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Phenolics Isolation and Chemical Profiling of *Livistona australis* (R.Br.) Mart. Fruit Extracts; Potent Inhibitors of *α*-Amylase and Pancreatic Lipase

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ABSTRACT

Great medicinal value and uses were reported for Palm family species. *Livistona australis* (R.Br.) Mart. is widely distributed as an ornamental plant in Egypt. The aqueous methanol extract of both pulps and seeds were subjected to chromatographic and spectral analysis as well as LC-ESI-MS technique for isolation and characterization of diagnostic constituents. Thirty-eight compounds were validated for the first time from the fruit parts (pulps and/or seeds) in which 14 compounds were purified and isolated from the pulps and 6 from the seeds. The isolated compounds were mainly phenolics (2 anthocyanins, 1 chlorogenic acid, 2 flavones and 9 flavonols isomers). In addition, some phenolic compounds were recognized putatively beside other minor compounds in both extracts. The phenolic rich extracts of fruit parts were *in vitro* evaluated for their inhibitory activities on alpha amylase and pancreatic lipase enzymes. The aqueous methanol extracts of pulps and seeds markedly inhibited alpha amylase enzyme with IC_{50} values of 153.42 ± 6.4 and 134.5 ± 6.1 µg/mL, respectively, higher than that of acarbose (230 ± 3.7 µg/mL). Also, both extracts exhibited significant pancreatic lipase hindrance with IC_{50} 23.03 ± 5.8 and 23.5 ± 4.9 µg/mL, respectively, at 300 ug/mL. The effects were greater than that of orlistat (IC_{50} 24.02 ± 2.1) at 100 ug/mL. Digestive enzymes inhibition activity play a major role in prevention and management of diabetes and obesity, these effects of fruit parts could be attributed to the presence of wide range of phenolic metabolites.

Keywords: *Livistona australis*, Fruit, LC-ESI-MS, Phenolics, Obesity, Diabetes.

Introduction

Triglycerides are one of the major energy sources that are high in calories. Suppression of triglycerides absorption is directly associated with the prevention of obesity and obesity-related diseases.[1] Lipids accumulation in the pancreas causes deterioration of insulin producing pancreatic βeta-cells which couldn't secrete insulin.[2] The elevated plasma glucose concentrations caused by either insufficient insulin or insulin resistance, or both. In addition, disturbances in metabolism of lipids, carbohydrates and proteins could lead to diabetes.[3] Chances of developing type 2 diabetes increases by gaining weight or being obese. Obesity and diabetes are top causes of mortality.[4,5] Insulin replacement therapy is the main treatment for patients with type 1 (DM). Whereas, management of type 2 diabetes includes several therapeutic approaches such as using oral hypoglycemic and inhibition of the digestive enzymes that hydrolyze carbohydrates and lipids.[6] However, major side-effects of the prescribed drugs are the main reason for an urgent need for alternative therapies that may have no side-effects.

Natural extracts are easily available, more affordable and more effective for the management of diabetes and obesity and have less side effects as compared to the synthetic drugs.[7] Digestive enzymes have been targeted as potential routes for modulation of blood glucose and lipid concentrations through inhibition of the enzymatic breakdown of complex carbohydrates and triglycerides. Thereby, the hindrance of their absorption by enzymes inhibition is a major approach for lowering the risk of obesity and related type 2 diabetis.[8]

From the Arecaceae family (Palmae), the genus *Livistona* comprises about 36 species distributed along Australia, Malesia, southern Arabia, southeastern Asia and Horn of Africa.[9] Varied pattern of phenolics distributed in the leaves and fruits of many *Livistona* species; phenolic acids, leucoanthocyanins, flavone *C*- and *O*-glycosides (apigenin, luteolin, tricin), flavonols (quercetin) and their sulphate salts.[10,11] Quercetin glucoside and caffoeylshikimic acid were reported in flowers of *L. chinensis*.[12] Whereas, flavanes characterized *Livistona* roots and fruits.[13,14] Phenylpropanoid class was found in the seeds of *L. saribus*.[15]

In traditional medicine, *Livistona* species were consumed extensively for curing carcinomas.[16] Meanwhile, several medicinal properties were recorded for leaves, fruits (pulp and seed) and roots. Different extracts of the palm revealed potent antioxidant,[14] analgesic, acute anti-inflammatory, antihyperglycemic, antitumor,[10,11,12] hemolytic,[17] hepatoprotective,[18] cardioprotective,[19] antimicrobial,[20] antiulcer and antihyperlipidimic activities.[21] *Livistona australis* (R. Br.) Mart is one among the four cultivated species grown in Egypt, known as Australian fan palm.[22] Various *C*- and *O*-glucosyl flavones together with little flavonols were identified in *L. australis* leaves.[23] The dried pulp of the fruit was studied for its lipophilic phytoconstituents, where oleic acid is the major fatty acid.[24] The leaves extract possessed ulceroprotective and antioxidatant activities.[23] On the other hand, more recent study reported the effectiveness of the fruit pulp in reducing lipids serum levels.[24]

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The present study is an update of our ongoing research on *L. australis* palm bioactive constituents and biological activities. 70% Methanol extracts of *L. australis* fruit pulps and seeds were investigated for the first time for their phenolic constituents and authenticated by LC-mass ESI. The extracts were biologically *in vitro* screened for their inhibitory effect on α-amylase and pancreatic lipase activities.

