Antioxidant and Estrogen Like Activity of the Seed of *Phoenix dactylifera* L. Palm Growing in Egyptian Oases

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Abstract: Developing the Egyptian desert, especially the Egyptian Oases, is considered as one of the most important National goals. Thousands species of medicinal, aromatic and food plants grow in the Egyptian desert, creating a base of raw materials to be explored for drug and nutraceutical production. This work focused on an important biologically active plant growing in the Egyptian Oases which is *Phoenix dactylifera* L. Family Palmae. The non-polar and polar successive extracts of the seeds of *Phoenix dactylifera* L. which is widely distributed in El-Dakhla Oases were tested for the antioxidant activity by an in-vitro bioassay technique according to the β-carotene bleaching method, as well as for the estrogen-like-activity. The effect of the extracts on nutritional parameters was also assessed. Acute toxicity test of the non-polar and polar extracts was carried out. The results showed that both extracts have antioxidant and estrogen like activity with different degrees. Variable significant changes in the nutritional parameters were noticed. The non-polar and polar extracts showed complete safety. The bioactive fractions were subjected to a phytochemical examination and the biologically active compounds were identified by using different chromatographic techniques. **Conclusion:** Both non-polar and polar extracts of the seeds of the studied seeds of *Phoenix dactylifera* showed antioxidant and estrogen like activity with different degrees which may be attributed to the presence of sterols, tannins and flavonoids. Both extracts showed complete safety. [Report and Opinion. 2009;1(3):1-8]. (ISSN: 1553-9873).

Key words: Palmae, *Phoenix dactylifera* L., Antioxidant, Estrogen like activity, Bioactive fractions.

Introduction

Egypt is characterized by the presence of a great number of palms abundantly distributed all over the country. Palms in general possess many economic uses, the fruits of some species can be considered as an important crop used as nutrient, other species are used in the production of sugar, starch, fiber, wax, timber and oil which can be used in many pharmaceutical and food products. Palms have become increasingly important in commercial horticulture. It is important to note that Palmae, an ancient Family of 3400 species, its classification being based largely on morphological and anatomical characters of the leaf and fruit. A recent study on gross morphological characteristics classified and reorganized six subfamilies within the Palmae (Bolombery and Tony, 1982; Uhl and Dransfield, 1987).

Rare publications have been reported for the Family concerning its phytocjemical studies, probably because of the difficulty of collecting fresh material and getting it authenticated. Most work has been carried out on economically important plants such as *Phoenix dactylifera, Cocos nucifera* and other plams cultivated for food and oils (Al-Shabib and Marshall, 2003).

Phoenix dactylifera L. Family Palmae is known in Arabic as Nakl; fruit as Balah and Tamr; in English as Date palm, in French as Dattier. The plant grows in Egyptian desert, especially in Egyptian Oases.

Reviewing the folk medicine as well as the literature, some species of Palms have been reported to possess many important biological effects such as *Phoenix dactylifera* which is one of the most important economic plants of Palmae. The edible fruit of this species are cited as the most important products (Vayalil, 2002). Dates are cooling, tonic, diuretic, fattening, aphrodisiac, alexeteric and are useful in leprosy thrist, asthma, bronchitis, fatigue, tuberculosis, abdominal complains, fever, vomiting, wanderering of the mind and loss of consciousness. In addition, dates have demulcent, expectorant and laxative effect. Dates are also used internally to clear enigmatic or to regulate the urine and in vaginal pessaries with other ingredient to enhance fertility. Kernels of dates are used for healing ulcers of genital organs. Date Palm pollen grains have gonadotrophic activity.

The aim of the present work is to study the antioxidant and estrogen like activity of the non-polar and polar successive extracts of *Phoenix dactylifera* seeds as well as the phytochemical constituents of the bioactive fractions. The safety of the biologically active fractions was also determined.

MATERIALS AND METHODS

MATERIALS

Plant materials:
Samples of the seeds of *Phoenix dactylifera* L. Family Palmae were collected from plants growing in El Dakala Oases, dried and then powdered to No. 26 scieve and kept in dark well closed container. Voucher specimens are deposited in the herbarium of the National Research Centre, Giza, Egypt.

