JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Characterization of Apigenin and Luteolin Derivatives from Oil Palm (*Elaeis guineensis* Jacq.) Leaf Using LC−ESI-MS/MS

Noor Idayu Tahir,[†,‡] Khozirah Shaari,[‡] Faridah Abas,[§] Ghulam Kadir Ahmad Parveez,[†] Zamzuri Ishak,[†] and Umi Salamah Ramli*[,†]

[†]Advanced Biotechnology & Breeding Centre, Malaysian Palm Oil Board, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

[‡]Laboratory of Natural Products, Institute of Bioscience, and [§]Faculty of Food Science & Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT: The palm oil industry generates several byproducts, and more than half of the dry weight of the waste is of oil palm leaf whereby the tissue is underutilized. Recently, several research studies found promising potential of oil palm fronds as a source of nutraceutical due to its bioactive properties. However, the chemical composition of the tissue is still not deciphered. Using reversed-phase liquid chromatography (LC) electrospray mass spectrometry (ESI-MS), glycosylated apigenin and luteolin were separated and identified from oil palm (*Elaeis guineensis* Jacq.) leaf and structures of the constituents were elucidated by collision-induced dissociation (CID) tandem MS. From 28 derivatives of the flavones, 9 compounds were conjugated with hydroxymethylglutaric (HMG) acid. Improved knowledge on oil palm especially on bioactive component of the leaf tissue will allow correlation of its beneficial effects and further promotes efficient utilization of this agriculture byproduct.

KEYWORDS: *byproducts, oil palm leaf, liquid chromatography (LC), electrospray mass spectrometry (ESI-MS), flavones*

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a perennial plant of the family Arecaceae and is considered the most productive oil crop with 42.41 million metric tonnes production in 2008 to 2009 contributing to 36% of total world oil production.[1] It is utilized for food and nonfood uses, for example, in the oleochemical industry. The palm oil industry generates several wastes during harvesting, pruning, replanting and processing in the mills, and at least 53% of the dry weight of these wastes is from oil palm leaves.[2] Oil palm fronds, which are the mature leaf, are pruned during harvesting rounds and are accessible as byproducts of oil palm plantations[3] while both the fronds and young unopened leaves or spear leaves are obtained after felling during replanting. The tissue is conventionally used for soil conservation and nutrient recycling by leaving them on the plantation floor to decompose. Several reports promote the utilization of oil palm fronds as ruminant livestock feed,[4−7] which is especially economical when the livestock are integrated in the oil palm plantation.[8] Despite these uses the tissue is underutilized[9] compared to its counterparts such as the empty fruit bunch and kernel shells that are commercialized as animal feed,[10] boiler fuel and carbon source for oil palm mills[11] and heavy industries such as cement manufacturing.[12]

Recently, oil palm leaves were reported containing bioactive agents such as antioxidants[13,14] and antihyperglycemic[15] and have organ-protective effects against hypertension.[16] Oil palm frond extract was evaluated for potential activity and found to possess highest antioxidant capacity compared to lemongrass, papaya shoots and green chili besides inducing vascular relaxation via endothelium-dependent mechanisms.[17] The high-fiber oil palm fronds provide cheap sources of energy to ruminant animals, and most importantly, these resources are abundant and renewable. They contain unsaturated fatty acids[18] and do not pose any risks as sources for ruminant feeds.[16]

Flavonoids are natural plant components and possess protective effects against human ailments such as cancer and cardiovascular disease.[19,20] Distinguished by a double bond between the C2 and C3 positions with B-ring attachment to C2 and usually no substituent present at C3,[21] flavones are grouped into the number of their hydroxyl groups.[22] Flavones and their glycosides exist as *O*- or *C*-glycosides in plants,[23] and the presence of *C*-glycosides in the plant tissues was established by their resistance to acid hydrolysis.[24] These compounds exhibit diverse biological properties including antioxidative, antitumor and antibacterial[25] such as reported for apigenin[26,27] and luteolin[28,29] in inhibiting growth of cancer cells. Apigenin and luteolin are potentially useful for the development of therapeutic treatments of methicillin-resistant *Staphylococcus aureus* (MRSA) infections[30] and are also promising remedies in skin photoaging.[31] Apigenin, luteolin and their derivatives are previously reported in the leaves of date palm (*Phoenix dactylifera*) and Thatch palm (*Howea forsteriana*) of Palmae family (Aracaceae)[32] and can be found naturally in fruits, vegetable and herbs such as belimbi fruit (*Averrhoa belimbi*), celery (*Apium graveolens*),[33] alfalfa (*Medicago sativa* L.),[34] pennyroyal (*Mentha pulegium*), thyme (*Thymus vulgaris*)[35] and Mas Cotek (*Ficus deltoidea*).[20]

Plant chemicals have been successfully studied and identified by utilizing high performance liquid chromatography (HPLC) with diode array and mass spectrometric detection (HPLC-DAD−MS).[36] Screening of apigenin and luteolin derivatives using mass spectrometry is widespread due to its speed and sensitivity[23] and its tandem MS capacity in generating

Received: March 19, 2012
Revised: October 17, 2012
Accepted: October 18, 2012
Published: November 1, 2012

informative product ions for structural elucidation.[37] This platform allows systematic separation of complex samples, characterization and identification of phytochemicals for a multitude of purposes such as quality control and biological activity of metabolite of interests. In this study, separation and identification of 28 compounds of apigenin and luteolin derivatives from oil palm leaf is performed using reversed-phase HPLC-DAD−ESI/MS and tandem mass spectrometry (MS/MS) and represents the first report of characterization of oil palm leaf chemicals. The designation of oil palm leaf phytochemicals identity is established by MS/MS due to lack of commercial reference standards for most of the major constituents of oil palm leaf. This information is relevant for better insight into the oil palm metabolome especially for the leaves, which is the most abundant byproduct of the industry. Due to significant biological properties, apigenin and luteolin from oil palm might be potentially useful as functional food and may become a target for metabolic engineering as accomplished in tomato[38] while increasing oil palm defense against pathogens[39] such as *Ganoderma* spp. and *Fusarium oxysporum*.[40]