Materials and Methods

General experiment

Sheets of Whatman filter paper No.1 and 3 MM (Whatman Ltd. Maidstone, Kent, England) (46 x 57 cm), were used for separations. Polyamide 6S (150 x 5 cm) glass column (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) and Sephadex LH-20 (60 x 1.5 cm and 35 x 2.5 cm) glass columns (Pharmacia, Uppsala, Sweden) were used for the isolation of pure compounds. Sugar authentic samples (E. Merck, Darmstadt, Germany). Solvents used for analysis were distilled water, methanol (HPLC grade, Fisher Chemical, UK), acetic acid (AcOH) (Adwic, Cairo, Egypt) and n-butanol (Sigma-Aldrich, St. Louis, MO, USA). Solvent systems were 15% HOAc (H_2O: AcOH, 85:15) and BAW (n-BuOH: AcOH: H_2O, 4:1:5, upper layer). Rotatory evaporator (Buchi, G. Switzerland) were used in laboratory works. UV were recorded on Shimadzu UV-240 and NMR on Jeol EX-500 spectrophotometer. LC-ESI-MS by means of Waters Alliance 2695 (HPLC) and Waters 3100 (mass spectrometry). All chemicals used for the bioassays were obtained from Sigma-Aldrich (Merck, USA).

Plant material and extraction

Fruits of *L. australis* were collected from Al Zohria Gardens, Gezira Island, Giza, Egypt in June 2018. Dr. Mohamed El Gibaly authenticated the species and a voucher specimen with no. 950 was deposited in CAIRC (National Research Centre herbarium) Giza, Egypt. Fruit pulps and seeds were detached manually by knife, air dried for three days and crushed. The powdered dried pulp (300 g) and seeds (400 g) of *L. australis* were extracted by percolation in 70% methanol/water at 40-60°C till exhaustion to give 48 g pulp extract (PE) and 60 g of seed extract (SE) dried residues. Assessment for inhibitory effects of alpha-amylase and pancreatic lipase enzymes were performed on part of each extract.

Phytochemical investigation

Isolation of phenolics

The residues of PE and SE were defatted using chloroform to yield 46 g and 58 g, respectively. Each extract was applied on a polyamide column chromatography. The elution was initiated with water and fractions of 100 mL were collected. The polarity decreased by addition of methanol till 100% obtained. Comparative (Whatman No. 1) and preparative (Whatman Grade No. 3 MM) paper chromatography techniques were accomplished using the three solvent systems; BAW, 15% AcOH and distilled water, for separation and purification of phenolic compounds. The chromatograms were inspected under visible and UV lights along with exposure to ammonia vapours. Final purification of the compounds was performed on Sephadex LH-20 column chromatography. The compounds obtained were **1** (39 mg), **2** (45 mg), **3** (56 mg), **4** (67 mg), **5** (47 mg), **6** (8.5 mg), **7** (8 mg), **8** (7 mg), **9** (29 mg), **10** (15 mg), **11** (3 mg), **12** (6 mg), **13** (11 mg), **14** (12 mg). Establishment of the compounds structures put through acid hydrolysis (2N HCl, 2 hours, 100 °C), UV, NMR and ESI-MS. Furthermore, comparing with reference standards.[25]

LC-ESI-MS phenolics profiling

The constituents of PE and SE were charted using LC-ESI-MS as stated via Hussein *et al*.[26] Authentic samples previously isolated and identified at the department of Phytochemistry and Plant Systematics were used as reference compounds.[26-31] The isolated compounds were also used as reference samples. Their retention times and mass pattern were compared with known peaks. Other peaks were identified by matching their fragmentation pattern with literature reports.

Biological Assessment

Alpha amylase bioassay

The *α*-amylase bioassay method was adopted and modified as described by Miller.[32] Briefly, a starch solution (0.5% w/v) was obtained by stirring potato starch in 20 mmol L^{-1} sodium phosphate buffer and 6.7 mmol L^{-1} sodium chloride. The enzyme solution was prepared by mixing 25.3 mg of α-amylase (10 U mg) with 100 mL distilled water. Extracts were dissolved in a buffer to give solutions with varying concentrations from 1000 µg mL^{-1} to 31.25 µg mL^{-1}. The colorimetric reagent was prepared by mixing a potassium sodium tartrate solution (12 g potassium sodium tartrate tetrahydrate in 8 mL 2M NaOH) and 96 mmol L^{-1} 3,5-dinitrosalicylic acid solution. Both control (acarbose) and extracts were added with starch solution and left to react with α-amylase solution in alkaline conditions at 25°C. The generation of maltose was evaluated by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. Acarbose was used as positive control. This reaction was detected at 540 nm using ELX 808 (Bio Tek Instrumental, Italy). Percentage of inhibition was calculated by using the following expression: 100 - [{A sample / A control} x 100].

