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Phytochemical study, nutritional evaluation and *in vitro* antiobesity potential of fruits pericarp and seeds of *Livistona carinensis* and *Thrinax parviflora*

Mohamed S. Hifnawy[a], Marwa Yousry Issaa[b], Hesham El-Seedi[b,c], Amr M. K. Mahrous[a,*], Rehab M. S. Ashour[a,*]

[a] *Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini 11562, Egypt.*[b] *Chemistry Department, Faculty of Science, El-Menoufia University, 32512 Shebin El-Kom, Egypt.*[c] *Medicinal Chemistry Department, Division of Pharmacognosy, Uppsala University, Box 574, 75123 Uppsala, Sweden.*

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Abstract

Overweight is a significant health hazard. It increases the risk of development of various diseases. The nutritional value, phytochemical composition and in-vitro antiobesity potential of the seeds and fruits pericarp of *Livistona carinensis* (*L. carinensis*) and *Thrinax parviflora* (*T. parviflora*) were assessed.

The phytochemical screening of both plants showed the presence of tannins, carbohydrates, sterols, flavonoids and anthraquinones. GLC (Gas liquid Chromatography) analysis of lipids allowed the identification of stigmasterol as the major phytosterol in seeds and pericarp of *L. carinensis* (9.01% and 10.81%), respectively while *β-sitosterol* is the major in seeds and pericarp of *T. parviflora* (13.79% and 9.19%), respectively. Palmitic acid was the major fatty acid methyl ester in the pericarp of *L. carinensis* (35.01%), followed by myristic acid in the pericarp of *T. parviflora* (20.68%). Concerning unsaturated fatty acids linoleic acid is the major in seeds of *T. parviflora* (53.93%) followed by seeds and pericarp of *L. carinensis* (18.49% and 11.10%), respectively, while oleic acid is the major in pericarp of *T. parviflora*. Quantitative estimation of constituents revealed that *L. carinensis* seeds showed the highest concentration of total polyphenolics (39.02±1.24 mg/g gallic acid equivalents), flavonoids (10.75±0.40 mg/g quercetin equivalent) and tannins (15.82±0.62 mg/g tannic acid equivalents). While the highest steroidal content is found in *T. parviflora* pericarp (11.51±0.44 mg/g *β-sitosterol* equivalent). Both plants pericarps possess higher content of carbohydrates than that of seeds while the protein content is higher in *T. parviflora*, pericarp and seeds than that of *L. carinensis*. Calcium and iron are found prominent in *T. parviflora* pericarp (6.47 and 2.07 mg/gm, respectively) while the highest concentration of potassium was found in *L. carinensis* pericarp (21.43 mg/gm). The free radicle scavenging activity using DPPH is found in the following order *T. parviflora* seeds (13.79 µg/ml) > *T. parviflora* pericarp (9.72 µg/ml) > *L. carinensis* pericarp (8.95 µg/ml) > *L. carinensis* seeds (7.37 µg/ml) compared to ascorbic acid as standard (7.80 µg/ml). In addition, seeds and pericarp of *L. carinensis* showed the highest *α*-glucosidase inhibitory activity with IC_{50} values 0.93 and 1.17 mg/ml, respectively followed by the seeds and pericarp of *T. parviflora* (IC_{50} =1.46 and 1.76 mg/ml, respectively), compared to acarbose (IC_{50} = 0.72 mg/ml). Moreover, seed extracts of *L. carinensis* and *T. parviflora* showed powerful inhibitory activity against pancreatic lipase (IC_{50} = 10.95 and 9.33 µg/ml, respectively) compared to orlistat (6.82 µg/ml).

In conclusion, seed extracts of *L. carinensis* and *T. parviflora* showed the most powerful *α*-glucosidase and lipase inhibitory activities, these recommend their incorporation in anti-obesity preparations. Their pericarps showed significant antioxidant activity with high calcium, iron and potassium contents in addition to their moderate *α*-glucosidase inhibitory activities which recommend their daily intake in weight control programs.

Keywords: *Livistona carinensis*, *Thrinax parviflora*, anti-obesity, *α*-glucosidase inhibition, pancreatic lipase.

1. Introduction

Obesity and its related diseases as type-II diabetes mellitus and coronary heart diseases possess a worldwide concern. It is now considered as a major lifestyle ailment specifically in developing countries due to several factors including fast food intake, reduction of physical activity and industrialization. Inhibition of fats and carbohydrates digestion through hinderance of pancreatic lipase and *α*-glucosidase enzymes with the purpose of reducing energy intake have been recently used in management of obesity (El-shiekh et al. 2019). *α*-glucosidase is the main enzyme that aids the digestion of carbohydrates. Hence, its inhibition can hinder the release of glucose from carbohydrates and delay the absorption of glucose, resulting in regulation of type II diabetes. Many phytoconstituents have been reported to inhibit *α*-glucosidase (Kumar et al. 2011). The free radical scavenging power is the main link to inhibit the oxidative stress related hyperglycemia. Some reports indicated that α-glucosidase inhibitory activity could be attributed to

* Corresponding author e-mail: Amrmkh@gmail.com, rehab.ashour@pharma.cu.edu.eg.

DPPH scavenging activity (Sekhon-Loodu,Rupasinghe 2019).

Moreover, pancreatic lipase inhibition has been widely used to assess natural drugs used as anti-obesity agents (Seyedan et al. 2015). Orlistat is a lipase inhibitor associated with numerous side effects as interference with the physiological process of gastrointestinal tract, disruption of the mineral and electrolyte balance system, affecting the absorption and pharmacokinetics of other medicaments regularly used by obese patients as sulfonylureas, oral contraceptives, statins, slow-released calcium channel blockers or drugs which have narrow therapeutic index and reduction of the liposoluble vitamins absorption (Qi 2018).

