum and comparing these data with published data of saponins (Idaka et al., 1991), the compound **2** could be identified as methyl protodioscin. It is worthwhile to mention that this is the first time to report the presence of dioscin and methyl protodioscin in the investigated plant pollens and even in the plant itself.

HPLC quantification revealed that methyl protodioscin was the major compound in the 70% ethanolic extract of pollens, amounting to 19.35% while dioscin was found to be 0.013%. The concentrations were calculated using linear regression equations (y = 278.22x + 458.35, R^2 = 0.9982) and (y = 144.22x + 252.263, R^2 = 0.956) obtained from the constructed plots of methyl protodioscin and dioscin, respectively.

Pollens were found to be rich in vit. C (109.13 ppm), followed by vit. A (53.71 ppm), and vit. E (40.60 ppm) was found in adequate amount as compared to WHO daily recommended doses. The total carbohydrates were calculated as 28.12%. Moreover, the analysis of amino acids revealed the presence of 16 amino acids, nine are essential representing 75.07% while the other seven are non-essential amino acids representing 24.93% of total amino acids. Lysine was the major amino acid with 55.67%. It cannot be synthesized by mammals, and plays a major role in absorption of calcium, building protein of muscles, and the production of hormones, enzymes, and antibodies synthesis, by the body (Louis, 2011). Lysine also has an anxiolytic action through its effect as serotonin antagonist (Smriga et al., 2002).

The mixture of saponins (dioscin (**1**) and methyl protodioscin (**2**)) isolated from pollens have ameliorated the prostatic hyperplasia exogenously induced in rats. In addition to its curative effect on stressed testicular tissue. However, the treatment with the 70% ethanolic extract and butanol fraction showed mild to moderate decrease in both stromal and epithelial hyperplasia, as well as, their moderate curative effect on testis.

The potential effect of pollens in the amelioration of induced benign prostatic hyperplasia-histological changes in rats, as well as, the moderate curative effect on stressed testicular tissue, were attributed to its saponin content especially dioscin and methyl protodioscin isolated. This may be related to the anti-inflammatory activity previously reported for both saponins (Lee et al., 2015). These results are in conformity with the traditional use of palm pollens as fertility enhancers as well as the reported protective effect of date palm pollens extract in atypical prostatic hyperplasia induced in rats (Elberry et al., 2011; Tahvilzadeh et al., 2016).

In conclusion, the use of pollens of *P. canariensis* as a dietary supplement is recommended not only as a good source of carbohydrates, proteins, vitamins and amino acids but also it has good prophylactic effect against prostatic hyperplasia. Further investigation of the mechanism of action of these saponins and how they contribute in the treatment of benign prostatic hyperplasia (BPH) must be conducted.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with

those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Author's contributions

MSH designed the study, supervised the whole work and contributed to critical reading of manuscript. AMK collected the plant sample, performed the experiments, and interpreted the results. AAS performed the biological study. RMSA participated in the study design, data interpretation, supervised the laboratory work and wrote the manuscript. All authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2018.09.003.

References

Adawy, S.S., Hussein, E.H., El-Khishin, D., Moharam, H., El-Itriby, H.A., 2002. Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars: II. RAPD and ISSR profiling. Arab. J. Biotechnol. 5, 225–236.

AOAC, 1995. Official methods of analysis. Association of Official Analytical Chemists, Arlington, VA, USA.

Asami, A., Hirai, Y., Shoji, J., 1991. Studies on the constituents of Palmae plants. VI. Steroid saponins and flavonoids of leaves of *Phoenix canariensis* hort. ex Chabaud, *P. humilis* Royle var. *hanceana* Becc., *P. dactylifera* L., and *Licuala spinosa* Wurmb. Chem. Pharm. Bull. 39, 2053–2056.

Block, R.J., Durrum, E.L., Zweig, G., 1958. Paper Chromatography and Paper Electrophoresis. Academic Press Incorporated Publishers.

Chung, Y.-L., Pan, C.-H., Wang, C.C.-N., Hsu, K.-C., Sheu, M.-J., Chen, H.-F., Wu, C.-H., 2016. Methyl protodioscin, a steroidal saponin, inhibits neointima formation *in vitro* and *in vivo*. J. Nat. Prod. 79, 1635–1644.

Council, N.R., 2010. Guide for the Care and Use of Laboratory Animals. National Academies Press.

Drury, R., Wallington, E., 1980. Preparation and fixation of tissues. Carleton's Histol. Tech. 5, 41–54.

DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, t.F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Elberry, A.A., Mufti, S.T., Al-Maghrabi, J.A., Abdel-Sattar, E.A., Ashour, O.M., Ghareib, S.A., Mosli, H.A., 2011. Anti-inflammatory and antiproliferative activities of date palm pollen (*Phoenix dactylifera*) on experimentally-induced atypical prostatic hyperplasia in rats. J. Inflamm. 8, 40.

Hifnawy, M.S., Mahrous, A.M., Ashour, R.M., 2016. Phytochemical investigation of *Phoenix canariensis* Hort. ex Chabaud leaves and pollen grains. J. Appl. Pharm. Sci. 6, 103–109.