MATERIALS AND METHODS

Standards and Reagents. HPLC grade methanol, acetonitrile, acetic acid, hydrochloric acid and L(+) ascorbic acid were purchased from Merck (Darmstadt, Germany). Purified water was prepared from Milli-Q system (Millipore Lab, Bedford, MA, USA). Vitexin (apigenin-8-*C*-glucoside) (>96.0%), isovitexin (apigenin-6-*C*-glucoside) (≥98.0%), orientin (luteolin-8-*C*-glucoside) (>95.0%) and isoorientin (luteolin-6-*C*-glucoside) (>98.0%) of HPLC grade; and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Materials and Extraction. Oil palm spear leaf and mature leaf (frond) tissues of commercial Tenera (Dura x Pisifera, DxP) variety were harvested from the Malaysian Palm Oil Board (MPOB) Kluang Research Station. Spear leaf is the unopened leaflet (noted as frond 0) while mature leaf is the 17th frond, counted from "frond 0" in a spiral manner from the apex to follow practice of agronomist for uniformity in sampling. The leaves were cut into small pieces of an inch length and shock-frozen in liquid nitrogen before being powderized in liquid nitrogen using mortar and pestle. The powder was then lyophilized using Labconco FreeZone Freeze-Dry System (Kansas City, MO, USA). Extraction of metabolites from leaf tissue was carried out using 0.1 g of the dried powder with 5 mL of 80% methanol. The mixture was vortexed for 30 s before sonication for 30 min at room temperature and later centrifuged at 4000 rpm for 15 min at 25 °C. The supernatant was collected and dried under a nitrogen stream before being reconstituted in 1 mL of water. The extracts were filtered with a 0.25 μm syringe filter for HPLC injection.

Acidic Hydrolysis. Acidic hydrolysis was performed on whole extracts of oil palm leaves and LC fractions containing peak of interest. The crude extracts and LC fractions were dried under a nitrogen stream before being reconstituted in 1 mL of water. Concentrated hydrochloric acid (1 mL, 12 N) was added to the fraction in a capped tube followed by 2 h incubation in a water bath at 85 °C. The extract was allowed to cool to room temperature and added with 400 μL of methanol. After sonication for 10 min the solution was dried under a nitrogen stream before being reconstituted in water.

Diphenylpicrylhydrazyl (DPPH) Radical Scavenging Activity. DPPH radical scavenging assay was performed as described previously[41,42] with slight modifications. DPPH solution of 100 μM was prepared in methanol. A volume of 1.9 mL of this solution was added with 100 μL of extract or standard solution. The mixture was incubated at room temperature for 30 min before the absorption (abs) was read at 517 nm in a U2800 Hitachi spectrophotometer against a methanol blank. Samples were prepared and read in duplicate. Percentage of DPPH radical scavenging activity (% DRSA) was calculated by the following equation:

$$\%\ \mathrm{DRSA} = \{[(\text{abs of DPPH solution added with methanol} - \text{abs of DPPH solution added with extract})] / (\text{abs of DPPH solution added with methanol})\} \times 100$$

while DRSA of oil palm leaf extract at 500 mg/L was expressed in vitamin C equivalent antioxidant capacity (VCEAC), μmol/L, using ascorbic acid standard curve that relates concentration of ascorbic acid to the amount DRSA.

Liquid Chromatography−Mass Spectrometry (LC−MS). Oil palm leaf extract was separated using C18 reversed-phase Acclaim 120, 5 μm particle size and 4.6 μm i.d. × 150 mm length column from Dionex (Sunnyvale, CA, USA) on Dionex UltiMate 3000 HPLC with a PDA-3000 photodiode array detector and a thermostatted column compartment which was maintained at 35 °C during HPLC analysis. Gradient elution was performed with water:0.1% acetic acid (solvent A) and acetonitrile:0.125% acetic acid (solvent B), with solvent B eluted up to 22% in 60 min. The flow rate was constant at 1.00 mL/min. After going through the detector, the flow was split to allow only 200 μL/min of eluent into the electrospray ionization (ESI) source of MS.

ESI-MS analysis was performed on MicrOTOF-Q quadrupole time-of-flight (Q-TOF) MS (Bruker Daltonik, Bremen, Germany). The source conditions were as follows: nebulizer gas (N_2) at 2.5 bar, dry gas (N_2) at 8.0 L/min, dry temperature at 180 °C, capillary at −3500 V and end plate offset at −500 V. The MS acquisitions were performed in the negative electrospray ionization mode, in the mass range of 50 to 1000 *m*/*z*. For tandem MS (MS/MS) data acquisitions were carried out in the automatic mode at 30 eV collision energy. Data acquisition was performed by HyStar Application version 3.2 while data processing was carried out with DataAnalysis Version 3.4 by Bruker Daltonik GmbH.

An adaptation of conventional nomenclature[43,44] is used in this work to explain fragment ions of glycoconjugates. Fragment ions denoted as ${}^{k,l}_{j}X_n$ represent ions still comprising the flavone aglycon with *k* and *l* denoting the cleavage positions within the carbohydrate rings. The total of carbons in the monosaccharide interglycosylated to the aglycon (hexose, 6; pentose, 5) is represented by *j* while *n* refers to the attachment position of the saccharide to the aglycon (C-6 or C-8).

Relative Concentration of Compounds. Concentration of identified compounds in the oil palm leaf extracts was performed using UV absorption data (area mAU·min) at 330 nm. Calibration curves were constructed using 200, 250, 300, 350, 400 and 450 mg/L concentration of vitexin (apigenin-8-*C*-glucoside) and orientin (luteolin-8-*C*-hexose), and the curve was used to quantify the 28 oil palm leaf constituents according to their proposed identities, by which the concentration of peaks **1**, **2**, **8**, **10**, **23**, **24**, **27** and **28** was estimated using the orientin calibration curve while the rest were subjected to that of vitexin.