Based on previous studies, traditional medicinal plants have been used in management of obesity (El-shiekh et al. 2019, Kazemipoor et al. 2012). Natural anti-obesity agents can enhance weight loss through various mechanisms (Kazemipoor et al. 2012). The search for several natural sources to inhibit pancreatic lipase and α-glucosidase with the aim of overcoming obesity with less side effects is greatly indicated. Palm oil from fruits have been reported as a natural adipocyte differentiation inhibitor (Saad et al. 2017).

The potent inhibitory potential of seeds from several date palm (*Phoenix dactylifera*) varieties versus key enzymes affecting obesity and diabetes have been previously reported (Masmoudi-Allouche et al. 2016). Several members of family Palmae revealed significant antihyperlipidemic effect (Uroko, 2019 and Vembu, 2012). Family Palmae is considered among the largest plant families, both in terms of number of species and in abundance (Gonzàlez et al. 1995). Human use of palms is widely distributed as edible food, medicinal and ornamental importance (Ahmad et al. 2013). Chemical characteristics of family Palmae, include the accumulation of polyphenols, some simple alkaloids (especially pyridine derivatives) and fatty acids, as well as steroidal saponins, oil palm and sterols. The palm genus *Livistona*, comprises about 36 species, native to southern and southeastern Asia, Australia and the Horn of Africa. The biological activity of genus *Livistona* includes anticancer, anti-osteoporosis, antioxidant and antibacterial (Yuan et al. 2009, Zeng et al. 2014) . The palm genus *Thrinax* consists of four species, endemic to Cuba and Caribbean countries (Riffle et al. 2012) and traditionally used in treatment of skin injury, kidney stones and nervous disorders (Calvo-Irabién,Soberanis 2008).

Reviewing the available literature, there is no published data regarding the fruits pericarp and seeds of *L. carinensis* and *T. parviflora* growing in Egypt. Therefore, it was deemed of interest to study these plants. As phytochemical analysis revealed that the two plants contain a large variety of several components with different anti-obesity and anti-oxidant effects on body metabolism and fat oxidation, so the study of anti-obesity efficacy was needed.

2. Material & methods

2.1. Plant material

Fruits pericarp and seeds of *Livistona carinensis* and *Thrinax parviflora* were collected in summer of the years 2017-2019 from El-Zoharia and Orman Botanic Gardens, Egypt, respectively. The plants were identified by Mrs. Therese Labib, botanical specialist and consultant at Orman and Qubba Botanical Gardens. Voucher specimens numbered (28072019 & 11112019), were placed in the Pharmacognosy Department Museum, Faculty of Pharmacy, Cairo University.

2.2. Proximate analysis

Crude fiber, moisture, total ash and acid insoluble ash were evaluated (Feldsine et al. 2002). Each value was an average of three determinations.

2.3. Phytochemical screening

The powdered air-dried fruits pericarp and seeds of *L. carinensis* and *T. parviflora*, were subjected, separately, to preliminary phytochemical screening tests (Wagner et al. 2013).

2.4. Study of the lipid content

Two hundred grams of air-dried powdered fruits pericarp and seeds of the two plants were extracted, separately, till exhaustion with petroleum ether. Each extract was evaporated under vacuum to give 4.5 g and 2.8 g of the fruits pericarp and seeds of *L. carinensis*, respectively, while 0.8 g and 3.8 g were obtained from the fruits pericarp and seeds of *Thrinax parviflora*, respectively. The saponifiable and unsaponifiable fractions were obtained from the fraction of petroleum ether, then the produced fatty acids were subjected to methylation (Finar 1973; Furniss 1989). GLC conditions used for unsaponifiable matter (USM) were applied on a capillary column (30 m x 0.32 mm I.D. x 0.25 µm film), the column was packed with HP-5 (5% phenyl methyl siloxane), and 2 µl was the injection volume. Programmed temperature was used for the analysis, where the initial temperature used was 80 °C for 5 min. then the temperature increased by the rate of 8 °C/min. to reach 280 °C, and the temperatures for injector and detector (FID) were 240 °C and 300 °C, respectively. The flow rate of nitrogen was 20 ml/min. Fatty acid methyl ester (FAME) was analyzed with the same carrier gas but at flow rate of 30 ml/min and on the same column used for USM. The initial temperature was 120 °C increased by the rate of 4 °C/min. to 240 °C, the temperature of injector and detector (FID) were, respectively, 250 °C and 280 °C. Identification of compounds was done by comparing the retention times of their peaks with the retention times of the authentic compounds (Sigma Chemical Co. St. Louis, MO. USA) similarly analyzed. Peak area measurement using a computing integrator was used for quantification.

2.5. Total polyphenolics content

Total polyphenolics content was determined according to the procedure reported in the European Pharmacopeia, using Folin-Ciocalteau colorimetric method (Singleton et al. 1999). Standard gallic acid (Sigma Co., St. Louis, MO., USA) was used at concentrations 0.1 to 1 mg/ml, a calibration curve (R^2 =0.9449) was prepared. Total phenolics were expressed as mg of gallic acid equivalent (mg GAE), and three replicates were carried for each concentration.

