Comparative Investigation of Minerals, Amino acids, Lipoidal Contents and General Analysis of *Chamaerops humilis* L. Organs Growing in Egypt

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Abstract: In this paper, a comparison of nutritional value of the pollen grains and the fruits of *chamaerops humilis L.* and comparison of lipoidal content of the pollen grains, the fruits and the leaves was carried out. The preliminary phytochemical screening of the petroleum ether extracts of different organs of *chamaerops humilis (L)* revealed that the petroleum ether of the leaf, the pollen grain, the fruit are rich in lipid content and sterols and/or triterpenes. Total protein, fats, ash, carbohydrates and moisture percentages were determined in pollen grains and fruits. Minerals analysis show that Potassium was the major composition of macro-elements in both organs followed by sodium.
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1. Introduction

Arecaceae family are flowering plants. Arecaceae family (palm family) have been important to human beings throughout history and about 200 genera with around 300 species are currently known. [1,2] Arecaceae family(palm family) have been important to human throughout much of history. many common products and foods are derived from palms for example the pejibaye palm (*Bactris gasipaes* Kunth), also known as peach palm the fruit of this palm can be used as a cereal for human consumption and has high food values for some nutrients[3] chamaerops is a genus of flowering plants in the family Arecaceae (palm family), comprising asingle species *chamaerops humilis* (European fan palm or Mediterranean dwarf palm).

Chamaerops humilis L. is a shrub-like clumping palm, with several stems growing from a single base. *chamaerops humilis L.* is a medicinal plant which belong to the Arecaceae family. [4-6] The seed of *C. humilis* showed high content of lipids, which implies an importance of the species for feeding wild life during winter, and a high degree of unsaturation [7] but no studies were done on fruits, pollen grains and leaves. Traditional medical practices survey carried out in Western Algeria (Tlemcen department) and Morocco revealed that *chamaerops humilis L.* is taken as stipe or leaf extracts for the treatment of diabetes, digestive disorders, spasm, toning and gastrointestinal disorders diseases. [8-10] Sterols, also known as steroid alcohols, are a subgroup of the steroids and an important class of organic molecules. They occur naturally in plants, animals, and fungi, with the most familiar type of animal sterol being cholesterol.

2. Material and Methods

Plant material

Samples of *chamaerops humilis L.* used in this study were collected during the years (2012-2013) from Orman zoo in Giza, Cairo, Egypt. The plants were kindly identified by Dr. Mohamed Gibali, Senior Botanist and madam tries.

The Leaves, the pollen grains and the fruits were air-dried, powdered and stored for chemical and biological studies.

Preparation of the extract

For the investigation of lipoidal matter content the air-dried powdered leaves, pollen grains and fruits of *chamaerops humilis L.* (50 g for each) were exhaustively extracted with petroleum ether and the solvent was evaporated under reduced pressure, then saponification of petroleum ether extracts of the leaves, the pollen grains and the fruits to separate sap and unsap matters. (1g) of defatted powder of the pollen grains and the fruits for amino acid analysis. (10g) of dried powder of the pollen grains and the fruits for minerals analysis. (20g) of dried powder of the pollen grains and the fruits for nutritional value analysis.

Study of the Lipoidal Content

The lipoidal content of the petroleum ether extract of the leaves, the pollen grains and the fruits of *chamaerops humilis* L. was investigated by GLC after saponification.

1. **Preparation of unsaponifiable (USM):** [11]

The petroleum ether extracts of the leaves, pollen grains and fruits of *chamaerops humilis L.* (1.5 g, 2.2 g a residues of pollen grains and leaves respectively and 1.5 g oily residue of fruits) were separately saponified by heating under reflux with 100 ml 10 %

alcoholic KOH and 40 ml benzene for 24 hours to ensure complete hydrolysis. The saponified solutions were then concentrated under reduced pressure and suspended in 100 ml Distilled H_2O followed by extraction with diethyl ether (10×50 ml) till exhaustion. the combined ethereal extracts in each case, were washed several times with Distilled H_2O till free from alkalinity the dried over anhydrous sodium sulphate, followed by evaporation to dryness. The solvent-free residues obtained represent the unsaponifiable matters (USM).

2. **Isolation of the fatty acids from the saponifiable fractions:**

The aqueous alkaline solution left after separation of the unsaponifiable matters was, in each case, acidified with dilute HCL (10%) to librate the free fatty acids (FA). These were extracted with diethyl ether (5×50ml). the combined ethereal extracts were separately washed with Distilled H_2O till free from acidity then dried over anhydrous sodium sulphate and evaporated to dryness.

