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Nutritional Evaluation and GC/MS Analysis of Lipophilic Fractions of *Livistonaaustralis* Leaves and Fruits

Seham S. El-Hawary[1], Asmaa I. Owis[2], Sara O. Abo El-Ela[2] and Ahlam Elwekeel[2*]

[1]*Pharmacognosy Department, Faculty of pharmacy, Cairo University, Cairo 11562, Egypt*
[2]*Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62111, Egypt*

Abstract

Livistonaaustralis R. Br. mostly known as cabbage-tree,is one of the palm species belonging to Family Arecaceae. The chemical composition of lipophilic fractions of leaves and fruits was evaluated by gas chromatography-mass spectrometry (GC/MS). The results showed that the major compounds detected in the unsaponifiable matter were acetyl eugenol and phytol (37.58, 16.44 %, respectively) while methyl esters of (Z)-9-octadecenoic acid and methyl caprylate constitutes (12.24, 4.44 %, respectively) of fatty acid composition present in leaves and fruits. Assessment of nutritional values revealed that total protein content was 6.74, 5.13 g %, crude fiber content was 39.72, 22.28 g %, and total lipid content was 2.57 2.93 g %, for leaves and fruits respectively. Five minerals; Na, Ca, K, Fe and P were measured in both organs. Four aflatoxins; B1, B2, G1 and G2 were also analyzed, and the results revealed that leaves were free from aflatoxins.

Keywords: *Livistonaaustralis*; GC/MS; FAME; phytol; acetyl eugenol

1. Introduction

The genus *Livistona* (family Arecaceae or Palmae) comprises about 36 species, native to Australia, Malaya, New Guinea, and the Asian[1]. The most common species *is Livistonachinenses* which is used in folk medicine for treatment of various tumors in Southern China [2].*Livistonaaustralis* R. Br., commonly known as cabbage-tree palm is a tree growing in Egypt with about 25 m in height. Its leaves are plaited like a fan, with spikes florescence, cream-white flowers and brown spherical berries [3, 4]. Previous phytochemical investigation of *L. australis* leaves revealed the isolation of a pyranone derivative, 3-hydroxy-2-(4-hydroyphenyl)-6- methyl-4-H-pyran-4-one [5] and tricin 7-O- glucopyranoside-2"-sulphate sodium salt in addition to 14 flavonoid from leaves. Kassem et al. 2014 analyzed the lipophilic fraction of *L. australis* leaves and reported that phytol was the most abundant compound in unsaponifiable fraction (USM) while palmitic acid was the major fatty acid detected in saponifiable fraction (FAME) [3]. To the best of the authors' knowledge, there was no data concerning the analysis of the lipophilic fraction of the fruits neither the evaluation of the nutritional values of both leaves and fruits.So, the objectives of this work were to comparatively study leaves and fruits of *L. australis* through evaluation of USM and FAME using GC/MS analysis in addition to determination of their nutritional values through measuring total protein, crude fiber, total lipids and some minerals in addition to detection of aflatoxins in both organs by LC/MS-MS Analysis.

2. Experimental

2.1. Plant material

Leaves and fruits of *Livistonaaustralis* were collected on Jan. 2018 from Orman garden, Giza, Egypt and identified by Eng. TreaseLabib, Former-Head of El Orman Botanical Garden and Dr.Reem Samir Hamdy, Lecturer of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University,

*Corresponding author e-mail: ahlam_hasham2000@yahoo.com; (**Ahlam Elwekeel**).
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Giza, Egypt. A voucher specimen (BUPD-71-2018) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Egypt.

2.2. Analysis of lipophilic fraction

2.2.1. Extraction of lipophilic fraction and preparation of USM and FAME

500 grams of *L. australis*fruits and leaves were separately extracted with 70% ethanol three times then the collected extracts were evaporated under reduced pressure using rotatory evaporator to afford crude extract 95.4 and 23.4 g respectively. The crude extracts were separately suspended in distilled water and fractionated using *n*-hexane to give 5.3 and 3.5 g of hexane fraction of fruits and leaves, respectively.

Two grams of hexane extract of leaves and fruits were refluxed separately with 20 ml 10% alcoholic potassium hydroxide for 6 h. then the reactions were cooled and mixed with 20 ml distilled water, the USM of both extracts were then extracted with diethyl ether (3 × 20 ml). The diethyl ether extracts were combined and washed with distilled water till free from alkalinity. The extracts were dried over anhydrous calcium chloride and filtered. Finally, USM were dried under vacuum using a rotary evaporator, and kept for GC/MS analysis.

The aqueous mother liquor remaining after extraction of USM of both extracts were acidified with 10% HCl to liberate free fatty acids. The two mixtures were extracted with diethyl ether (3 × 20 ml), extracts were combined and dehydrated over anhydrous calcium chloride. Diethyl ether extracts were evaporated under pressure to afford FAME of leaves and fruits [6].