2.6. Flavonoids content

Total flavonoid content was determined spectrophotometrically based on measuring the yellow

color intensity produced after aluminum chloride reagent was complexed with flavonoids (Geissman 1962). A standard calibration curve (R^2 =0.9767) was composed utilizing several dilutions of authentic quercetin (Sigma Co., St. Louis, MO., USA) equivalent to 0.025 to 1 mg/ml. Three replicates were carried out for each concentration.

2.7. Tannins content

Tannins content was determined according to the procedure reported in the European Pharmacopeia, by Folin-Denis colorimetric method (Earp et al. 1981) using tannic acid (Sigma Co., St. Louis, MO., USA) as standard. Using tannic acid concentrations ranging from 0.1-2 mg/ml, a standard calibration curve (R^2 =0.9925). The values of tannins content were expressed as mg of tannic acid equivalent (mg TAE). Three replicates were carried out for each concentration.

2.8. Steroids content

The total steroidal content was determined based on measuring the greenish color intensity generated after Libermann-Burchard's reagent complexes with steroidal compound using standard *β*-sitosterol (Daksha et al. 2010). Standard calibration curve (R^2 =0.9006) was established using several dilutions of standard *β*-sitosterol (E-Merk, Darmstadt, Germany) equivalent to 0.03-2 mg/ml. Three replicates were carried out for each concentration.

2.9. Nutritional evaluation

2.9.1. Determination of the carbohydrates, protein and minerals contents.

Determination of total carbohydrates was estimated using the phenol–sulphuric acid colorimetric method (Dubois et al. 1956). Concentrated sulphuric acid (5 mL) was added to the mixture of the sample (1 mL) and 1 mL phenol solution (5% w/v). The sample was left at room temperature for 30 min. before measuring absorbance at 485 nm utilizing a spectrophotometer. The total amount of carbohydrate was estimated based on a standard calibration curve prepared using different dilutions of glucose. Three replicates were done for each concentration

Determination of protein content was estimated using the Micro-Kjeldal method that includes oxidation or digestion of the sample with concentrated sulfuric acid. The rate of oxidation of organic matter is also increased under most conditions by addition of copper (Cu), or selenium (Se) that serves as catalysts. The reaction was followed by steam distillation. The ammonia which is formed at high PH was received in solution of 4% boric acid then titrated using 0.1N HCL.

Determination of minerals content: Advanced microwave digestion system was used for digestion of samples. The concentration of Ca and Fe in sample were determined by using Industry Coupled Plasma (ICP-AES), Thermo Sci, model: ICAP6000 series. Argon gas was used for excitation of the mineral atom. The blank values for each element were deduced from the sample values. Potassium: The concentration of K in sample was determined by using Atomic Absorption Spectrometry (Metcalfe 1987).

2.9.2. Vitamins:

The vitamins HPLC analysis was carried out using these conditions; the column hypersil-EDS-C_{18} (4.6 mm x 250 mm), methanol was used as mobile phase at 1 ml/min flow rate. The volume of injection was 5 µl. Vitamin A, E and C were estimated using UV detector at wavelength 325, 292 and 254 nm, respectively (Nöll 1996; Pyka,Sliwiok 2001; Romeu-Nadal et al. 2006).

2.10. In-vitro biological study

2.10.1. Preparation of plant material

One and half kg random samples of fruits pericarp and seeds of the two plants were collected, separately, air-dried in shade, reduced to fine powder (sieve number 36) and kept in tightly closed amber colored glass containers at low temperature then extracted using 70% ethanol.

2.10.2. DPPH assay

DPPH assay is non-enzymatic assay. It is well trusted and accepted tool to assess free radical scavenging activity. Assay is based on the decrease of the ethanolic DPPH solution (Sigma Co., St. Louis, MO., USA) in the presence of the antioxidant, which disturbs free radical chain oxidation to form a stable end product diphenyl-β-picrylhydrazyl (DPPH). This causes the purple-colored DPPH to lose its chromophore (α, α-diphenyl-β-picrylhydrazyl) (Gulati et al. 2012).

20 ul of different extracts (conc. 0.5 mg/ml) were added to 96-well plate, then, 280 µl of 0.5 mM DPPH was added. The mixture was strongly shaken and incubated for 30 minutes at room temperature in dark; after that the absorbance of control and ethanolic extracts were measured at 540 nm using a microplate reader UV-spectrophotometer. Vitamin C (Sigma Co., St. Louis, MO., USA) served as reference standard with conc. 100 µg/ml.

The reduction in the absorbance revealed an increase in DPPH radical-scavenging activity. The inhibition percent was determined using the equation:

DPPH radical scavenging (%) = (1 - AS/AC) X 100

Where AS = The absorbance of the sample

AC = The absorbance of the control

The experiment was repeated three times, and the mean values were calculated. IC_{50} values were calculated as the sample concentration required to scavenge 50% of DPPH free radicals. The lowest IC_{50} value indicates the strongest ability of the sample to act as DPPH radical scavenger.

2.10.3. α-glucosidase inhibitory assay

The *α*-glucosidase inhibitory activity was estimated using the standard method with minor modification (Shai et al. 2011). In a 96-well plate, reaction mixture including 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl α-glucosidase (Sigma Co., St. Louis, MO., USA) (1 U/ml), and 20 µl of several concentrations of the two plants different extracts (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was preincubated at 37°C for 15 min. After that, 20 µl para-nitrophenyl-α-D-glucopyranoside P-NPG (5 mM) (Sigma Co., St. Louis, MO., USA) was added as a substrate and then incubated for 20 min. at 37°C. The reaction was stopped by the addition of 50 µl Na_2CO_3 (0.1 M). The released p-nitrophenol absorbance was measured at 405 nm using Multiplate Reader. Acarbose (Sigma Co., St. Louis, MO., USA) at different concentrations (0.1–0.5 mg/ml) was used as a standard drug (positive control). Negative control group was done concurrently without the addition of test substance and every experiment was done

three times. The results were calculated as percentage inhibition, using the formula,

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100$$

Where, As: the test substance absorbance; Ac: the control absorbance.