3. **Preparation of the fatty acid methyl esters (FAME):** [12]

Aliquots of the fatty acid mixtures (0.5 g) were, separately, dissolved in 50 ml methanol, 2.5 ml of sulphuric acid were added and the mixture refluxed for one hour on a boiling water bath. The cooled mixture was diluted with 20ml of H2O and then extracted with ether (5×50 ml). the combined ethereal extracts, in each case, were washed with water till neutral to litmus paper, dried over anhydrous sodium sulphate and the solvent was evaporated under reduced pressure. The dried residue was, in each case, saved in a dessicator for GLC analysis.

4. **GLC analysis of the USM and FAME:**

USM analysis was carried out on Hewlett-Packard HP-6890 N network GC system equipped with a FID detector. Analysis was performed on an HP-5 column; using N2 as a carrier gas, injection temperature 250oC, detector temperature 300oC. Aliquots, 2 µL each, of 2% chloroformic solutions of the analyzed USM and reference samples were co-chromotographed. Identification of the components was based on comparison of the retention times of their peaks with those of the available authentic samples. The relative amount of each component was calculated via peak area measurement by means of computing integrator.

FAME were analysed on a pye Unicam 304 series gas chromatograph equipped with a FID detector. Analysis was performed on 10% PEGA (on chromosorb W-AW, 100-120 mesh) column; using N2 as carrier gas, injection temperature 250oC, detector temperature 280oC. Aliquots, 2 µL each, of 2% chloroformic solutions of the analyzed FAME and reference fatty acid methyl esters were co-chromatographed. Identification of the fatty acids (FA) was carried out by comparing the retention times of their methyl esters with those of the available reference fatty acids similarity analyzed.

- Preparation of samples for General chemical analysis (total protein, crude fat, ash, crude fibre and moisture:

Percentage of total protein, crude fat, ash, crude fibre and moisture were determined according to the methods outlined by AOAC (1980).[13]

Total protein was determined by the Micro-Kjeldahl method, which involves digestion or oxidation of the sample with conc. H2SO4 followed by steam distillation. Ammonia formed at high pH was received in 4% boric acid solution then titrated with 0.1N HCl. The percent of protein was obtained by multiplying N% by the factor of 6.25.

Determination of total crude fat depends on the difference in weight between the original sample and the hexane-extracted sample in Soxhlet apparatus.

Total ash obtained by heating a crucible containing the sample in a muffle furnace at 550 oC overnight (till constant weight.

Total crude fibre (edible fibre) was determined by boiling the sample (after extraction of the fats, as they form gelatinous layer with the alkali used in the test) for 30 minutes with dilute acids followed by boiling for another 30 minutes with dilute alkali to get rid of proteins, starches and carbohydrates, leaving only some carbohydrates which resist acid and alkali and some ash minerals. Ashing will then cause the fibre only to volatilize, while the ash will not.

Total carbohydrates content (as % of dry weight) was obtained by difference.

Total moisture was estimated (for the fresh samples) by heating at 105 ^oC overnight (till constant weight).

-Amino acids determination:

Determination of all amino acids other than tryptophan was carried out according to the method described by Winder and Eggum (1966). [14]

- Preparation of the protein for amino acid analysis: [15]

One g of the defatted powdered of each of the pollen grains and the fruits were stirred in10% sodium chloride solution for one hour then filtered. An equal volume of 10% trichloroacetic acid (TCA) solution was added to the filtrate of each sample. The protein was precipitated as white flocculent amorphous precipitate, collected by centrifugation, washed with 5% TCA solution, then with ether and absolute ethanol and dried in a vacuum desiccator. The crude dried protein was dialyzed by parchment membrane. The non-dialyzed fraction was collected and lyophilized by freeze drying.