Animals:
Rats: Twenty-four female immature white albino rats with average body weight of 45.5 ± 0.3g were obtained from the animal house of National Research Centre, Giza, Egypt. The animals were kept individually in stainless steel cages at room temperature. Water and food were given ad-libitum.

Mice: Adult normal male and female albino mice of 21-25 g body weight were used in acute toxicity test.

Diets:
A balanced diet composed of 10% protein, 10% corn oil, 23.5% sucrose, 47% maize starch, 5% fiber, 3.5% salt mixture (Briggs & Williams, 1963), and 1% vitamin mixture (Morcos, 1967) was prepared for feeding the rats all over the experimental period.

METHODS

Phytochemical screening of the seeds under investigation: The powdered seed was screened for carbohydrates and/or glycosides, reducing sugars, tannins, flavonoids, alkaloids and/or nitrogenous bases, saponins, unsaturated sterols and/or triterpenes and coumarins using chemical and chromatographic techniques (Harborne, 1984)**.**

Preparation of the successive extracts with selective organic solvents: The air-dried powdered seeds under investigation were extracted successively in a continuous extraction apparatus until exhaustion with petroleum ether (40-60 °C), ether, methanol and 50% aqueous methanol. The solvents were stripped off under reduced pressure and dried to constant weight in vacuum desiccator over anhydrous calcium chloride. The extracts of both methanol and 50% anhydrous methanol were pooled together as polar extract. Those of petroleum ether and ether were mixed to be the non-polar fraction.

Determination of antioxidant activity: Antioxidant activity of non-polar and polar extracts was determined according to the β-carotene bleaching method using D,L α-tocopherol as standard (Velioglu *et al*., 1998). 1 ml of β-carotene solution (0.2 mg/ml in chloroform) was transferred to different round bottom flasks (100 ml) containing 0.02 ml of linoleic acid and 0.2 ml Tween 20. Each mixture was then dosed with 0.2 ml of 80% MeOH (as control), or 50 mg/L of D,L α-Tocopherol (as standard) or corresponding plant extracts. After evaporation to dryness under vacuum at room temperature, 50 ml of oxygenated distilled water was added and the mixtures were shaken to form a liposome solution. The mixtures were then subjected to thermal auto-oxidation at 50 °C for 2h. The absorbance of the solution was measured immediately at 470 nm after their preparation (t = 0 min) and at the end of the experiment (t = 120 min) using UVPC spectrophotometer. All samples were assayed in triplicate. Antioxidant activity (AA) was calculated as percent inhibition relative to control using the following equation (Al-Saikhan *et al*., 1995).

$$AA = (R_{control} - R_{sample} / R_{control}) X\ 100$$

Where $R_{control}$ and R_{sample} were the bleaching rates of β-carotene in reactant mixture without antioxidant and with plant extract, respectively.

Studying the estrogen like activity and safety of the extracts: The estrogen like activity was determined according to Ali *et al*. (1998). Rats were divided into four groups (6 rats/group). Rats of first and second group were given daily oral dose of non-polar and polar extracts respectively (500mg/kg rat body weight for 10 days). Rats of the third group were daily subcutaneously injected with estradiol benzoate (2mg/kg rat body weight) for 10 days and served as reference group. Rats of the fourth group were run as control where no medication or extract was given. During the experimental period rats were fed on balanced diet. All rats were examined daily for opening of vagina as one parameter of estrogenic activity. Body weight and food intake were recorded once weekly. At the end of the experiment (10 days); total food intake, body weight gain and food efficiency ratio were calculated. Blood samples were withdrawn on heparin from eye vein orbital after an overnight fast. Blood samples were centrifuged for 10 min at 3500 rpm for separation of plasma for determination of 17-β estradiol using ELISA technique (as second parameter of estrogenic activity) and glucose (Tinder, 1969), cholesterol (Watson, 1960), total protein (Rheinhold, 1953), urea (Fawcett and Scott, 1960), creatinine (Houot, 1985) and activity of alanine transaminase (ALT) (Reitman and Frankel, 1957) and asprtate transaminase (AST) (Reitman and Frankel, 1957) adopting colorimetric assay. The uteri of the rats were excised and weighed. Uteri were dried to a constant weight in an oven at 100 °C and their dry weights were determined (as third parameter of estrogenic activity). The results obtained were expressed as the Mean ± SE and the significance of the results was analyzed statistically adopting Student's t-test.