2.10.4. Anti-lipase assay

Pancreatic lipase (PL) inhibitory activity was determined colorimetrically using ρ-nitrophenyl palmitate (PNPP) [10 mM, in isopropanol] as substrate and porcine pancreatic lipase (PPL) (Sigma Co., St. Louis, MO., USA) as described by (Kordel et al. 1991) with few modifications.

The solutions of enzyme were prepared prior to use, by suspending crude porcine PL type II (Sigma, EC 3. 1. 1. 3.) in Tris-HCl buffer (100 mMTris, PH 8) to produce a concentration of 2 mg/ml (200 unites/ml) and mixed gently. Methanolic extracts (1mg/ml) 25 µl were pre-incubated with 50 µl of PPL solution for 10 minutes at 37 ^{0}C in the absence of light before assaying the PPL activity. The reaction was then started by adding 10 µl solution of PNPP substrate (10 mM in isopropanol). Tris-HCl buffer was used to dilute the volume to 200 µl. After incubation for 20 min. at 37 ^{0}C, the absorbance was determined at 405 nm. The assay was accomplished using Orlistat (final concentration 100 µg/ml) as a positive control and 5% DMSO as blank, each experiment was performed in triplicates.

3. Results and discussion

The ethanolic extraction yield of fruits pericarp and seeds of *T. parviflora* (46.4 and 60.8 gm), respectively and *L. carinensis* (76.5 and 62.4 gm), respectively.

3.1. Proximate analysis

The quality and purity of crude drugs are determined by their ash values. The total ash values found in the seeds of *L. carinensis* and *T. parviflora* are 2.36±0.11 and 1.46±0.06, respectively. Results are consistent with that reported about the seeds of *L. chinensis* 1.31±0.01. Moreover, the results reported for *L. chinensis* fruits pericarp (6.92±0.01) are similar to those of *L. carinensis* fruits pericarp (7.17±0.33) while *T. parviflora* showed a higher value (12.89±0.62) (Nwosu et al. 2012). In addition, the total ash in both organs of the two plants is higher than the insoluble acid ash, indicating the presence of large amount of soluble crystals. In general the insoluble acid ash is used in the detection of calcium oxalate crystals (Priya et al. 2013). The total moisture content of fruits pericarp and seeds in *L. carinensis* are found to be 9.40±0.39, and 11.75±0.52, respectively, while in *T. parviflora* they are 13.99±0.68 and 10.16±0.43, respectively. These values are in accordance with that reported for *L. chinensis* pulp (11.14 ±0.02) while they are much lower than that reported for *L. chinensis* seeds (35.35±0.05) (Nwosu et al. 2012). The lower moisture content found the higher the stability as this lower the chances of microbial infections. Total crude fiber for fruits pericarp of *L. carinensis* and *T. parviflora* are 22.70±1.03 and 15.20±0.66%, respectively. This is much lower than the value reported for the pulp of *L. chinensis* 36.66±1.46% while that of the seeds of *L. carinensis* and *T. parviflora* are 49.20±2.36 and 58.60±2.39%, respectively (Nwosu et al. 2012). Results of pharmacopoeial constants of the fruits pericarp and seeds are tabulated in table 1. These variations in pharmacopoeal constants may be probably due to difference in plant species, origin and growth phase. These parameters were studied as they are helpful in identification and authentication of both plants.

Table 1. Pharmacopoeial constants of the fruits pericarp and seeds.

Analysis	g%			
	Pericarp		Seeds	
Plant	L. *carinensis*	T. *parviflora*	L. *carinensis*	T. *parviflora*
Moisture content	9.40±0.39	13.99±0.68	11.75±0.52	10.16±0.43
Total ash	7.17±0.33	12.89±0.62	2.36±0.11	1.46±0.06
Acid insoluble ash	3.90±0.17	4.15±0.19	1.45±0.06	1.10±0.05
Crude fiber	22.70±1.03	15.20±0.66	49.20±2.36	58.6±2.39

3.2. Phytochemical screening

The fruits pericarp and seeds of both species revealed the presence of tannins, carbohydrates, flavonoids and sterols while crystalline sublimate, steam volatile substances, alkaloids and cardiac glycosides are absent. Anthraquinones are present only in the fruits pericarp of both plants. Results of phytochemical screening are represented in table (2)

Table 2. Results of phytochemical screening of the fruits pericarp and seeds of *L. carinensis* and *T. parviflora*

Test	*Livistona carinensis*		*Thrinax parviflora*	
	Fruits pericarp	Seeds	Fruits pericarp	Seeds
Crystalline sublimate (Claus and Tyler, 1967)	-	-	-	-
Steam volatile substances (Wagner *et al.*, 1983)	-	-	-	-
Carbohydrates and/or glycosides (Coutts and Snail, 1973)	++	+	++	+
Tannins (Evans, 2002)	++	++	++	++
Flavonoids 1-Aglycones (Geissman, 1962)	±	+	+	-
2-Glycosides (Peach and Tracey, 1955)	+	+	+	±
Saponins (Walform*et al.*, 1940)	-	-	-	-
Sterols and/or Triterpenes (Leibermann and Burchard, 1890) (Salkowski, 1872)	±	++	+	±
Alkaloids (Evans, 2002)	-	-	-	-
Anthraquinones 1-Free (Borntrager, 1880)	+	±	+	±
2-Combined (Borntrager, 1880)	+	-	+	±
Cardiac glycosides (Feiser and Fieser, 1959)	-	-	-	-