- Amino acid analysis:

The protein (1mg) of each sample was hydrolysed with 6N HCl at 105°C for 24 hr in a sealed tube. [16] after cooling and filtering, the residue left after filtration was washed with distilled water and the combined filtrate were completed to 25 ml in volumetric flask. A portion of the filtrate (5ml) of each samples was evaporated to dryness at room temperature in desiccator under vacuum. The residue of each sample was dissolved in 5 ml buffer (0.2 sodium citrate, pH 2.2) and the solution was filtered through 0.22 µm membrane. Twenty microliters of each sample of the final filtrate were injected in the instruments capsule for quantitative determination of the amino acids. [17] The cationic exchange resins UL-tropac sodium and special programmed buffer system were used (citrate buffer 0.2 N with three different pH, at 3.2, 4.25 and 6.45 at rate of 32 ml/ hour). The effluent of each sample was met by a stream of ninhydrin reagent at rate 25ml/hour. The quantitative estimation of the amino acids depends on the colorimetric determination of blue color. [18] Amino acids were identified by comparison with the standard chromatograph of known authentic amino acids.

- preparation of Mineral element for analysis:

Mineral elements were determined by atomic absorption spectrometry adobting the reported methods. [25] Dry ashing was carried out by ashing 1 g of the pollen grains and the fruits samples in a muffle furnace at 500°C overnight. The ash of each sample was allowed to cool then was dissolved in 5 ml 20% HCl (with slight warming). The solution of each was then filtered through an acid-washed Whatman filter paper no. 1 in a volumetric flask 50 ml, washing the filter paper several times with deionized water. Dilution of each sample with deionized water to specific volume was then carried out after which each element was determined spectrophotometrically in the samples solution. The concentration of each element was determined from the standard calibration curve, obtained using known concentrations of standard solutions prepared from stock solutions. For Ca and Mg, sample, blank and standard solutions contained 1% w/v Lanthanum to avoid anionic interferences.

3. Results

Determination of nutritional value of the pollen grains and the fruits (total protein, total ash, total carbohydrates, total crude fat and total moisture) revealed that total protein, fats and ash percentages were higher in the pollen grains higher than that of the fruits, carbohydrates and moisture content were higher in the fruits more than those of the pollen grains. Total carbohydrates is the highest value in the fruits and the pollen grains followed by total protein in the both organs (Table 1).

Amino acid analysis show that each organ contains 16 amino acids, 9 are essential while other 7 are non essential amino acids. Total percentage of non essential amino acids in both organs, 48.39% and 61.99% for the pollen and the fruit respectively while percent of essential amino acids is 51.60% and 38% for the same organs respectively. proline was the highest percent of amino acids in the fruits (1486.5134mg /100g) followed by aspartic acid (1041.84 mg/100g). Glutamic acid (1516.48mg/100g) was highest percent of amino acids in pollen grains followed by aspartic acid (1116.96mg/100g) (Table 2). Minerals analysis revealed that Potassium was the major composition of macro-elements in both organs followed by sodium. Nickle was the minor macro-element in both organs. Among microelements copper represent the highest percent in both organs (Table 3).

The yield of lipoidal matter was 1.5gm, 2.2gm of the pollen grains and the leaves respectively and 1.5gm oily residue of the fruits.

Saponification of petroleum ether extract of the leaves, the pollen grains and the fruits of *chamaerops humilis L.* resulted about (1.18gm, 0.34gm and 0.068gm respectively) (unsap) and (0.09gm, 0.24gm and 1.309gm respectively) (sap).

Results of GLC analysis of the identified unsaponifiable matter of different organs of *Chamaerops humilis(L.)* show that Sterols present in the pollen grains are cholesterol and stigmasterol but β-sitosterol and campesterol present in the leaves and hydrocarbons present in the leaves is C28 (Table 4).

Results of GLC analysis of the identified Fatty acid methyl esters show that the pollen grains contain 8 fatty acids, 6 fatty acids in the fruits and the leaves contain 10 fatty acids, so the leaves is the most organ rich by fatty acids (Table 5).

4. Discussion

From the determination of nutritional value of pollen grains and fruits of *chamaerops humilis* L. the resulted data suggested that both pollen grains and fruits can be used as high source for nutrition. The percentages of macro- and micro-elements in pollen grains were higher than that of fruits and Total amino acids percent in pollen grains were much higher than these of fruits except proline

The Pollen grains is the most rich by fatty acids. Glutamic acid was highest percent of amino acids in the both organs followed by proline then aspartic acid. Aspartic acid is of highly importance that plays an important role in synthesis of RNA and DNA. [19] While glutamic acid was found to play an important role in immunoglobulin production and synthesis of antibodies[20], also it was found to be used as a drug for improving the symptom of benign prostate hyperplasia. [21,22] Tyrosine that present in a moderate amount found to stimulate the hypothalamus to secrete the ghonadotrophic hormone[23]and increase sperm capacity. [24].