RESULTS AND DISCUSSION

Diphenylpicrylhydrazyl (DPPH) Radical Scavenging Activity of Oil Palm Leaf Extracts. From DPPH radical scavenging assay, oil palm leaves and their hydrolyzed extracts showed antioxidant capacity as a function of concentration (Figure 1). Only oil palm frond showed more than 50% antioxidant activity compared to other extracts while the activity of hydrolyzed spear leaf extract was slightly higher than that of spear leaf, suggesting that certain compounds to some extent react better after losing their conjugated form. However, activities of spear leaf and its hydrolyzed extract did not surpass the DRSA of oil palm fronds or its hydrolyzed extract. The DRSA of the four samples was expressed in vitamin C equivalent antioxidant capacity (VCEAC), μmol/L, with frond

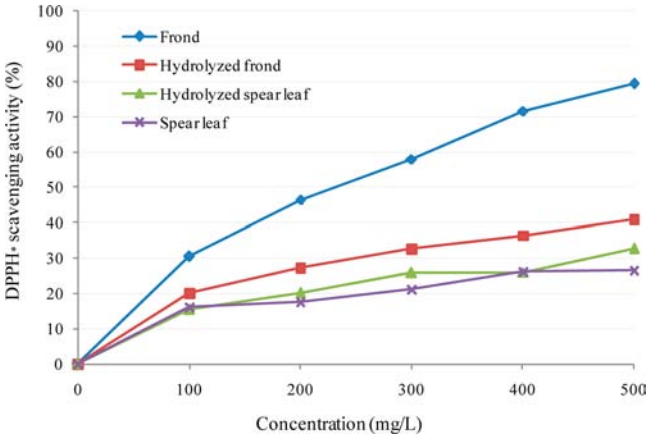


Figure 1. Diphenylpicrylhydrazyl (DPPH) radical scavenging activities of oil palm leaves and their hydrolyzed extracts.

tissue exerting the highest value of 22.38 ± 0.03 VCEAC per μmol/L (Table 1).

Table 1. DRSA of Oil Palm Leaf Extract at 500 mg/L Expressed in Vitamin C Equivalent Antioxidant Capacity (VCEAC)[a]

tissue	VCEAC per μmol/L
frond	22.38 ± 0.03
hydrolyzed frond	11.53 ± 0.01
hydrolyzed spear leaf	9.18 ± 0.00
spear leaf	7.52 ± 0.03

[a]Values reported are means of 2 replicates ± standard error of the means.

Liquid Chromatography–Mass Spectrometry of Oil Palm Leaf Extracts. Aqueous methanolic oil palm spear leaf and frond extractables were separated and monitored using HPLC-DAD before being analyzed by MS. Similar analyses were also performed onto hydrolyzed samples of the two tissues, and their LC–MS chromatograms were overlaid for comparison (Figure 2). Results from UV detection gave a general idea of the classes of compounds contained in complex oil palm leaf samples. Compound peaks observable in the ion chromatogram from the MS and UV chromatographic profile of the oil palm leaf constituents at 270 and 340 nm and information such as retention time (t_R), mass-to-charge ratio (m/z), MS/MS data and maximum absorption at UV/vis facilitate the identification of the corresponding peaks (Table 2). Information on relative abundance of ions in comparison to their precursors also facilitated the position recognition of sugar moieties in the compound structure.

The identities of apigenin and luteolin derivatives were initially resolved by means of comparison of retention time and UV/vis absorption to commercial standards such as vitexin, orientin and their isomers. However, the standards were unavailable for most of the derivatives, hence peak identities were assigned according to their molecular formula generated by isotope pattern and their fragmentation pattern via collision-induced dissociation (CID) on high-resolution Q-TOF mass spectrometer and by analyzing their products after chemical reactions, e.g., acid hydrolysis. From LC–MS analysis of the oil palm leaf extracts, luteolin and apigenin derivatives are found to be the major constituents. All collected peaks showed two ranges of absorption maxima of 270–309 nm (band II) and 336–348 nm (band I). Resistance of several compound peaks to hydrolysis gave a clue of their nature of carbon–carbon bond[45] conjugation in addition to their UV absorption range. Their structure assignments are discussed below.

Identification of Luteolin Derivatives. Peak numbers **1**, **2**, **8**, **10**, **23**, **24**, **27** and **28** were attributed as luteolin derivatives based on their fragmentation patterns in MS/MS and UV spectra. Peak **1** was fragmented into m/z 519.1167, 489.1035, 399.0654 and 369.0617. These fragments exhibit losses of 90, 120, 210 and 240 amu respectively (Figure 3). Fragmentation of *C*-hexosyl conjugate at the 6-C position of luteolin (aglycon; Agly) results in ion $[^{0,3}{}_6X_6]^-$ at m/z 519.1167, $[(M - H) - 90]^-$ and further cleavage of the hexose produces $[^{0,2}{}_6X_6]^-$ ion at m/z 489.1035 $[(M - H) - 120]^-$. Availability of $[Agly + 83]^-$ and $[Agly + 113]^-$ ions is most probably due to fragmentation of both 6-*C*- and 8-*C*-hexosyl with breakage at the 0 and 3″ positions for both hexoses for m/z 399 and further loss of CH_2O from any of the hexosyl ring to result in m/z 369 ion.