Pancreatic lipase bioassay

The inhibition of pancreatic lipase was determined as described according to Conforti *et al*.[33] Type II crude porcine pancreatic lipase were used for preparation of aqueous pancreatic lipase solution (1 $mgmL^{-1}$). Then a 5 mmol L^{-1} solution of 4-nitrophenyl octanoate (NPC) in dimethylsulfoxide was set as a substrate. The composition of the reaction mixture was: 100 µl of 5 mmol L^{-1} NPC, 4 mL of tris-HCl buffer (pH = 8.5), 100 µl of extract and 100 µl of enzyme solution. The mixture was incubated at 37 °C for 25 min before the substrate was added. In the control, the extract was replaced with the same volume of dimethylsulfoxide. The absorbance was measured in cuvettes at 412 nm using ELX 808 (Bio Tek Instrumental, Italy). A blank sample without the enzyme was measured for each extract. For comparison, orlistat was tested at a final concentration of 20 μgmL^{-1}. Inhibition percentage was obtained using expression: 100 - [{A sample / A control} x 100].

Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviations (SDs). The IC_{50} (concentration necessary for 50% inhibition of enzyme activity) was calculated by constructing a linear regression curve showing extracts concentrations (from 75 to 600 µg/mL) for α-amylase and (from 12 to 100 µg/mL) for pancreatic lipase on the x-axis and percentage of inhibition on the y-axis.[34] All analyses were carried out using the SPSS v. 22.0 (IBM, Chicago, USA). Microsoft Excel 2010 was used for graph construction.

Results and Discussion

Phenolics isolated from PE and SE

Chromatographic investigation of PE and SE led to the isolation of fourteen compounds (Figure 1). After the physical investigation (colour reactions and R_f values), the compounds were exposed to chemical (acid hydrolysis) and spectral (UV, 1H- and ^{13}C-NMR, ESI mass) analyses. Further confirmation was carried out through co-chromatography with reference compounds and comparing their spectral data with previous reports. The compounds isolated were delphinidin-3-*O*-rutinoside (1), cyanidin-3-*O*-rutinoside (2),[35] 4-*O*-caffeoylquinic acid (3),[36] baicalein 7-*O*-glucoside (4),[37] quercetin 3-*O*-glucoside (5),[38] luteolin 7-*O*-glucoside (6),[39] quercetin 3-*O*-rhamnoside (7),[38] quercetin 7-*O*-rhamnoside (8),[40] quercetin 7-*O*-arabinoside (9),[41] isorhamnetin 7-*O*-glucoside (10),[42] kaempferol (11),[39] kaempferol 4´-*O*-methylether (12),[43] quercetin (13),[44] isorhamnetin (14).[45] Compounds 1-14 were isolated from PE whereas, compounds 1-3 and 6-8 were isolated from the SE (Table 1). Except for compounds 5, 6 and 13, all compounds were isolated for the first time from *L. australis*.

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LC-ESI-MS characterization of chemical constituents of PE and SE

Thirty six compounds were recognized and detected in PE and SE using LC-ESI-MS in the negative ion mode. Thirteen peaks (1, 2, 4, 5, 8, 10, 16, 18, 19, 22, 27, 29 and 30) were common for both extracts (Table 1, Figure 2A, 2B). Excluding the isolated phenolics (peaks 18, 23, 27-30 and 32-37); 10 flavonoids, 5 hydroxycinnamic acids, 3 chlorogenic acid isomers, 3 benzoic acids and 3 organic acids were tentatively identified. The isolates 1 and 2 are anthocyanins and cannot ionized in the negative ion mode.

Flavonoids

The detected quercetin derivatives; 1 (quercetin-*O*-sulfate),14 (quercetin coumaroyl hexoside), 19 (quercetin di-*O*-hexoside sulphate) and 21 (quercetin tri-*O*-hexoside). A significant fragment ion at *m/z* 301 coupled with the product ion at *m/z* 300 were representative in all previous compounds. They were characterized for the deprotonated quercetin aglycone $[M-H]^-$ and its radical anion due to loss of H radical, respectively.[46] Peaks 1 (*m/z* 381) and 19 (*m/z* 705) corresponded to sulphated forms of quercetin isomers. The loss of 80 amu was indicative for sulphate moiety $[M-H-SO3]^-$. For compound 19, the cleavage of 324 amu (162+162) from the fragment *m/z* 625 was correlated to the loss of di-hexosyl moiety linked at the same position $[M-H-SO3-2(162)]^-$.[47] The spectrum of compound 21 (*m/z* 787) displayed a subsequent loss of three hexosyl units attached directly to the aglycone giving fragments at *m/z*: 625 (loss of 162), 463 (loss of 2x162), 301 (loss of 3x162).[27] Quercetin coumaroyl hexoside (14) constructed a quasimolecular peak at *m/z* 609. Successive loss of coumaric acid $[M-H-146]^-$ and hexosyl moiety $[M-H-146-162]^-$ coincided with the daughter ions at *m/z* 463 and 301, respectively. Moreover, the fragments at *m/z* 163 [coumaric acid-H]$^-$ and 119 (coumaric acid-H-COO) tentatively characterized the compound.[48]