Hifnawy, M.S., Mahrous, A.M., Sleem, A.A., Ashour, R.M., 2017. Comparative chemical and biological studies of leaves and pollens of *Phoenix canariensis* hort. ex Chabaud. Future Med. Chem. 9, 871–880.

Huang, T., Chen, C., Wefler, V., Raftery, A., 1961. A stable reagent for the Liebermann-Burchard reaction. Application to rapid serum cholesterol determination. Anal. Chem. 33, 1405–1407.

Idaka, K., Hirai, Y., Shoji, J., 1991. Studies on the constituents of Palmae plants. V. Steroid sapnins and flavonoids of leaves of *Phoenix rupicola* T. Anderson, *P. loureirii* Kunth, *P. reclinate* NJ Jacquin, and *Arecastrum remanzoffianum* Beccari. Chem. Pharm. Bull. 39, 1455–1461.

Jain, S.M., Al-Khayri, J.M., Johnson, D.V., 2011. Date Palm Biotechnology. Springer Science and Business Media.

Jeyaraj, D.A., Udayakumar, T.S., Rajalakshmi, M., Pal, P.C., Sharma, R.S., 2000. Effects of long-term administration of androgens and estrogen on rhesus monkey prostate: possible induction of benign prostatic hyperplasia. J. Androl. 21, 833–841.

Kroyer, G., Hegedus, N., 2001. Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. Innov. Food Sci. Emerg. 2, 171–174.

Krueger, R., 2001. Date palm germplasm overview and utilization in the USA. In: Proceedings International Conference on Date Palms.

Lee, H.J., Park, J.S., Yoon, Y.P., Shin, Y.J., Lee, S.K., Kim, Y.S., Hong, J.-H., Son, K.H., Lee, C.J., 2015. Dioscin and methylprotodioscin isolated from the root of *Asparagus cochinchinensis* suppressed the gene expression and production of airway MUC5AC mucin induced by phorbol ester and growth factor. Phytomedicine 22, 568–572.

Lee, M.-Y., Shin, I.-S., Seo, C.-S., Lee, N.-H., Ha, H.-K., Son, J.-K., Shin, H.-K., 2012a. Effects of *Melandrium firmum* methanolic extract on testosterone-induced benign prostatic hyperplasia in Wistar rats. Asian J. Androl. 14, 320.

Lee, Y.C., Lin, J.T., Wang, C.K., Chen, C.H., Yang, D.J., 2012b. Antiproliferative effects of fractions of furostanol and spirostanol glycosides from yam (*Dioscorea pseudojaponica* Yamamoto) and diosgenin on cancer and normal cells and their apoptotic effects for mcf-7 cells. J. Food Biochem. 36, 75–85.

Lepor, H., 2004. Pathophysiology, epidemiology, and natural history of benign prostatic hyperplasia. Rev. Urol. 6, S3–S10.

Louis, S., 2011. Goodman and Gilman's Pharmacological Basis of Therapeutics.

Nöll, G.N., 1996. High-performance liquid chromatographic analysis of retinal and retinol isomers. J. Chromatogr. A 721, 247–259.

Okoronkwo, N.E., Mba, K.C., Nnorom, I.C., 2017. Estimation of protein content and amino acid compositions in selected plant samples using UV-Vis spectrophotometeric method. Am. J. Food Sci. Health 3, 41–46.

Pyka, A., Sliwiok, J., 2001. Chromatographic separation of tocopherols. J. Chromatogr. A 935, 71–76.

Romeu-Nadal, M., Morera-Pons, S., Castellote, A., Lopez-Sabater, M., 2006. Rapid high-performance liquid chromatographic method for Vitamin C determination in human milk versus an enzymatic method. J. Chromatogr. B 830, 41–46.

Smriga, M., Kameishi, M., Uneyama, H., Torii, K., 2002. Dietary L-lysine deficiency increases stress-induced anxiety and fecal excretion in rats. J. Nutr. 132, 3744–3746.

Tahvilzadeh, M., Hajimahmoodi, M., Rahimi, R., 2016. The role of date palm (*Phoenix dactylifera* L) pollen in fertility: a comprehensive review of current evidence. J. Evid. Based Complement. Altern. Med. 21, 320–324.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., Kaur, H., 2011. Phytochemical screening and extraction: a review. Int. Pharm. Sci. 1, 98–106.

Wahba, H.E., Sarhan, A.Z., Salama, A.B., Sharaf-Eldin, M.A., Gad, H.M., 2017. Effect of seasonal variation on the growth and chemical composition of *Cynara cardunculus* L. plants. J. Mater. Environ. Sci. 8, 318–323.

Wang, Z., Zhou, J., Ju, Y., Zhang, H., Liu, M., Li, X., 2001. Effects of two saponins extracted from the *Polygonatum zanlanscianense* pamp on the human leukemia (HL-60) cells. Biol. Pharm. Bull. 24, 159–162.