Peaks **1** and **2** share analogous MS/MS products of m/z 399 and 369 ions. Abundance of m/z 369 and 399 ions in the MS/MS spectra for these peaks is significant for the existence of typical $[Agly + 83]^-$ and $[Agly + 113]^-$ ions in *C*-glycosylated flavones, is in agreement with previously published data[46,47] and is coherent with the presence of two *C*-glycosylated sugar to flavones, in this case, luteolin ($C_{15}H_{10}O_6$, 286 MW). Peak **1** was therefore established as luteolin-di-*C*-hexose. Peak **2** is identified as luteolin-*C*-hexose-*C*-deoxyhexose based on the presence of the $[Agly + 113]^-$ and $[Agly + 83]^-$ ions and due to neutral loss of 120 amu for a hexose. Peak **2** exhibited different fragment ions from peak **3** that has similar m/z value. Peak **8** (m/z = 447.2883, $[M - H]^-$, t_R = 32.8 min) and peak **10** (m/z = 447.0959, $[M - H]^-$, t_R = 33.6 min) were confirmed as isoorientin (luteolin-6-*C*-glucoside) and orientin (luteolin-8-*C*-glucoside), respectively, by comparing their retention times (t_R) and MS/MS fragmentation pattern to authentic commercial standards of isoorientin and orientin.

Peak **23**, **24**, **27** and **28**, which are detected in oil palm frond extract, are also of luteolin derivatives by deducing their MS/MS product ions. Peaks **23** and **24** share a similar m/z to peak **2**, and both have quite similar MS/MS fragment ions except for m/z 447.1111, which is only found in fragments of peak **23**. Ions at m/z 447.1111 and 429.0776 denote for $[(M - H) - 146]^-$ and $[(M - H) - 146 - 18]^-$, which indicate the presence of *O*-glycosylated deoxyhexose with interglycosidic linkage in the compound, probably in the 2″ position of the hexose.[46] The identity of peak **23** is established as luteolin-6-*C*-hexose-2″-*O*-deoxyhexose. The presence of *C*-glycosylated hexose at position 6-C of luteolin would raise more $[(M - H) - 120]^-$ ions of m/z 473 during MS/MS. By observing the relative abundance in MS/MS products of peaks **2** and **24**, the specific position of hexose and deoxyhexose can be deduced. $[(M - H) - 44]^-$, $[(M - H) - 74]^-$, $[(M - H) - 104]^-$ and $[(M - H) - 134]^-$ are characteristics of *C*-deoxyhexose fragmentations,[48] however these ions were only observed at trace level and are not comparable to $[(M - H) - 120]^-$ ions. Peaks **2** and **24** are thus identified as isomers of luteolin-6-*C*-hexose-8-*C*-deoxyhexose.

Peaks **27** and **28** shared a similar single distinct fragment ion of m/z 285, $[M - H]^-$ (Figure 4). Based on the molecular formula calculated using the isotopic pattern ($C_{15}H_{10}O_6$) and their UV absorptions, both compounds are deduced as *O*-

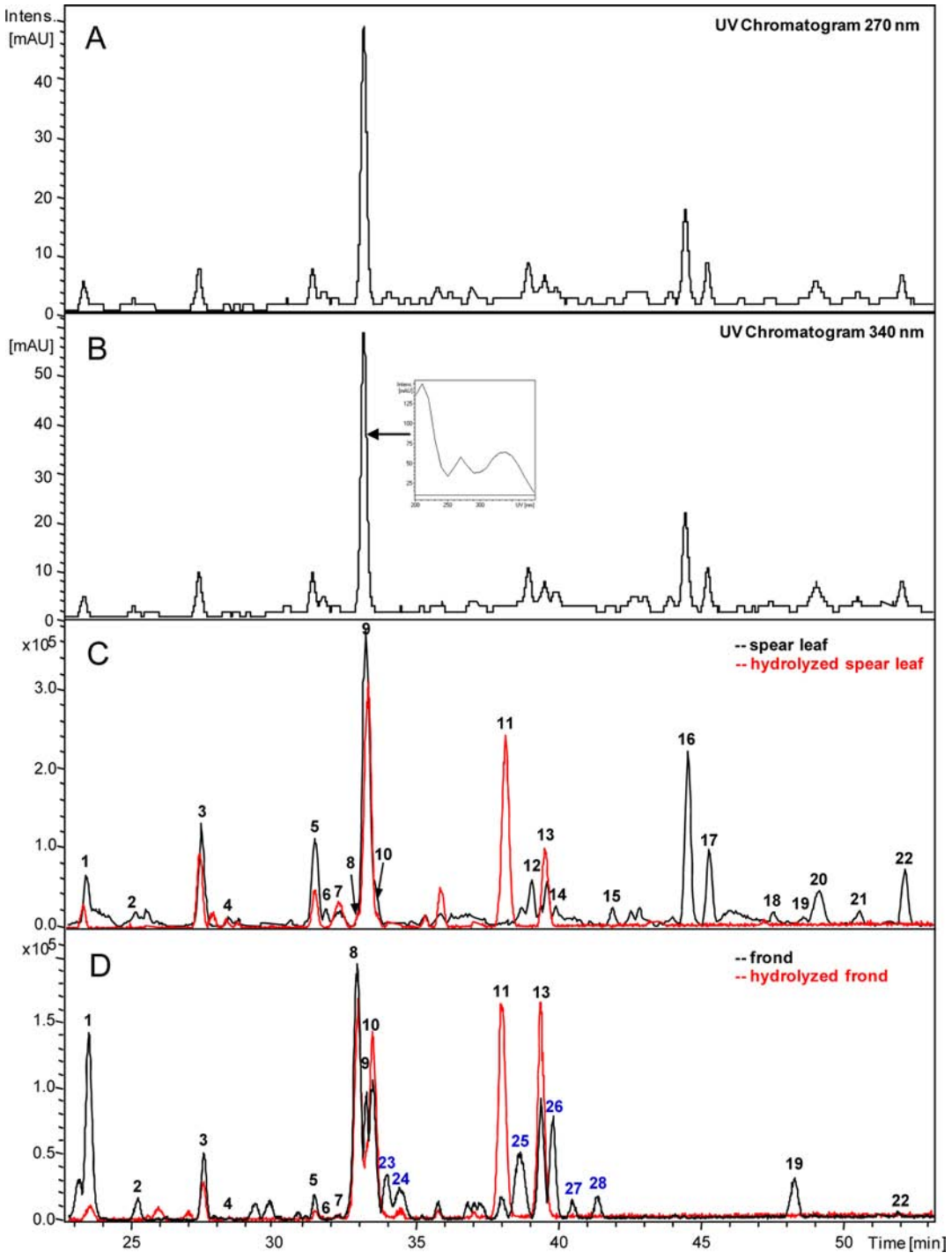


Figure 2. UV chromatograms at 270 nm (A) and at 340 nm (B); and ion chromatograms of oil palm spear leaf extract (C) and oil palm frond extract (D) overlaid with their hydrolysis products, with numbered peaks. Image in box in B is UV spectrum of peak **9**.

glycosylated derivatives of luteolin. Peak **27** is established as luteolin-*O*-hexose-*O*-deoxyhexose by the loss of 308 amu while peak **28** is identified as luteolin-*O*-hexose, which loses 162 amu during MS/MS.