The compounds originated from kaempferol contributed the deprotonated aglycone fragment at *m/z* 285; 15 (kaempferol-*O*-hexsoide), 22 (kaempferol-*O*-hydroxyferuloylglucoside) and 24 (acetyl kaempferol-*O*-(X´´-acetyl hexsoide)). Compound 15 (*m/z* 447) lost a hexosyl unit $[M-H-162]^-$ while 22 (*m/z* 639) exhibited fragmentation signal at *m/z* 447 $[M-H-192]^-$ before losing the hexosyl residue, correlated to a dehydrated hydroxyferulic acid moiety.[47] Peak 24 (*m/z* 531) possessed MS fragment at 489 $[M-H-42]^-$; pointing to the existence of CH_3CO. Further loss of 204 amu (acetyl hexosyl residue) putatively established the compound.[49]

On the other hand, fragments of methoxylated flavone (11) were predicted as *m/z* 359 $[M-H]^-$, 344 $[M-H-15]^-$ $-CH_3$, 329 $[M-H-30]^-$ $-2CH_3$, 301 $[M-H-30-28]^-$ $-2CH_3$-CO. It could be deduced as trihydroxytrimethoxy flavone isomer.[28]

The main fragment of peak (20) at *m/z* 593 was synchronized with luteolin-C-deoxyhexoside-X´´-*O*-hexoside. The presence of fragment at *m/z* 431 $[M-H-162]^-$ was attributed to *O*-hexoside linkage. Other two characteristic fragments at *m/z* 357 $[Aglycone+71]^-$ ($[M-H-162-74]^-$) and 327 $[Aglycone+41]^-$ ($[M-H-162-104]^-$) suggested the identity of mono-C-deoxyhexosyl luteolin.[26] Peak (31) was corresponded to tricin-*O*-deoxyhexoside-*O*-hexoside (*m/z* 637). The fragments at *m/z* 491 $[M-H-146]^-$ and *m/z* 475 $[M-H-162]^-$ indicated the linkage of the deoxyhexosyl and hexosyl units at different positions of the aglycone.[26]

Hydroxycinnamic acids

The hydroxycinnamic acids; caffeic (2), ferulic (13 & 16) and sinapic (12 & 17), were simply esterified with a hexoside moiety to produce pseudomolecular ion peaks at 341, 355 and 385 *m/z*; respectively. Caffeic acid-*O*-hexoside (2) displayed *m/z* 179 [caffeic acid-H]$^-$, 161 [caffeic acid-H_2O]$^-$ and 135 [caffeic acid-CO_2]$^-$.[50] Ferulic acid-*O*-hexoside (13 & 16) showed 193 [ferulic acid-H]$^-$, 175 [ferulic acid-H_2O]$^-$ and 149 [ferulic acid-CO_2]$^-$.[28] Sinapic acid-*O*-hexsoide (12 & 17) presented 223 [sinapic acid-H]$^-$, 205 [sinapicacid-H_2O]$^-$ and 179 [sinapic acid-CO_2]$^-$.[51]

Quinic acid and its hydroxycinnamic acids esters

Peak (6) was in agreement with the cyclohexanecarboxylic acid; quinic acid (*m/z* 191) by fragment ion peak at *m/z* 127 $[M-H-CO-2H_2O]^-$.[52] Three chlorogenic acids were detected. They derived from esterification of quinic acid either by caffeic acids (peaks 3 and 26) or caffeic and coumaric acids (peak 25). They shared the common fragment ions at *m/z* 191 [quinic acid-H]$^-$ and 179 [caffeic acid-H]$^-$. Tri-*O*-caffeoylquinic acid (26) exhibited *m/z* 677 and fragmented to give *m/z* 515 $[M-H-162]^-$ and 353 $[M-H-2(162)]^-$ which are identical to the base peaks of compounds 3 (dicaffeoylquinic acid) and 18 (4-*O*-caffeoyl quinic acid); respectively. A characterized caffeoyl residue was affirmed through *m/z* 173 [quinic acid-$H-H_2O$]$^-$.[28,53] Besides, compound 25 (*m/z* 499) yielded ion peaks at *m/z* 353 and 337 due to losing a coumaroyl $[M-H-146]^-$ and caffeoyl $[M-H-162]^-$ residues, respectively. An ion at $[M-H]^-$ 163 related to deprotonated coumaric acid coexisted with caffeoylcoumaroylquinic acid isomer.[54]