Table (1): general chemical analysis

Item Type of analysis	Pollen grain	Fruits (pericarp)
Total protein (as % of dry weight)	14.7	7.31
Total crude fat (as % of dry weight)	1.61	.36
Total ash (as % of dry weight)	8.4	3.87
Total carbohydrates (as % of dry weight, by difference)	70.12	81.56
Total moisture (as % of fresh weight)	5.17	6.9

Table (2): Amino acids analysis

Items		**RRT*** **	**Organ**	
			Pollen grains	**Fruits**
Amino acids content (relative%)**	Aspartic acid	0.64	1116.96	1041.84
	Thereonine*	0.84	493.44	202.96
	Serine	0.89	573.44	283.6
	Glutamic acid	1.0	1516.48	870.8
	Proline	1.13	407.20272	1486.5134
	Glycine	1.42	300.16	261.44
	Alanine	1.48	684.48	477.2
	Valine *	1.78	579.2	291.52
	Methionine*	1.99	139.52	84.24
	Isoleucine *	2.09	640.64	225.36
	Leucine *	2.15	882.56	506.96
	Tyrosine	2.33	744.32	476.56
	Phenylalanine*	2.41	790.24	435.52
	Histidine *	2.82	477.76	265.84
	Lysine *	3.03	723.36	398.08
	NH_4^+	3.26	1780.48	1553.36
	Arginine *	3.54	971.04	592.4
	%of essential amino acid	51.60%		38%
	% of non essential amino acid	48.39%		61.99%

*Essential amino acids **percent mg/100 g of powder *** Relative retention time to glutamic acid

Table (3): Mineral elements analysis

tems			**organ**	
			Pollen grain	**Fruit**
Mineral content (mg/100gm)	**Macroelements***	**Mg**	283	110
		Na	458	355
		Ca	434	243
		K	3923	2295
	Microelements*	**cu**	3	3
		mn	6	2
		Ni	1	1
		Fe	31	20
		cr	2	2

*concentration (mg/100gm)

Table 4: Results of GLC analysis of the identified unsaponifiable matter of different organs of *chamaerops humilis (L.)*:

Items		RRT*	organs		
			Pollen	Fruits	leaves
Hydrocarbon & sterols content (Relative%)	cholesterol	0.82	1.8	1.0	-
	stigmasterol	0.88	1.5	0.5	-
	β-sitosterol	0.46	-	-	2.9
	campesterol	1	-	-	69.7
	C28	0.67	-	-	6.5
	% of identified Hydrocarbons		-	-	6.5
	% of identified sterols		3.3	1.5	72.6

*Retention time relative to campesterol.

Table 5: Results of GLC analysis of the identified fatty acid methyl esters of the different organs of *chamaerops humilis (L.)*:

Items	RRT*	organs		
		Pollen	Fruits	leaves
Myristic acid $C_{14:0}$	0.65	7.6	1.1	-
Palmatic acid $C_{16:0}$	0.81	15.0	10.3	-
Stearic acid $C_{18:0}$	0.97	3.2	1.4	0.9
Oleic acid $C_{18:1}$	1	13.6	43.7	-
Linoliec acid $C_{18:2}$	1.04	8.7	29.8	-
Pentadecanoic acid 15:0	0.74	2.9	1.3	-
Arachidic acid $C_{20:0}$	1.22	3.0	-	-
Tetracosanoic acid 24:0	1.34	2.3	-	-
Carbamic acid	0.41	-	-	2.2
2,4,6,8,10Tetradecapentaenoic acid	0.45	-	-	1.2
pentadecanoic acid	0.60	-	-	13.5
2,4,6-Decatrienoic acid	0.65	-	-	1.9
docosanoic acid	0.70	-	-	1.2
9-octadeconoic acid	0.78	-	-	6.5
octadeconoic acid	0.80	-	-	6.8
Eicosanoic acid	0.99	-	-	2.5
1,2-Benzenedicarboxylicacid	1.18	-	-	1.4

*Retention times relative to the major fatty acid (Oleic acid).