Identification of Apigenin Derivatives. The consistent presence of m/z 383 $[Agly + 113]^-$ and 353 $[Agly + 83]^-$ ions in MS/MS spectra for peaks **3**, **4**, **5**, **6**, **7**, **9**, **10**, **12**, **14**, **15**, **16**, **17**, **20**, **21**, **25** and **26** in addition to neutral losses of 60, 90 and/or 120 amu fits the assignment of identity for the compounds as *C*-glycosylated apigenin derivatives.[49] Peak **3** (m/z = 593.1552, $[M - H]^-$, t_R = 27.3 min) was fragmented into m/z 503.1369, $[(M - H) - 90]^-$, 473.1090, $[(M - H) - 120]^-$, 383.0783, $[(M - H) - 210]^-/[Agly + 113]^-$, and 353.0665, $[(M - H) - 240]^-/[Agly + 83]^-$, wherein the losses are comparable to peak **1**. Thus, its identity was determined as apigenin-di-*C*-hexose. Peak **4** (m/z = 563.1409, $[M - H]^-$, t_R = 28.4 min) was fragmented into m/z 473.1093, $[(M - H) - 90]^-$; 443.0979, $[(M - H) - 120]^-$; 383.0803, $[(M - H) - 180]^-/[Agly + 113]^-$ and 353.0672, $[(M - H) - 210]^-/[Agly + 83]^-$ (Figure 5).

Ion $[^{0,3}{}_5X_8]^-$ at m/z 503.1189 occurs due to 0, 3″ breakage of an 8-*C*-pentosyl of the aglycon. Both ions $[^{0,3}{}_6X_6]^-$ and