Benzoic acids

The phenolic acids derived from benzoic acid; 2-hydroxy benzoic acid (salicylic acid) (8) and 3,4-dihydroxy benzoic acid (protocatechuic acid) (10) presented pseudomolecular ions at *m/z* 137 and 153, respectively. Their retention times and mass fragmentations proved their identification, based on reference samples.[31,55] Peak 9 (*m/z* 331) after the loss of a hexosyl unit gave rise to an anion fragment of gallic acid at *m/z* 169 $[M-H-162]^-$ characteristic for galloyl-*O*-hexoside.[56]

Organic acids

In addition to quinic acid, two other organic acids were detected as gluconic/galactonic (4) and citric/iso-citric (5) acids. Their deprotonted fragment ions were *m/z* 195 and 191 with daughter ions *m/z* 129 $[M-H-2H_2O-CH_2O]^-$ and 111 $[M-H-CO_2-2H_2O]^-$; respectively.[28]

Impact of PE and SE on α-amylase

The effect of PE and SE on α-amylase enzyme activity is illustrated in Figure (3). The assessment revealed that PE and SE inhibited the enzyme by 73.6 ± 6.8% and 67.9 ± 5.8%, respectively for each extract compared to acarbose reference standard by 49 ± 2.8% inhibition activity at 300 ug/mL. SE possessed the lowest IC_{50} (134.5 ± 6.4 µg/mL) followed by that of PE (153.42 ± 6.1 µg/mL) compared to acarbose IC_{50} (230 ± 3.7% µg/mL). Both extracts exhibited potent suppression effect on α-amylase activity higher than that produced by acarbose at the same concentration.

Alpha-amylase is a pancreatic enzyme that hydrolyzes starch into mixture of maltose, maltotriose and oligoglucans which are further broken down by glucosidase into glucose that enters the blood stream upon absorption. These caused postprandial hypergylcemia (PPHG). Therefore, according to Eichler et al., PE and SE could retard the absorption of glucose through their powerful inhibition of α-amylase leading to profound control of obesity and type 2 diabetes.[57]

Impact of PE and SE on pancreatic lipase inhibition

The effect of PE and SE on pancreatic lipase activity is illustrated in Figure (3). The bioassay revealed that PE and SE exert pancreatic lipase inhibition of 95.8 ± 5.5% and 90 ± 6.2% at 300 ug/mL, respectively. Both extracts were more active than positive control orlistat (81 ± 3.6% enzyme inhibition) at 100 ug/mL. Almost similar IC_{50} for both extracts were recorded (23.03 ± 5.8 and 23.57 ± 4.9 ug/mL for PE and SE, respectively), which were markedly better than that of orlistat (24.02 ± 2.1 ug/mL). Pancreatic lipase plays a key role for triglycerides absorption in the small intestine. This enzyme is secreted from the pancreas and hydrolyzes triglycerides into glycerol and fatty acids.[58] Orlistat strongly inhibits the activity of pancreatic lipase. Nevertheless, it may cause serious adverse effects on the gastrointestinal, nervous, endocrine, renal systems and also affected with the absorption and efficiency of many drugs and vitamins.[59]

The present phytochemical study of *L. australis* fruit pulp and seed extracts revealed some bioactive phenolics (anthocyanins, chlorogenic acids, hydroxycinnamic acids, flavones and flavonols derivatives). They are well represented as main components of the chemical finger

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print of the palm fruit extracts .The obtained inhibitory results of PE and SE could be referred to the presence of phenolic contents. According to Martinez-Gonzalez *et al*., phenolics regulate the carbohydrate and lipids digestion and control their absorption offering an anti-diabetic and anti-obesity effects.[60] Anthocyanins could suppress the reaction of both enzymes through different mechanisms.[61] Chlorogenic acids attenuate starch hydrolysis and relay the postprandial blood glucose through alpha amylase inhibition activity.[62] Hydroxycinnamic acids are considered powerful inhibitors of pancreatic lipase. Conversely, hydroxybenzoic acids are of less potency.[63] Polyphenolics commonly occur in edible plants as glycosylated derivatives. However, they were studied in their form of aglycones due to the fact that their glycosidic form interacts with the digestive enzymes of the small intestine mainly as aglycones. Quercetin is among the most potent pancreatic lipase inhibitors. It displayed an inhibitory activity higher than that exerted by orlistat, against the enzyme.[64] While, kaempferol reversibly repress pancreatic lipase activity in a competitive manner and revealed with orlistat a synergistic inhibition.[65] A relevant potent inhibitory activity against alpha amylase could be assigned to baicalin which is represented in PE and SE as its 7-*O*-glucosidic form. Furthermore, based on the previous reports on detected and isolated phenolics from *L. australis* palm fruit extracts, they could produce a synergistic role to the overall repressions effects of PE and SE against the activities of digestive enzymes.[6]