2.2.2. Conditions for GC/MS analysis of USM and FAME

The analyses of USM and FAME were carried out using gas chromatography-mass (GC/MS) spectrometry instrument stands with the following specifications, Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TR-5 MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 60 ^{o}C for 1min; rising at 4.0 ^{o}C/min to 240 ^{o}C and held for 1 min. The injector and detector were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 1 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40-450. The chemical constituents identified by its retention indices [relative to *n*-alkanes (C8-C22), mass spectrum matching to authentic standards (when available). Wiley spectral library collection and NSIT library database].

2.3. Nutritional Evaluation

Fresh leaves and fruits of *L. australis*were separately washed and cleaned with distilled water. One part of each organ was separately kept fresh for the total protein analysis while the other part was dried, grounded and used for evaluation of crude fiber, mineral profiling, total fats and aflatoxin.

2.3.1. Determination of total protein content

Total protein content were measured as total nitrogen using micro Kjeldahl method as describe in AOAC (2000)[7]

2.3.2. Determination of Crude fiber content

The crude fiber content of both samples was determined by filter bag method according to A.O.A.C. (2000) by using Fibertherm; Gerhardt, FT12 for analysis of crude fiber[8].

2.3.3. Determination of total lipid content

Total lipids (free and conjugated with proteins) were determined according to the method of A.O.A.C.(2000) [9] by using a rapid soxhlet extraction system, Gerhardt Soxtherm System. Leaves and fruits samples were treated with boiling HCl to free the conjugated lipids. The digestion solution was filtered and the fat remaining in the filter left to dry, extracted with petroleum ether and the solvent evaporated. The dried residue was weighed, and total lipid content (%w/w) was calculated.

2.3.4. Determination of mineral content

Minerals such as sodium, calcium, potassium, iron and phosphorus were measured in both organs using Inductively Coupled Plasma (ICPA-AES, Thermo Sci, model: ICAP6000 series) and Advanced Microwave Digestion System according to the reported method [10].

2.3.5. Analysis of aflatoxins

Four aflatoxins B1, B2, G1 and G2 were analyzed by LC/MS according to the reported method[11].

3. Results and discussion

The hexane fractions of *L. australis* leaves and fruits were saponified to afford USM which was used for identification of hydrocarbons and terpenoids and FAME which was used for identification of fatty acids. Identification of the compounds present in both subfractions was based on comparing their retention times and mass fragmentation patterns with those of

the data base libraries (Wiley spectral library collection and NSIT library database).

GC/MS analysis of USM of *L. australis* leaves (Table 1) revealed the presence of 22 compounds, the major compound detected was phytol (16.44%); phytol is a diterpene alcohol obtained from chlorophyll and shown antinociceptive and antioxidant activities [12] as well as anti-inflammatory and antiallergic effects [13]. Previous study on the lipophilic fraction of the fruit pulp reported phytol (7.98%) as the major compound detected in USM fraction [3]. The second major compound was alkane derivative named 2-phenyl tridecane (11.41%) in addition to, four phenyl derivatives of tridecane namely 6-phenyl tridecane, 5-phenyl tridecane, 3-phenyl tridecane, 4-phenyl tridecane with area 9.41, 7.20, 9.76 and 6.92 %, respectively. 1-Nonadecene and 1-docosene were the major unsaturated hydrocarbon detected with area 5.40 and 5.24 %, respectively. Octacosanol, a long chain fatty alcohol was also present (6.32 %). The other identified compounds were two nitrogenous compounds, three phenyl derivatives of dodecane, 2-methylenecholestan-3-ol, two unsaturated fatty alcohols; 1-eicosanol and 1-heptatriacotanol.Pentadecane, 2-phenyl, eicosane, 7-phenyl, isochiapin b, 1,2-benzenedicarboxylic acid and 4,8,12,16-tetramethylheptadecan-4-olide were detected also in USM of leaves.

GC/MS analysis of USM of the fruits (Table 2) revealed the identification of 20 compound, the major compounds detected were volatile phenolic constituents; acetyl eugenol (37.58%); followed by eugenol 29.61%. They possess many biological activities as antifungal, anticancer, anti-inflammatory, and antioxidant [14-16]. Caryophyllene (11.66 %) and caryophyllene oxide (6.31%) were also detected.