Acute toxicity test: Acute lethal toxicity test of non-polar and polar extracts was carried out according to Goodman *et al*., (1980). The 24 h mortality counts among equal sized groups of mice (8 animals/group) receiving progressively increasing oral dose levels of the different extracts were recorded.

Identification of the bioactive constituents of non-polar fraction: Three grams of the non-polar extract was saponified by refluxing with alcoholic potassium hydroxide 10%, after dilution with water, the unsaponifiable fraction was extracted with ether. The ether was evaporated; the unsaponifiable fraction was kept for TLC and GLC analysis. The aqueous mother liquor was acidified with 10% hydrocholoric acid and the liberated fatty acids were extracted with ether. Ether was evaporated and the residue was kept for studying the total fatty acids (EL-Said and Amer, 1965).

The unsaponifiable matter was analyzed by both TLC and GLC: For analysis through TLC, silica gel "G" plate was used as adsorbent and Benzene : Ethyl acetate (86:4) (v/v) as solvent system. The developed chromatoplates were detected with vanillin-sulphuric acid reagents, and heated at 100 °C for 5 minutes (Stahl, 1969).

The unsaponifiable fraction was analyzed by GLC adopting the following conditions: Column: 10% OV-101 packed column; Stationary phase: Chromosorb W-HP; Detector temperature: 290°C; Injector temperature, 28°C; Carrier gas N_2; flow-rate 30 ml/min; air flow-rate: 300ml/min; H_2 Flow-rate 30ml/min; Detector FID; Chart speed: 0.5 cm/min; Oven program: Initial temperature, 70°C; Final temperature, 270°C; total time, 85 min. Identification of hydrocarbons and sterols contents of the unsaponifiable matter was carried out by comparison of their retention times with co-injected authentic reference compounds. Quantification was based on peak area integration.

Analysis of fatty acids: The fatty acid fraction previously prepared was subjected to methylation (Vogel, 1961) and analysis by GLC of the methyl ester adopting the following conditions: Stationary phase, 10% diethylene glycosuccinate packed column; oven temperature, 170°C; detector temperature, 300°C; injector temperature, 250°C; Carrier gas, N_2; flow-rate, 30ml/min; air flow-rate, 350ml/min; H_2 flow-rate, 350ml/min; detector, FID; Chart speed, 2cm/min. Identification of the fatty acid methyl ester was carried out by direct comparison of retention times of each of the separated compounds with authentic samples of the fatty acid methyl esters analyzed under the same conditions. Quantification was based on peak area integration.

Results and Discussion

Developing the Egyptian Oases through the use, as well as protection and conservation of its endogenous medicinal and food plants is a National and International goal. This work focused on the biological and phytochemical study of the seed of *Phoenix dactylifera* palm growing in these areas, with the aim of discovering novel therapeutic agents or functional food ingredients.

Phytochemical screening of seed revealed the presence of carbohydrates, and/or glycosides, sterols and/or triterpenes, tannins, flavonoids, alkaloids and/or nitrogenous bases.

The antioxidant activity of the non-polar and polar extracts of the seeds of *Phoenix dactylifera* L. are shown in table (1). The synthetic antioxidant D,L α-tocopherol showed the highest antioxidant activity (92%). The non-polar extract showed higher antioxidant activity (57.8%) than the polar extract (53.9%).

In the estrogen like activity experiment, the biochemical parameters of the different experimental groups are shown in table (2). The treatment of immature female rats with date seed extracts or estradiol showed significant increased in the plasma level of estrogen compared to control. The maximum elevation was due to estradiol treatment (97%, $p < 0.001$), followed by the polar extracts (46%, $p < 0.001$), while the non-polar extracts showed the lowest elevation (35%, $p < 0.001$). This result indicated that both extracts have estrogen like activity but still below the estradiol effect. Rats treated with estradiol produced significant increase in plasma creatinine, urea and plasma activity of AST compared to control, while the biologically active extracts produced no changes in these parameters. Estradiol and the extracts did not produce any significant change in plasma glucose, total cholesterol, total protein and activity of ALT. These results reflect the safety of administration of date seed bioactive extracts compared to estradiol.

Table1: Antioxidant activity of the studied extracts and tocopherol as standard.