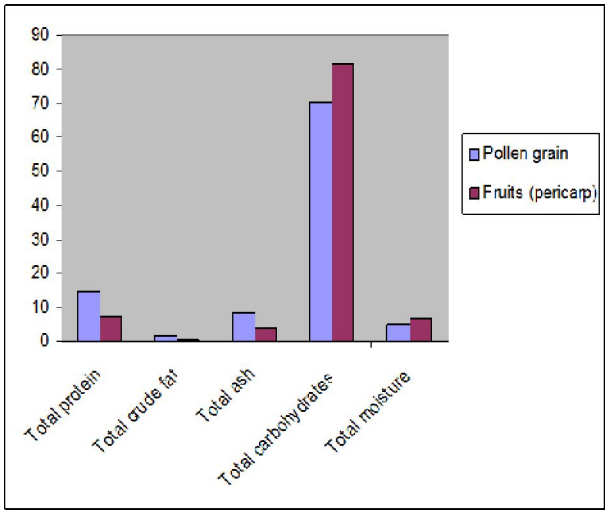

Fig. (1): a graph comparing the protein, fat, ash, carbohydrates and moisture percentages in pollen grains and fruits.

Table (6): shows the contribution of the levels of essential nutrients in plant under investigation to their RDA Recommended Dietary/Daily Allowances) values for adults established by the Food and Nutrition Board 1980). [26]

Essential nutrient		RDA value for adults	Percentage coverage of daily needs for 100g of the dried powder	
			Pollen grains	fruits
Protein		34-56 g/day	26.2-43.2 %	13.05-21.5%
Essential amino acids	**Thereonine**	27mg/g protein	124.3 %	102.8 %
	Methionine	25mg/g protein	37.96 %	46.09%
	Valine	32mg/g protein	123.12 %	124.62 %
	Isoleucine	25mg/g protein	174.32 %	123.31 %
	Leucine	51mg/g protein	117.72 %	135.98%
	Phenylalanine and Tyrosine	47mg/g protein	114.37 % 107.73%	126.76% 138.70%
	Histadine	18mg/g protein	180.55 %	202.03 %
	Lysine	55mg/g protein	89.46 %	99.01 %
	Arginine	27mg/g protein	244.65 %	300.14%
carbohydrates		130 g/day	53.93 %	62.73 %
Minerals	**Mg**	240-420 mg/day	117.9-67.3 %	26.1-45.8%
	Ca	1,000-1,300 mg/day	33.3-43.4 %	18.6-24.3 %
	fe	8-18 mg/day	172.2-387.5%	111.1-250%

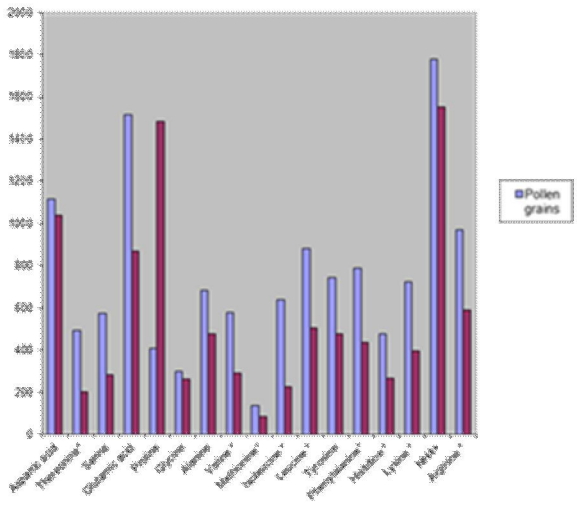

Fig. (2): A graph comparing the concentrations of the different amino acids in pollen grains and fruits.

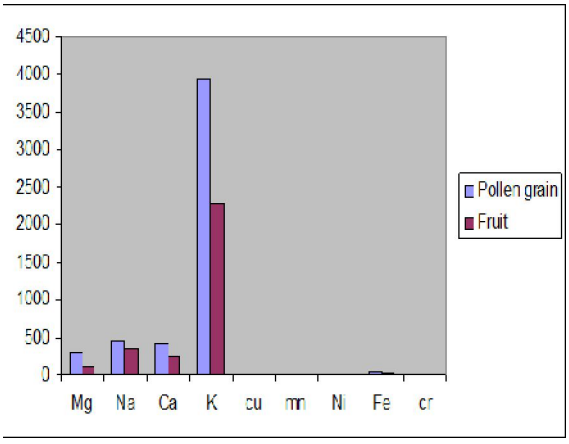

Figure. (3): A graph comparing the concentrations of the different macro and microelements.

Conclusion

It was obviously that pollen grains and fruits posses a high nutritional value, since each 100g of the dried plant covers at least 25 % of adults daily

requirements for most nutrients. Fruits of *chamaerops humilis* L. can be edible due to its high nutritive values.

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