dx.doi.org/10.1021/jf303267e | *J. Agric. Food Chem.* 2012, 60, 11201−11210

Table 2. Peak Assignments of Luteolin and Apigenin Derivatives in Oil Palm Leaf

peak	t_R, min	λ_{max}, nm	[M − H], m/z	MS/MS fragments, m/z (% rel abundance)	empirical formula	structure assignment	concn (mg/L) equiv to vitexin/orientin: spear leaf tissue	concn (mg/L) equiv to vitexin/orientin: frond tissue
1	23.2	270, 348	609.1520	519.1167 (7.0), 489.1035 (30.8), 399.0654 (3.3), 369.0617 (7.1)	$C_{27}H_{30}O_{16}$	luteolin-6,8-di-*C*-hexose	495.18	78.14
2	24.8	269, 339	593.1517	473.1107 (11.8), 399.1451 (38.0), 369.1310 (6.8), 337.1040 (5.6)	$C_{27}H_{30}O_{15}$	luteolin-6-*C*-hexose-8-*C*-deoxyhexose	19.73	23.23
3	27.3	270, 337	593.1552	503.1369 (2.5), 473.1090 (12.7), 383.0783 (1.3), 353.0665 (3.1)	$C_{27}H_{30}O_{15}$	apigenin-6,8-di-*C*-hexose	624.60	104.40
4	28.4	270, 337	563.1409	473.1093 (12.6), 443.0979 (17.2), 383.0803 (5.2), 353.0672 (6.2)	$C_{26}H_{28}O_{14}$	apigenin-6-*C*-hexose-8-*C*-pentose	52.43	3.37
5	31.3	270, 337	563.1400	473.1078 (13.4), 443.0987 (10.4), 383.0771 (5.6), 353.0672 (7.2)	$C_{26}H_{28}O_{14}$	apigenin-6-*C*-pentose-8-*C*-hexose	362.40	37.51
6	31.8	270, 337	725.1950	563.1424 (7.2), 473.1057 (11.2), 443.1010 (46.4), 383.0821 (27.2), 353.0696 (48.4)	$C_{32}H_{38}O_{19}$	apigenin-6-*C*-hexose-8-*C*-pentose-7-*O*-hexose	34.29	6.01
7	32.1	270, 337	563.1414	473.1088 (18.7), 443.1000 (10.0), 383.0777 (7.5), 353.0675 (7.4)	$C_{26}H_{28}O_{14}$	apigenin-6-*C*-pentose-8-*C*-hexose	40.89	8.00
8	32.8	270, 339	447.2883	357.0592 (43.9), 339.0545 (19.8), 327.0487 (100.0), 297.0368 (76.2)	$C_{21}H_{20}O_{11}$	isoorientin (luteolin-6-*C*-hexose)	143.18	638.05
9	33.1	270, 337	563.1397	473.1080 (8.6), 443.0986 (9.0), 383.0771 (2.6), 353.0666 (3.5)	$C_{26}H_{28}O_{14}$	apigenin-6-*C*-hexose-8-*C*-pentose	3076.17	214.86
10	33.6	269, 339	447.0959	357.0623 (16.1), 339.0544 (9.3), 327.0535 (100.0), 297.0413 (41.7)	$C_{21}H_{20}O_{11}$	orientin (luteolin-8-*C*-hexose)	68.59	196.91
11	37.8	270, 338	431.0979	341.0594 (4.3), 311.0502 (66.0), 283.0647 (100.0)	$C_{21}H_{20}O_{10}$	vitexin (apigenin-8-*C*-hexose)	213.86	47.91
12	39.0	270, 336	737.1991	593.1548 (1.3), 473.1099 (8.0), 413.0868 (11.6), 383.0758 (81.0), 353.0657 (100.0), 161.0426 (1.8), 99.0445 (2.4)	$C_{33}H_{38}O_{19}$	apigenin-6,8-di-*C*-hexose-*O*-hydroxymethyl glutaric acid	57.89	nd
13	39.7	270, 339	431.1001	413.0947 (0.2), 341.0663 (23.0), 311.0568 (72.6), 283.0507 (100.0)	$C_{21}H_{20}O_{10}$	isovitexin (apigenin-6-*C*-hexose)	268.00	0.20
14	39.9	270, 336	737.1995	593.1549 (0.9), 473.1052 (6.6), 413.0790 (9.1), 383.0753 (55.5), 353.0655 (100.0), 161.0438 (3.4), 99.0413 (2.7)	$C_{33}H_{38}O_{19}$	apigenin-6,8-di-*C*-hexose-*O*-hydroxymethyl glutaric acid	222.29	nd
15	41.7	270, 330	707.1839	617.1513 (2.1), 563.1402 (3.5), 473.1054 (3.4), 443.0988 (40.6), 383.0774 (2.3), 353.0623 (1.6)	$C_{32}H_{36}O_{18}$	apigenin-6-*C*-hexose-8-*C*-pentose-*O*-hydroxymethyl glutaric acid	66.86	nd
16	44.3	270, 330	707.1817	617.1485 (1.8), 563.1424 (2.7), 473.1115 (4.2), 443.0967 (35.8), 383.0750 (0.4), 353.0649 (0.7), 161.0412 (1.0), 99.0443 (0.3)	$C_{32}H_{36}O_{18}$	apigenin-6-*C*-hexose-8-*C*-pentose-*O*-hydroxymethyl glutaric acid	378.74	nd
17	45.2	270, 330	707.1824	647.1621 (0.6), 617.1492 (3.6), 563.1411 (1.0), 545.1302 (1.2), 443.0981(5.6), 383.0785 (0.5), 353.0660 (0.6)	$C_{32}H_{36}O_{18}$	apigenin-6-*C*-pentose-8-*C*-hexose-*O*-hydroxymethyl glutaric acid	155.83	nd
18	47.6	270, 339	577.1581	269.0455 (100.0)	$C_{27}H_{30}O_{14}$	apigenin-*O*-hexose-*O*-deoxyhexose	26.14	nd
19	48.7	270, 339	575.1443	473.1079 (20.7), 431.0969 (42.6), 413.0915 (19.0), 353.0677 (9.1), 341.0688 (9.6), 311.0566 (100.0), 161.0449 (14.0), 99.0436 (2.9)	$C_{27}H_{28}O_{14}$	apigenin-*C*-hexose-*O*-hydroxymethyl glutaric acid	100.91	1465.83
20	49.2	270, 339	721.2030	577.1575 (43.9), 559.1468 (6.9), 457.1090 (10.4), 413.0798 (1.8), 353.0583 (0.2), 341.0667 (0.4), 311.0539 (1.0), 161.0398 (3.6), 99.0462 (0.6)	$C_{33}H_{38}O_{18}$	apigenin-6-*C*-hexose-8-*C*-deoxyhexose-*O*-hydroxymethyl glutaric acid	547.43	nd
21	51.4	270, 330	707.1843	647.1608 (2.6), 617.1479 (10.8), 563.1353 (5.8), 545.1395 (4.4), 443.0994 (15.0), 353.0665 (3.4), 161.0466 (3.6)	$C_{32}H_{36}O_{18}$	apigenin-6-*C*-pentose-8-*C*-hexose-*O*-hydroxymethyl glutaric acid	12.77	nd
22	52.2	270, 336	575.1392	473.1091 (36.9), 431.0966 (53.5), 413.0855 (18.3), 353.0622 (2.3), 341.0674 (27.2), 311.0565 (100.0), 161.0462 (38.8), 99.0451 (4.0)	$C_{27}H_{28}O_{14}$	apigenin-*C*-hexose-*O*-hydroxymethyl glutaric acid	284.57	846.94
23	34.0	270, 340	593.1505	473.1011 (29.3), 447.0877 (6.31), 429.0776 (18.3), 357.0623 (8.7), 339.0505 (3.7)	$C_{27}H_{30}O_{15}$	luteolin-6-*C*-hexose-2″-*O*-deoxyhexose	nd	47.91

dx.doi.org/10.1021/jf303267e | J. Agric. Food Chem. 2012, 60, 11201−11210

Table 2. continued

peak	t_R, min	λ_{max}, nm	$[M - H]^-$, m/z	MS/MS fragments, m/z (% rel abundance)	empirical formula	structure assignment	concn (mg/L) equiv to vitexin/orientin: spear leaf tissue	concn (mg/L) equiv to vitexin/orientin: frond tissue
24	34.5	269, 338	593.1579	473.1063 (53.1), 429.0907 (44.9), 369.0559 (4.4), 357.0622 (59.4), 327.0520 (44.0)	$C_{27}H_{30}O_{15}$	luteolin-6-*C*-hexose-8-*C*-deoxyhexose	nd	136.45
25	38.7	270, 339	577.1605	457.1012 (5.7), 413.0913 (100.0), 383.0761 (0.4), 353.0679 (0.7), 341.0686 (2.7), 311.0569 (6.9)	$C_{27}H_{30}O_{14}$	apigenin-6-*C*-hexose-8-*C*-deoxyhexose	nd	557.80
26	39.9	270, 339	577.1618	457.1146 (12.17), 413.0911 (64.9), 353.0678 (2.8), 341.0685 (6.3)	$C_{27}H_{30}O_{14}$	apigenin-6-*C*-hexose-8-*C*-deoxyhexose	nd	1111.86
27	40.4	269, 338	593.1491	285.0427 (49.6)	$C_{27}H_{30}O_{15}$	luteolin-*O*-hexose-*O*-deoxyhexose	nd	17.45
28	41.3	270, 337	447.0864	285.0427 (100.0)	$C_{21}H_{20}O_{11}$	luteolin-*O*-hexose	nd	21.41