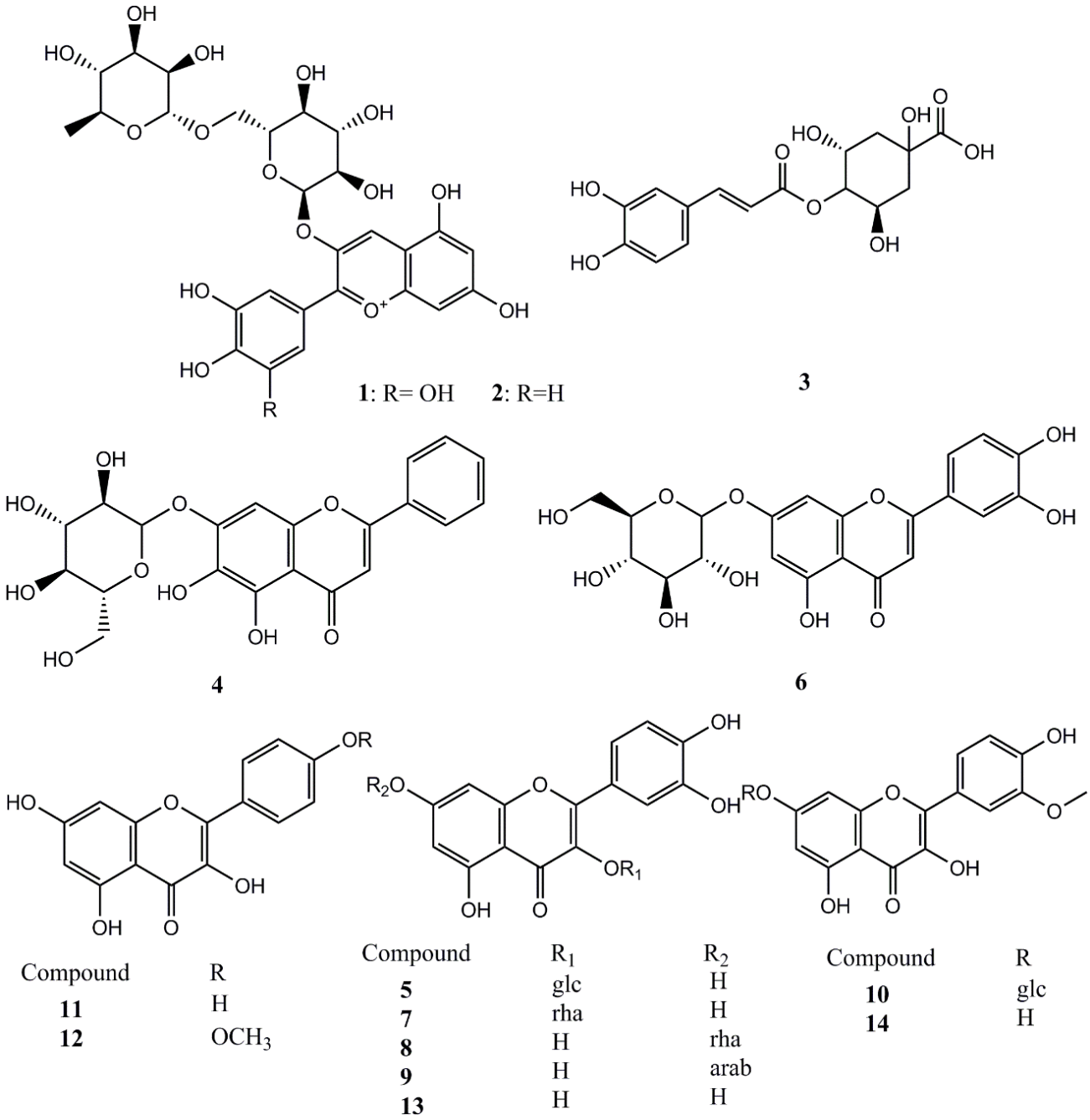

Figure 1: Chemical Structures of the isolated compounds from PE and SE

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Table 1: Chemical compounds tentatively identified in PE and SE