+: Present ++: Strongly present -: Not detected ±: Trace

3.3. Lipid content

GLC analysis of lipids, revealed the identification of the unsaponified content in a 69.37% and 80.93% in the pericarp and seeds of *L. carinensis*, respectively, while 76.08% and 82.45% were identified in the pericarp and seeds of *T. parviflora*, respectively. Seeds of *T. parviflora* contained much higher percent of phytosterols (29.53%) and triterpenes (7.12%). Concerning the triterpenes, *α*-amyrin is the major triterpene of the unsaponified contents

(7.12 and 5.52% in the seeds of *T. parviflora* and *L. carinensis*, respectively). Stigmasterol is the major phytosterol in the fruits pericarp of *L. carinensis* representing 10.81% while β-sitosterol was the major in seeds (13.79%) and fruits pericarp (9.19%) of *T. parviflora*. *β-sitosterol* was previously reported as major compound in the unsaponifiable matter of *L. chinensis* pulp (43%) also stigmasterol and campsterol were detected (Kadry et al. 2009). The analysis of FAME (Fatty acid methyl ester) by GLC revealed the identification of 98.90% and 89.66% fatty acids of the total lipids of fruits pericarp and seeds of *L. carinensis*, correspondingly, while the total lipids displayed 99.30% of fruits pericarp and 99.29% of seeds of *T. parviflora*.

The saturated fatty acid palmitic acid constituted the highest percentage of FAME in the fruits pericarp of *L. carinensis* with a yield of 35.01%, followed by myristic acid in the fruits pericarp of *T. parviflora* by 20.68%. Concerning the unsaturated fatty acids; linoleic acid is the major constituent of the FAME with percent of 53.93% in the seeds of *T. parviflora* while in seeds of *L. carinensis* it is found to be 18.49%. Previous report on *L. decipiens* pulp showed that oleic acid was the major FAME constituting 53.40% of the total identified compounds while palmitic acid was the major in *L. chinensis* pulp 47.40%. On the other hand, linoleic acid concentration in *L. chinensis* pulp was found in relatively high concentration (12.16%) compared to that reported for *L. decipiens* (0.31%) (Kadry et al. 2009). The presence of the unsaturated fatty acids oleic and linoleic acid in *L. carinensis*, and *T. parviflora* is of great importance to help the reduction of the risk of heart diseases and lower cholesterol levels among other health benefits as anti-inflammatory and anti-cancer (de Morais et al. 2017). Results of GLC analysis of lipids were tabulated in tables (3&4)

Table 3. Results of GLC analysis of the identified unsaponified content of *L. carinensis* and *T. parviflora*

Authentic	RRt*	Percentage					
		L. carinensis			*T. parviflora*		
		Leaves	Pericarp	Seeds	Leaves	Pericarp	Seeds
n-Tridecane C-13	0.639	--	--	--	0.237	--	--
n-Tetradecane C-14	0.724	4.327	--	0.646	0.114	--	--
n-Pentadecane C-15	0.781	13.651	--	0.431	0.293	0.099	--
n-Hexadecane C-16	0.848	4.141	--	0.806	0.334	--	--
n-Heptadecane C-17	0.914	18.576	0.908	0.918	3.776	0.178	0.148
n-Octadecane C-18	1	24.680	1.150	0.721	1.757	0.113	1.423
n-Nonadecane C-19	1.058	5.858	5.740	3.174	2.718	1.111	3.481
n-Eicosane C-20	1.231	10.806	1.283	2.595	13.201	0.795	1.424
n-Henicosane C-21	1.254	3.760	16.537	6.337	7.810	1.497	10.450
n-Docosane C-22	1.349	1.826	4.120	2.741	2.711	1.625	2.288
n-Tricosane C-23	1.408	0.932	4.114	2.164	3.211	1.951	2.375
n-Tetracosane C-24	1.481	1.680	2.619	5.359	7.656	2.726	4.728
n-Pentacosane C-25	1.537	0.788	3.272	3.793	0.567	2.758	2.341
n-Hexacosane C-26	1.658	1.076	5.109	8.948	1.466	5.136	5.374
n-Heptacosane C-27	1.772	1.682	5.265	5.228	6.574	9.139	8.543
n-Octacosane C-28	1.868	1.042	--	9.414	4.283	17.615	3.231
n-Nonacosane C-29	1.948	--	--	3.288	--	5.502	--
Cholesterol	1.979	--	--	2.764	2.060	2.392	--
Campsterol	2.003	--	3.158	--	3.667	2.111	7.106
Stigmasterol	2.033	0.506	10.809	9.010	11.192	8.993	8.634
β-sitosterol	2.162	1.044	5.288	7.070	8.725	9.193	13.786
α-amyrin	2.362	0.753	--	5.522	0.828	3.144	7.121
% Total identified compounds		97.128	69.373	80.930	83.181	76.078	82.453
Percentage of total hydrocarbons		94.825	50.118	56.564	56.709	50.245	45.806
Percentage of total phytosterols		1.550	19.255	18.844	25.644	22.689	29.526
Percentage of total triterpenes		0.753	--	5.522	0.828	3.144	7.121