Extract type	Percentage antioxidant activity
Non-polar extract	57.8
Polar extract	53.9
DL α-tocopherol	92

Table (2): Biochemical parameters of different experimental groups of the estrogenic test.

Plasma parameters	Group (Mean ± SE)			
	Control	Estradiol	Non-polar extract	Polar extract
Glucose (mg/dl)	51.5 ± 1.839	57.2 ± 1.782	55.8 ± 0.946	52.5 ± 1.384
% Change		2	8	0.4
Total Cholesterol (mg/dl)	63.7 ± 1.782	65.8 ± 1.992	64.8 ± 1.621	61.3 ± 1.819
% Change		3	2	-4
Total protein (g/dl)	6.9 ± 0.087	6.7 ± 0.097	6.6 ± 0.198	6.7 ± 0.108
% Change		-3	-4	3
Creatinine (mg/dl)	0.58 ± 0.014	0.66* ± 0.032	0.61 ± 0.008	0.61 ± 0.009
% Change		14	-4	5
Urea (mg/dl)	24 ± 1.183	29.8** ± 0.946	26.7 ± 1.054	26.3 ± 1.115
% Change		24	11	10
AST (IU/l)	137.5 ± 1.258	148** ± 1.064	139 ± 1.712	139.5 ± 1.607
% Change		8	1	2
ALT (IU/l)	56.3 ± 1.475	60.3 ± 1.744	58.2 ± 1.514	58.2 ± 1.661
% Change		7	3.4	3
17-β Estradiol (pg/ml)	34.8 ± 1.621	68.5*** ± 1.543	47*** ± 1.154	50.8*** ± 0.946
% Change		97	35	46

Values significantly differ from control:
*: $p < 0.05$, **: $p < 0.010$, ***: $p < 0.001$.

The effect of administration of the date seed extracts or estradiol on uterine weight and opening of vagina are shown in table (3). The administration of non-polar and polar extracts or estradiol to immature femal rats produced significant increase in uterine weight ($p < 0.001$) compared to control. Estradiol administration produced the maximum increase (99%), while the non-polar extract produced the lowest

increase (34%). Estradiol treatment produced the maximum increase in the rate of opening of vaginal orifice followed by the polar extract, while the non-polar extract produced the lowest effect. These results clarified the estrogen like activity of both date seed extracts, which was below the estradiol effect.

Table (3): The effect of administration of date seeds extracts or estradiol on uterine weight and opening of vagina.

Parameters	Control	Estradiol	Non-polar extract	Polar extract
Uterine weight (mg) (Mean±SE)	20.5 ± 0.764	40.7* ± 1.358	27.5* ± 0.764	31.8* ± 0.946
% Change		99	34	55
Degree of opening vagina	0/6	4/6	2/6	3/6

Values significantly differ from control: *: $p < 0.001$.

Nutritional parameters of the different experimental groups are seen in table (4). The administration of the date seed extracts or estradiol showed significant lower values of the final body weight and food efficiency ratio ($p < 0.001$) in spite of the significant increase in total food intake in the group given estradiol and polar extract ($p < 0.005$ and $p < 0.025$ respectively) compared to control. The worst effect on nutritional parameters was attributed to the non-polar extract. The reduction in nutritional parameters may be a bad effect in growing stage however it may have beneficial use in obese subjects, especially that the safety of the extracts have been proven on both liver and kidney function in the present study. The polar extract may increase the energy expenditure thereby reducing the body weight in spite of the significant increase of food intake.

The acute lethal toxicity test revealed that both non-polar and polar extracts were very safe up to 12g/kg mice body weight which corresponds to 93g/70kg man body weight for human when the dose of mice was extrapolated to corresponding estimates in human adopting interspecies dosage conversion scheme (Paget and Barnes, 1974). This reflects the highest safety of the bioactive extracts.

Table (4): Nutritional parameters of different experimental groups of the estrogenic test.