The results of GC/MS analysis of FAME of *L. australis* leaves (Table 3) revealed the identification of 26 compounds. The major fatty acids detected were the saturated fatty acids (25.86 %) represented by hexadecanoic acid, octadecanoic acid, and heptadecanoic acid methyl esters with 8.71, 3.61 and 2.83 %, respectively. While the unsaturated fatty acids constituting (20.34 %) and the major compounds detected were methyl ester of (*Z*)-9-octadecenoic acid, and 6, 9, 12-octadecatrienoic acid (13.05, 2.95 %, respectively). Seven fatty acids, with peak area more than one, were detected and identified as methyl esters of dodecanoic acid, nonanedioic acid, tetradecanoic acid, 7,10-octadecadienoic acid, 17-methyl octadecanoic acid, *cis*-13-eicosenoic acid, and methyl icosanoate. While the results of GC/MS analysis of FAME of *L. australis*fruits (Table 4) showed 44 compounds. The major fatty acids detected were the saturated acids (33.56 %) represented by the methyl esters of octanoic acid (methyl caprylate, 4.44 %), while the unsaturated fatty acids (28.06 %) were represented by methyl esters of 7,10-hexadecadienoic acid, 2-methoxy-10-undecenoic acid and (*Z*)-9-hexadecenoic acid (4.41, 4.26, 4.02 %, respectively). Three fatty acids were detected with area 3-4 % which were methyl ester of (*Z*)-9-octadecenoic acid, dimethyl ester of nonanedioic acid, and methyl esters of 9,10-dihydroxy-octadecanoic acid. Three fatty acids were detected with area 2-3% which were methyl esters of 11-octadecenoic acid, (*Z, Z*)-9,12-octadecadienoic acid and tetradecanoic acid. Nine fatty acids with area between 1 and 2 %, were detected as methyl esters of nonanoic acid, 9-oxo-nonanoic acid, dodecanoic acid, pentadecanoic acid, *cis*-10-heptadecenoic acid, heptadecanoic acid, methyl 9-*cis*,11-*trans*-octadecadienoate and (Z)-7-methyly-6-hexadecenoic acid and dimethyl esters of octanedioic acid. The other detected compounds were less than 1%.

The leaves of *L. australis* are sweet and edible so their nutritional values were assessed and compared with the fruits as shown in Table 5 which includes measuring total protein, fats, fiber content as well as some minerals as Na, Ca, K, Fe and P. The results revealed that total fats were measured as 2.57, 2.93 %, and total protein was recorded as 6.74, 5.13 % for leaves and fruits respectively. Leaves and fruits contained low amount of total fats and total protein, but leaves were little higher than fruits. The crude fiber content of the leaves and fruits were 39.72, 22.28 % respectively so the leaves were considered as a good source as fibers. The mineral content of leaves and fruits showed that leaves were rich in Na and P (140, 100 mg/ 100 g dry sample) respectively while fruit were rich in Ca and K (210 and 200 mg/100g dry sample). So, the leaves are a good source for Na which is necessary for proper fluid balance, nerve transmission, and muscle contraction while the fruits are rich in Ca which is necessary for healthy bones and teeth; helps muscles relaxation and contraction; important in nerve functioning, blood clotting, blood pressure regulation, immune system health while K is necessary for proper fluid balance, nerve transmission, and muscle contraction[17]. Also, four aflatoxins (B1, B2, G1 and G2) were analyzed in the leaves and fruits. Aflatoxins are toxic metabolites produced by different species of toxigenic fungi. Humans can be exposed to aflatoxins by the periodic consumption of contaminated food, contributing to an increase in nutritional deficiencies, immunosuppresion and hepatocellular carcinoma [18]. The results revealed that the leaves were negative against tested aflatoxins while G1and G2 detected in fruits with 22 and 8 ppm in the dry samples. From the nutritional analysis of leaves and fruits, it was concluded that the leaves and fruits were rich with fibers and minerals while protein and fat contents were low.

4. Conclusions

This study revealed that lipophilic fraction of Livistona australis leaves was rich in phytol and the unsaturated fatty acid; (Z)-9-octadecenoic acid while the lipophilic fraction of the fruits was rich in volatile oil constituents as eugenol and acetyl eugenol. Moreover, the nutritional analysis of both organs showed that they were rich in fibers and minerals (sodium & phosphorus for leaves and potassium & calcium for fruits) so they can be used as dietary supplements.

5. Conflicts of interest

There are no conflicts to declare.

6. References

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التقييم الغذائى وتحليل المستخلصات المحبة للدهون باستخدام كروماتوغرافيا الغاز المقرون بتقدير الكتلة لأوراق وثمار نبات ليفستونا

سهام صلاح الدين الهوارى [1]، أحلام الوكيل [2]، سارة أسامة ابو العلا [2]، أسماء أبراهيم عويس [2]

[1]قسم العقاقير-كلية الصيدلة - جامعة القاهرة - القاهرة – مصر

[2]قسم العقاقير-كلية الصيدلة - جامعة بنى سويف – بنى سويف- مصر

ليفستونا استرلاس تُعرف في الغالب باسم شجرة الملفوفوهي أحد أنواع النخيل التي تنتمي إلى عائلة Arecaceae. التركيب الكيميائى للمستخلصات الدهنية لكلا من أوراق وثمار نبات ليفيستونا أسترلاس قد تم تقييمه باستخدام جهاز كروماتوجافيا الغاز المقرون بتقدير الكتلة وقد اظهرت النتائج ان acetyl eugenol وphytol هما المركبين ذو النسبة الكبيرة فى الجزء غير المتصبن للاوراق والثمار بينما (Z)-9-octadecenoic acid و methyl caprylate قد تم تقدير هما فى الجزء المتصبن. تقدير القيمة الغذائية للأوراق والثمار اوضح ان محتوى البروتينات يساوى 6.74و 5.13.جم % كم ان الالياف تقدر22,28و 39,72جم % وكمية الدهون 2,57و 2,93 جم % لكل من الاوراق والثمار بالترتيب وايضا تم تقدير بعض العناصر فى النبات.