* RRt: Relative retention time to n-Octadecane C-18 with Rt= 14.597 min. -- : not detected

Table 4. Results of GLC analysis of the identified FAME of *L. carinensis* and *T. parviflora*

Authentic	RRt*	Percent					
		L. carinensis			*T. parviflora*		
		Leaves	Pericarp	Seeds	Leaves	Pericarp	Seeds
Caprylic acid (Octanoic acid) C_8 (0)	0.351	--		0.853	--	--	--
Capric acid (Decanoic acid) C_{10} (0)	0.401	--	1.146	1.473	3.840	--	--
Undecylic (Undecanoic acid) C_{11}	0.554	3.063	--	--	0.870	5.299	0.602
Lauric acid (Dodecanoic acid) C_{12} (0)	0.659	14.927	3.959	28.615	2.243	8.812	--
Tridecylic acid (Tridecanoic acid) C_{13}	0.749	28.003	8.535	--	--	2.497	14.598
Myristic acid (Tetradecanoic acid) C_{14} (0)	0.804	18.551	5.948	11.211	0.523	20.678	--
Pentadecylic (Pentadecanoic acid) C_{15} (0)	0.935	3.335	3.804	--	--	3.119	3.098
Palmitic acid C_{16} (0)	1	3.361	35.004	12.918	32.950	14.564	--
Palmitoleic acid C_{16} (1)	1.050	2.501	--	--	--	--	--
Heptadecanoic acid C_{17}(0)	1.071	3.643	2.308	--	--	15.521	--
Stearic acid C_{18} (0)	1.194	1.581	2.490	3.070	0.308	7.278	--
Oleic acid C_{18} (1)	1.232	1.322	9.771	12.363	34.371	10.827	24.258
Linoleic acid C_{18} (2)	1.267	1.318	11.106	18.485	14.697	6.886	53.925
α-Linolenic acid C_{18} (3)	1.313	3.849	10.977	0.676	6.408	3.818	2.813
Arachidic acid C_{20}(0)	1.367	1.547	3.849	--	--	--	--
Percentage of identified fatty acids		87.001	98.899	89.663	96.209	99.299	99.295
Percentage of unsaturated F.A.		8.990	31.855	31.524	55.476	21.531	80.997
Percentage of saturated F.A.		78.011	67.044	58.139	40.733	77.768	18.298

* RRt: Relative retention time to Palmitic acidC_{16}(0) with Rt= 21.176 min. --- : not detected.

3.4. Total polyphenolics, flavonoids, tannins and steroids

3.4.1. Spectrophotometric determination of total polyphenolics

L. carinensis seeds showed the highest concentration of total polyphenolics constituting 39.02±1.24 mg/g expressed in gallic acid equivalents followed by the *T. parviflora* seeds (30.45±0.97 mg/g) expressed in gallic acid equivalents. Our results are much higher than the results reported about *L. speciosa* seed polyphenolic content which was found to be 2.35 mg/g gallic acid equivalent (Takolpuckdee 2016). Results are shown in table (5).

Table 5. Total polyphenolics content in fruits pericarp and seeds of *L. carinensis* and *T. parviflora*.

Plant	Organ	Absorbance	Concentration mg/g expressed in gallic acid equivalents
Livistona carinensis	Pericarp	1.3937	20.97±0.83
	Seeds	1.7089	39.02±1.24
Thrinax parviflora	pericarp	1.3756	25.30±0.91
	Seeds	1.5593	30.45±0.97

Values are mean of triplicates ± Standard error M±S.E (n=3)
Mean values of significant variation at p <0.05

3.4.2. Total flavonoids content

The total flavonoids concentration, calculated as quercetin equivalent, was higher in *L. carinensis* seeds (10.75±0.40 mg/gm) than in the seeds of *Thrinax parviflora* (0.13±0.01 mg/gm), while that of fruits pericarp of *T. parviflora* (6.88±0.26 mg/gm) was greater than *L. carinensis*, fruits pericarp (1.13±0.04 mg/gm). On contrary of total polyphenolics reported on *L. speciosa* seed its total flavonoids content was found at very high concentration (39.27 mg/g rutin equivalent) much higher than that found on both species under study (Takolpuckdee 2016). Results of total flavonoids content are presented in table (6).

Table 6. Total flavonoids content in *L. carinensis* and *T. parviflora*, fruits pericarp and seeds.

Plant	Organ	Absorbance	Concentration mg/g expressed in quercetin equivalents
Livistona carinensis	Pericarp	0.0907	1.13±0.04
	Seeds	0.0984	10.75±0.40
Thrinax parviflora	Pericarp	0.0953	6.88±0.26
	Seeds	0.0899	0.13±0.01

Values are mean of triplicates ± Standard error M±S.E (n=3)
Mean values of significant variation at p <0.05

3.4.3. Tannins content

The *L. carinensis* seeds exhibited the highest tannins content with concentration of 15.82±0.62 mg of tannic acid equivalents / gram fresh weight while the concentration in fruits pericarp was 13.55±0.42 mg/gm. *T. parviflora*, fruits pericarp and seeds tannins contents are 11.92±0.47 and 11.05±0.33 mg/g, respectively. *L. speciosa* seed is reported to contain only 4.26 mg/g tannic acid equivalent (Takolpuckdee 2016). Results of total tannins content are presented in table (7).