Peak No. (isolates)	*Rt*	M	$[M-H]^-$	Fragments	Tentative identification	PE	SE
1	2.14	382	381	301, 299	Quercetin-*O*-sulphate	+	+
2	2.67	342	341	179, 161, 135	Caffeic acid-*O*-hexoside	+	+
3	2.93	516	515	353, 191, 179, 173, 135	Dicaffeoyl quinic acid	+	-
4	3.74	196	195	129	Gluconic/galactonic acid	+	+
5	3.87	192	191	111	Citric/iso-citric acid	+	+
6	5.07	192	191	191, 173, 127, 111, 93, 85	Quinic acid*	+	-
7	8.54	218	217	97, 80	Unknown	+	+
8	10.68	138	137	119, 92, 62	Salicylic acid*	+	+
9	14.68	332	331	169, 151, 89	Galloyl hexoside	-	+
10	16.02	154	153	109, 108, 91	3,4-Dihydroxy benzoic acid (protocatechuic acid)*	+	+
11	16.82	360	359	344, 329, 301, 197, 137	Trihydroxytrimethoxy flavone isomer	-	+
12	18.83	386	385	223, 205, 179	Sinapic acid-*O*-hexsoide isomer	-	+
13	20.03	356	355	193,175, 149	Ferulic acid-*O*- hexsoide	-	+
14	20.43	610	609	463, 301, 163, 119	Quercetin Coumaroylhexsoide	+	-
15	21.49	448	447	285	Kaempferol O-hexoside	+	-
16	21.63	356	355	193, 135	Ferulic acid-*O*- hexsoide isomer	+	+
17	22.16	386	385	223, 205, 179	Sinapic acid-*O*-hexsoide isomer	-	+
18 (**3**)	23.50	354	353	191, 179, 173 135	4-*O*-Caffeoylquinic acid	+	+
19	28.57	706	705	625, 301	Quercetin di-*O*-hexoside sulphate	+	+
20	30.98	594	593	431, 357, 327	Luteolin-*C*- deoxyhexoside -X´´-*O*-hexoside	-	+
21	31.64	788	787	625, 463, 301	Quercetin tri-*O*-hexoside	+	-
22	32.71	640	639	447, 285	Kaempferol-*O*- hydroxyferuloylglucoside	+	+
23 (**6**)	34.31	448	447	285, 179, 151	Luteolin 7-*O*-glucoside*	+	-
24	34.50	532	531	489, 285, 179	Acetyl kaempferol-*O*-(X´´-acetyl hexsoide)	-	+
25	36.45	500	499	353, 337, 191, 179, 163	Caffeoylcoumaroyl quinic acid	-	+
26	36.85	678	677	515, 353, 334, 179, 173	Tri-*O*-caffeoylquinic acid	-	+
27 (**5**)	37.11	464	463	301, 300, 271, 179, 151	Quercetin 3-*O*-glucoside*	+	+
28 (**4**)	37.52	432	431	341, 311, 269, 251, 241, 223	Baicalein 7-*O*-glucoside*	+	-
29 (**9**)	39.39	434	433	301, 300, 271, 255, 179, 151	Quercetin-7-*O*- arabinoside*	+	+
30 (**7**)	40.86	448	447	301, 300, 271, 179, 151	Quercetin 3-*O*-rhamnoside (quercetrin)*	+	+
31	41.39	638	637	491, 475, 329	Tricin-*O*-deoxyhexoside-*O*-hexoside	-	+
32 (**10**)	41.52	479	478	315	Isorhamnetin 7-*O*-glucoside*	+	-
33 (**8**)	43.39	448	447	301, 300	Quercetin 7-*O*-rhamnoside*	+	-
34 (**11**)	47.8	286	285	255, 227, 179, 151	Kaempferol*	+	-
35 (**14**)	48.73	316	315	301, 300, 179, 151	Isorhamnetin*	+	-
36 (**13**)	50.07	302	301	300, 255, 179, 151,243, 121	Quercetin*	+	-
37 (**12**)	53.81	300	299	284, 255, 151	Kaempferol 4´-*O*-methylether (Kaempferide)*	+	-