Parameters	Group (Mean ± SE)			
	Control	Estradiol	Non-polar extract	Polar extract
Initial body weight (g)	45.8 ± 1.077	45.3 ± 0.843	45.4 ± 0.615	45.2 ± 0.703
Final body weight (g)	74.2 ± 0.654	58.2*** ± 0.654	53.3*** ± 1.174	67.7*** ± 1.022
Body weight gain (g)	28.3 ± 0. 843	12.8*** ± 0.703	8*** ± 1.291	22.5*** ± 0.764
Total food intake (g)	47.9 ± 1.538	55.5** ± 1.875	44.5 ± 3.929	52.8* ± 1.352
Food intake (g/day)	4.8 ± 0.154	5.6** ± 0.187	4.5 ± 0.393	5.3* ± 0.135
Food efficiency ratio	0.592 ± 0.015	0.231*** ± 0.007	0.178*** ± 0.022	0.426*** ± 0.009

Values significantly differ from control:
*: $p < 0.025$, **: $p < 0.005$, ***: $p < 0.001$.

Chromatographic investigation of the non-polar extract of the seeds of *Phoenix dactylifera* revealed that the unsaponifiable fraction contains 66% hydrocarbons and 15.6% of terpenoidal compounds which are cholesterol, β-sitosterol, campasterol, stigmasterol and β-amyrin (in a percentage of 3.2%, 3.2%, 2.8%, 2.6%, 3.8% respectively). The fatty acids fraction after methylation and analysis by GLC revealed the presence of lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, stearic and oleic acid as 20%, 2.7%, 10.3%, 3.2%, 6.8%, 3.3%, 32.8% and 10% respectively. The results also revealed that the percentage of saturated fatty acids was 79.1 and that of unsaturated fatty acids was 10.

In the present study, the non-polar extract of the seed of *Phoenix dactylifera* which exhibited a remarkable antioxidant and estrogen like activity contains different sterols such as; campasterol, stigmasterol, β-amyrin and β-sitosterol. So, the antioxidant and estrogen like activity of the non-polar extract may be attributed to the presence of the fore-mentioned sterols. Some of the oxidation products of stigmasterol have been reported previously to have estrogenic activity (Newill *et al*., 2007). Phytosterols such as campasterol, β-sitosterol and β-amyrin have been shown previously to possess estrogenic activity (Nakari, 2005 and Nieminen *et al*., 2004). Antioxidant activity of β-sitosterol has been confirmed

previously in experimental cancer model (Jayaprakasha *et al*., 2007). Also plant sterols have been shown to reduce oxidative stress during hyperlipidemia (Fuhrman *et al*., 2007). Phytosterols may have antioxidant activity through acting as hydrogen donor (Gordan and Magos, 1983 and Oomah and Mazza, 1999). Previously we have proved that petroleum ether extract of the seeds of *Phoenix dactylifera*, Zaghlool, possess low in-vitro antioxidant activity (8%) (Mohamed and Al-Okbi, 2005). In the present study, polar extract of the seeds exhibited an antioxidant activity slightly lower than the non-polar extract and a remarkable significant estrogen like activity. These effects may be ascribed to the presence of flavonoidal compounds and tannins which were shown from the phytochemical screening of the powdered seeds. Several flavonoids (eg, isoflavones) are known as phytoestrogens, based on their ability to mimic estrogen in mammals (Girppo *et al*., 2007). Also Wong *et al*. (2007) showed that certain flavonoids possess estrogenic activity. Also phenolic compounds possess an antioxidant activity due to their redox properties which allow them to act as reducing agents, hydrogen donators and metal chelator thereby reduce lipid oxidation (Duthie *et al*., 2000 and Kluth *et al*., 2006), quench reactive oxygen species and protect from pro-oxidative damage (Kaindle *et al*., 2007). It was also cited that the antioxidant capacity of phytochemicals are related to total phenolics compounds including flavonoids (Wolfe and Liu, 2007 and Soobrattee *et al*., 2008). Tannins have reported to possess antioxidant activity (Tzulker *et al*., 2007 and Ito *et al*., 2008). As a matter of fact the antioxidant extracts may possess a protctive effect towards diseases in which free radicals are involved such as cancer, diabetes, chronic inflammation, cardiovascular and cerebrovascular diseases (Rice Evan and Diplock, 1993).

Conclusion: Both non-polar and polar successive extracts of the seeds of the studied date, *Phoenix dactylifera* showed an antioxidant and estrogen like activity with different degrees which may be attributed to the presence of sterols, tannins and flavonoids. Both extracts showed complete safety. Significant decrease in body weight gain and food efficiency ratio was noticed on administration of both bioactive extracts.

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