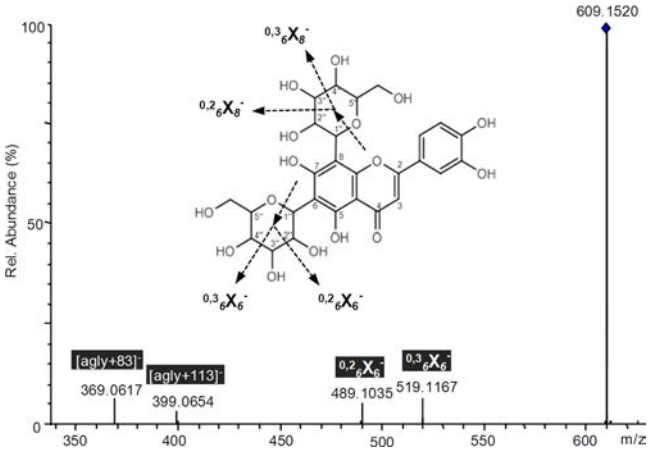


Figure 3. MS/MS spectrum of m/z 609.1520, $[M - H]^-$ and annotation of its fragments.

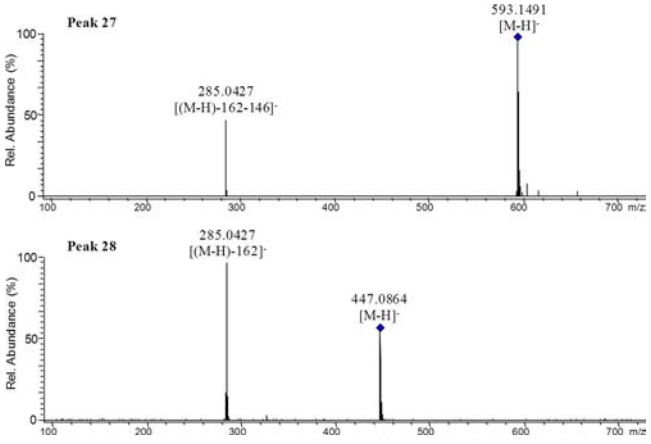


Figure 4. MS/MS spectra of peaks **27** and **28**.

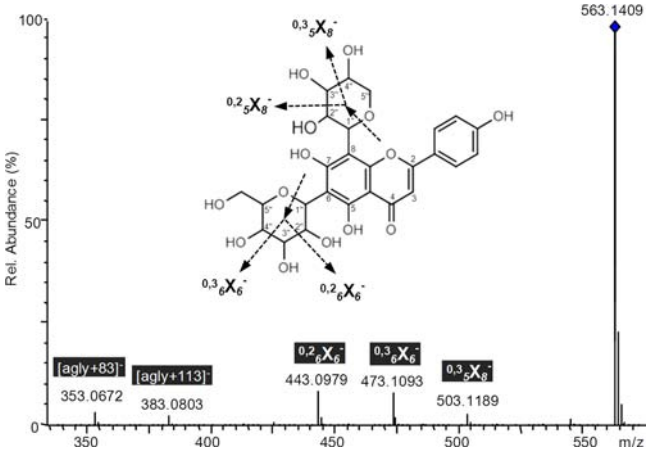
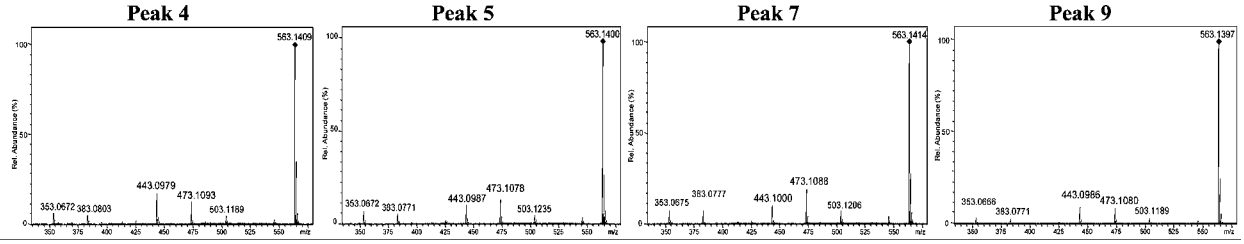


Figure 5. MS/MS spectrum of m/z 563.1409, $[M - H]^-$ and annotation of its fragments.

$[^{0,2}{}_5X_8]^-$ contribute to abundance of m/z 473.1093, $[(M - H) - 90]^-$, while m/z 443.0979, $[(M - H) - 120]^-$ ion is $[^{0,2}{}_6X_6]^-$ as a result of 6-*C*-hexosyl fragmentation of the molecule. Peaks **5**, **7** and **9** had similar m/z value and fragmentation patterns to peak **4** and are identified as isomers of apigenin-*C*-hexose-*C*-pentose. The relative abundance of the fragment ions demonstrates the type of sugar at the C-6 position,[50] and the substituents at the C-6 position are more

Table 3. MS/MS Spectra of m/z 563 Isomers with Fragment Ion Relative Abundance Information[a]



Fragment ion (m/z)	Rel. abundance (%)	Rel. abundance (%)	Rel. abundance (%)	Rel. abundance (%)
563 $[M-H]^-$	100.0	100.0	100.0	100.0
473 $[M-90-H]^-$	12.6	**13.4**	**18.7**	8.6
443 $[M-120-H]^-$	**17.2**	10.4	10.0	**9.0**
C-6 sugar	hexose	pentose	pentose	hexose
Proposed structure	*Apigenin-6-C-hexose-8-C-pentose*	*Apigenin-6-C-pentose-8-C-hexose*	*Apigenin-6-C-pentose-8-C-hexose*	*Apigenin-6-C-hexose-8-C-pentose*

[a]Higher CID collision energy (>30 eV) decimated m/z 473 and 443 ions and generated higher abundance of m/z 383 and 353.