*Compounds identified based on the reference samples

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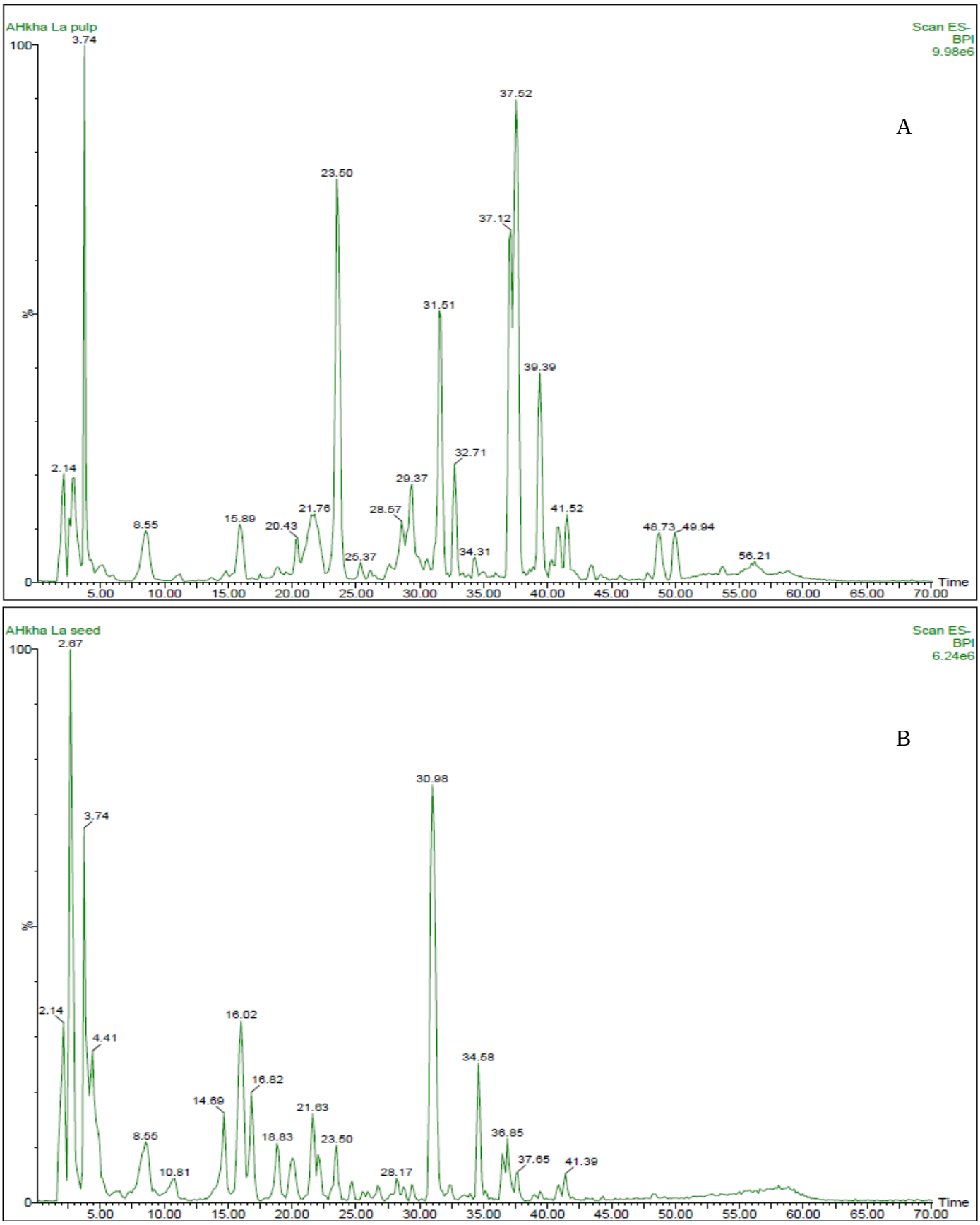

Figure 2: Base Peak Chromatogram of the negative ion mode LC-ESI-MS of *L. australis* fruit extracts; A: PE, B: SE

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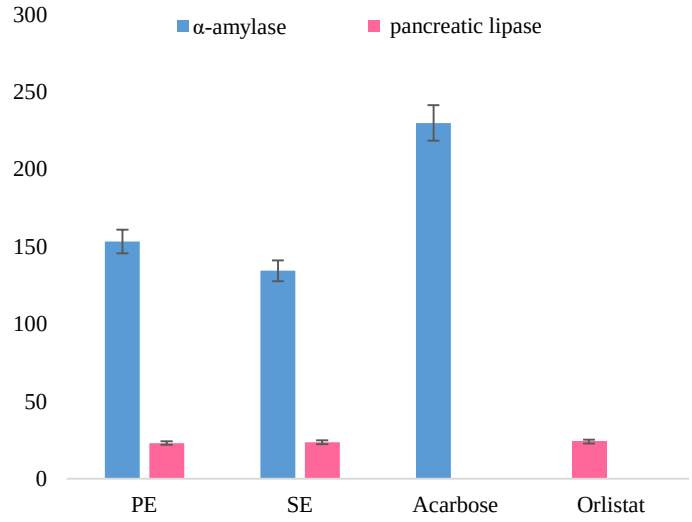

Figure 3: IC_{50} of PE, SE, acarbose at 300 ug/mL and orlistat at 100 ug/mL on α-amylase and pancreatic lipase.
Data are presented as (Mean ± S.D.), *: indicates significant difference at $p < 0.05$.

Conclusion

A total of 14 phenolic compounds comprising anthocyanins, chlorogenic acid, flavones and flavonols were isolated and identified from *L. australis* PE and/or SE. Furthermore, 24 different compounds were tentatively designated using LC–ESI–MS system from PE and/or SE. These compounds are reported for the first time in *L. australis* fruit. Both extracts effectively inhibited *α*-amylase and pancreatic lipase enzymes which are responsible for elevating blood glucose levels and gaining weight; proposed as safe and effective natural sources for treatment and management of diabetes and obesity which could enhance the health care benefits. These effects could be attributed to numerous phenolic phytoconstituents in fruits (pulps and seeds). Further *in vivo* investigations are required for controlling diabetes and obesity.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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