Table 7. The tannins content in fruits pericarp and seeds of *L. carinensis* and *T. parviflora.*

Plant	Organ	Absorbance	Concentration mg of tannic acid equivalents per gram.
Livistona carinensis	Pericarp	2.3274	13.55±0.42
	Seeds	2.6310	15.82±0.62
Thrinax parviflora	Pericarp	2.1059	11.92±0.47
	Seeds	2.0022	11.05±0.33

Values are mean of triplicates ± Standard error M±S.E (n=3)
Mean values of significant variation at p <0.05

3.4.4. Steroidal content.

T. parviflora, pericarp were rich in steroidal content (11.51±0.44 mg/g) compared to the pericarp of *L. carinensis*, (0.82±0.03 mg/g), while the seeds of *L. carinensis*, (4.75±0.18 mg/g), were

higher than that of *T. parviflora*, (0.13±0.01 mg/g). Results of total steroidal content are presented in table (8).

Table 8. Total steroidal content in *L. carinensis* and *T. parviflora*, fruits pericarp and seeds.

Plant	Organ	Absorbance	Concentration mg/g expressed in β-sitosterol equivalents
Livistona carinensis	Pericarp	0.1892	0.82±0.03
	Seeds	0.2045	4.75±0.18
Thrinax parviflora	Pericarp	0.2308	11.51±0.44
	Seeds	0.1865	0.13±0.01

Values are mean of triplicates ± Standard error M±S.E (n=3)
Mean values of significant variation at p <0.05

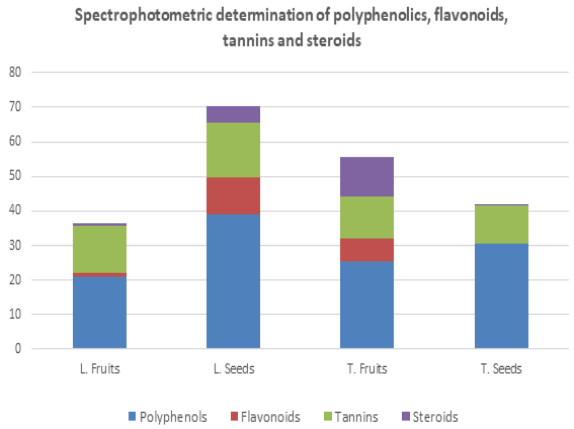

Figure 1. Spectrophotometric determination of total phenolics, flavonoids, tannins and steroids.

3.5. Nutritional evaluation

The worldwide mineral malnutrition is a serious issue in many countries especially in developing ones (Oyeyinka,Afolayan 2019). However, the potential of nutraceuticals to combat it has not been extensively studied. Fruits of *L. carinensis* and *T. parviflora* possess high content of total carbohydrates (30.22±1.19 and 32.08±0.98%, respectively) compared to that of the seeds (17.34±0.59 and 14.31±0.47%, respectively). This is in accordance with that previously reported about *L. chinensis* pulp (33.69 ± 0.05) and seeds (17.83 ± 0.03) (Nwosu et al. 2012), but the amount of carbohydrates is much lower than that reported for date pits that ranged from 70.9 - 86.9% (Hossain et al. 2014). In addition, the total protein content of edible plants is considered a measure of its nutritional value for herbivores (Al-Rowaily et al. 2019). The protein content showed significant variation, pericarp and seeds of *T. parviflora* (8.64±0.24 and 7.00±0.18%, respectively) comprises much more protein than that of *L. carinensis*, pericarp and seeds (0.13±0.01 and 0.27±0.01%, respectively). The results of protein content in *T. parviflora* seeds are higher than that previously reported about *L. chinensis* seed (4.44±0.16%) and *Phoenix dactylifera* seeds (5.64±0.21%), while the protein content of the pericarp are in accordance with that conveyed about *L. chinensis* pulp (9.04±0.03) (Nwosu et al. 2012).

Table 9. Mineral contents of *L. carinensis* and *T. parviflora*, fruits pericarp and seeds.

Mineral content (mg/100gm)	*L. carinensis*		*T. parviflora*		Daily needs**
	Pericarp	Seeds	Pericarp	Seeds	
Ca *	147.86 (14.786%)***	127.36 (12.736%)***	647.28 (64.728%)***	118.41 (11.841%)***	1000 mg
Fe *	10.27 (57.05%)***	14.62 (81.22%)***	207.31 (1151.72%)***	6.56 (36.44%)***	18 mg
K *	2143.28 (61.23%)***	614.79 (17.56%)***	1284.40 (36.69%)***	374.82 (10.70%)***	3500 mg

* Dry weight. ** The Daily Values are the nutrient amounts recommended per day for Americans 4 years of age or older (FDA Vitamins and Minerals Chart). *** % Percent of daily needs at consumption of 100 gm of crude powder.

There is a significant relation between obesity and several minerals intake including calcium, potassium and iron. Calcium regulates various cellular processes in the body as cell differentiation and proliferation in addition to bone formation. Moreover, the potent effect of dietary calcium supplementation in prevention or treatment of obesity has been reported (Zhang et al. 2019). Adequate daily potassium intake can in turn decrease the risk of obesity (Cai et al. 2016). Also, iron deficiency showed significant correlation (Sal et al. 2018).

The results of mineral contents are shown in table (9) and figure (2). Calcium and iron are found prominent in *T. parviflora*, fruits pericarp (6.47 and 2.07 mg/gm, respectively). While the highest concentration of potassium was found in *L. carinensis*, fruits pericarp (21.43 mg/gm).

This would recommend the use of the pericarp of both plants as a source of carbohydrates, calcium, potassium, and iron. In addition, the fruits pericarp and seeds of *T. parviflora* are also good protein sources.

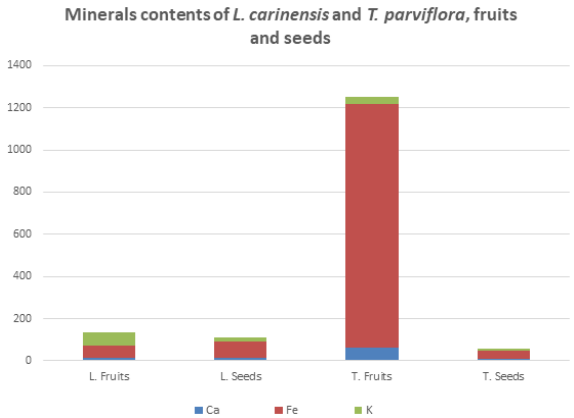

Figure 2. Mineral contents of *Livistona carinensis* and *Thrinax parviflora*, fruits and seeds.

3.6. DPPH assay

The results of the free radical scavenging activity are displayed in table (10). In the DPPH assay, the extracts (0.05- 1.0 mg/ml) scavenged the free radical in a concentration dependent pattern. The result also revealed

that at dose 1 mg/ml, *T. parviflora* fruits pericarp and seeds showed IC_{50} of 9.72 and 13.79 µg/ml, respectively while *L. carinensis* extracts, fruits pericarp and seeds inhibited DPPH radical by IC_{50} values 8.95 and 7.37 µg/ml, respectively compared to the standard, ascorbic acid IC_{50} = 7.80 µg/ml. The results are slightly higher when compared to the previously reported for *Phoenix dactylifera*, pulp and seeds (IC_{50} values 89.14 and 31.05 µg/ml, respectively) while the standard drug IC_{50} was 12.80 µg/ml (Masmoudi-Allouche et al. 2016).

3.7. α-glucosidase inhibitory assay

Seeds and fruits pericarp of *L. carinensis* showed the highest *α*-glucosidase inhibitory activity with IC_{50} values 0.93 and 1.17 mg/ml, followed by the seeds and fruits pericarp of *T. parviflora* (IC_{50} =1.46 and 1.76 mg/ml, respectively), compared to Acarbose IC_{50} = 0.72 mg/ml as standard drug. The results are in accordance with that previously reported about *Phoenix dactylifera*, pulp and seeds (IC_{50} equals 10.93 and 3.08 mg/ml, respectively) (Masmoudi-Allouche et al. 2016).

The potent *α*-glucosidase inhibitory activity of the seeds of *T. parviflora* may be attributed to the high content of linoleic acid (53.92%) and oleic acid (24.25%) which were previously reported to possess α-glucosidase inhibitory activity (Su et al. 2013).

3.8. Anti-lipase assay

The two plant extracts (0.5- 4.0 mg/ml) significantly inhibit the pancreatic lipase in a dose dependent pattern, at 4 mg/ml dose, the seeds extracts of *L. carinensis* and *T. parviflora* showed powerful inhibition activity against pancreatic lipase (PL) enzyme (IC_{50} = 10.95 and 9.335 µg/ml, respectively) compared to the standard drug Orlistat (0.1 mg/ml) with IC_{50} value 6.82 µg/ml. This is in accordance with that previously reported about *Phoenix dactylifera* seeds (IC_{50} ranging from 1.21 to 47.51 µg/ml) but for Orlistat, this is much higher than the previously reported (IC_{50} equals 0.92 µg/ml) due to the difference in substrates (Masmoudi-Allouche et al. 2016). Seeds extracts of *L. carinensis* and *T. parviflora* showed more powerful anti-lipase activity compared to the standard drug Orlistat.

The significant lipid lowering effect of *L. carinensis* and *T. parviflora* seeds may be due to their content of polyunsaturated fatty acids (linoleic acid).

Linoleic acid was reported to reduce body fat mass in specific regions in healthy, overweight and obese adults (Gaullier et al. 2007). Phenolic compounds derived from medicinal plants might be responsible for their lipase inhibitory potential (McDougall et al. 2009). Various natural products rich in polyphenolics have been reported as anti-obesity by inhibiting pancreatic lipase (McDougall et al. 2009, Yajima et al. 2005).

Table 10. IC_{50} values of *L. carinensis* and *T. parviflora*, fruits pericarp and seeds compared to standard drugs.

Parameter	Standard drug	*L. carinensis*		*T. parviflora*	
		Pericarp	**Seeds**	**Pericarp**	**Seeds**
DPPH	(Ascorbic acid) 7.80 µg/ml	8.95 µg/ml	7.37 µg/ml	9.72 µg/ml	13.79 µg/ml
α-glucosidase inhibitory	(Acarbose) 0.72 mg/ml	1.17 mg/ml	0.93 mg/ml	1.76 mg/ml	1.46 mg/ml
Anti-lipase	(Orlistat) 6.82 µg/ml	Nd	10.95 µg/ml	Nd	9.33 µg/ml

Nd: Not determined

There is no direct relation between the DPPH scavenging activity and the inhibition of *α*-glucosidase and pancreatic lipase enzymes. Further detailed *in vivo* studies are required to confirm the anti-obesity activity.

4. Conclusion

Seed extracts of *L. carinensis* and *T. parviflora* showed the most powerful α-glucosidase and lipase inhibitory activities which recommend their incorporation in anti-obesity preparations. While their fruits pericarp showed significant DPPH scavenging activity with high calcium, iron and potassium contents in addition to their moderate α-glucosidase and lipase inhibitory activities which recommend their daily intake in weight control programs.

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