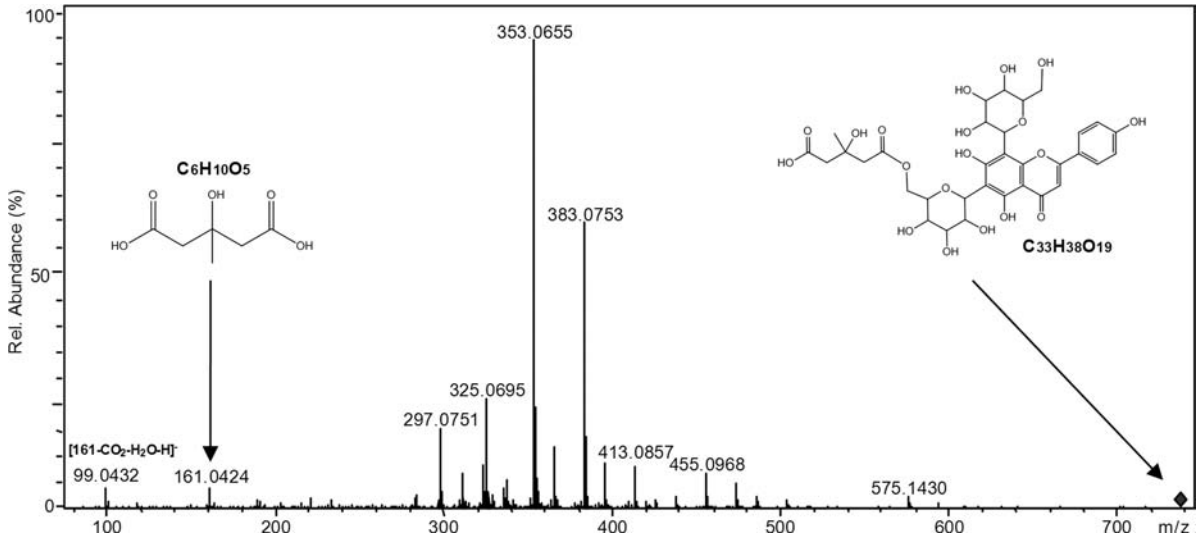


Figure 6. MS/MS spectrum of peak **12**, m/z 737.1991, $[M - H]^-$.

prone to cleavage in flavones.[51] Relative abundances of m/z 473, $[(M - H) - 90]^-$ and 443, $[(M - H) - 120]^-$ as results of CID fragmentation of the four m/z 563 isomers are studied to establish the substituent in the C-6 position of the apigenin structure for the four isomer peaks (Table 3). Their identities are proposed based on the relative abundance of the m/z 473, $[(M - H) - 90]^-$ and 443, $[(M - H) - 120]^-$ ions.

Peak **6** (m/z = 725.1950, $[M - H]^-$, t_R = 31.8 min) fragments into m/z 563.1424, $[(M - H) - 162]^-$; 473.1057, $[(M - H) - 162-90]^-$; 443.1010, $[(M - H) - 162-120]^-$; 383.0821, $[Agly + 113]^-$ and 353.0696, $[Agly + 83]^-$. A neutral loss of 162 amu from m/z 725 signifies loss of a hexose, while other fragments are congruent with fragmentation of an apigenin-*C*-hexose-*C*-pentose. Due to the absence of m/z 545 that is $[(M - H) - 180]^-$ ion that denotes fragmentation of an *O*-interglycosidic linkage, the position of *O*-glycosylation is suggested to be on the phenolic hydroxyl of apigenin, probably at the 7 position.[46] Furthermore, in a similar manner of deducing the positions of *C*-glycosylation of hexose and pentose for peaks **4**, **5**, **7** and **9**, the abundance of m/z 443, $[(M - H) - 162-120]^-$ which is higher than m/z 473 for peak **6** allowed the identification of the compound as apigenin-6-*C*-hexose-8-*C*-pentose-*O*-hexose.

The identities of peaks **11** and **13** (m/z = 431.1001, $[M - H]^-$) were confirmed as vitexin (apigenin-8-*C*-hexose) and isovitexin (apigenin-6-*C*-hexose) respectively, using authentic standards and their concurring fragmentation pattern: m/z 413, $[(M - H) - H_2O]^-$; 341, $[Agly + 71]^-$; 311, $[Agly + 41]^-$ and 283.0507, $[Agly + CH_2]^-$. Peaks **12** (m/z = 737.1991, $[M - H]^-$, t_R = 39.0 min) and **14** (m/z = 737.1951, $[M - H]^-$, t_R = 39.9 min) share similar m/z values, 737.19, $[M - H]^-$ at different retention times. The CID fragmentation resulted in several analogous fragments for both peaks: m/z 593, $[(M - H) - 144]^-$; 473, $[(M - H) - 144 - 120]^-$; and 413, $[(M - H) - 144 - 120 - 60]^-$ alongside m/z 383, $[Agly + 113]^-$; 353, $[Agly + 83]^-$; 161 and 99. The losses signify cleavages of *C*-sugars from the molecules while loss of 144 amu was due to the presence of a moiety that is *O*-linked to the molecules. The molecular formula for the precursor ion and fragments m/z 161 and 99 were generated using the isotopic pattern (Figure 6).

Similar phenomena of MS/MS spectra containing m/z 161 and 99 and loss of 144 amu from the precursor were observed in peaks **15**, **16**, **17**, **19**, **20**, **21** and **22**. Peaks **15**, **16**, **17** and **21** are of m/z 707 and share similar MS/MS fragments of m/z 563, $[(M - H) - 144]^-$; 545, $[(M - H) - 144 - H_2O]^-$; 473, $[(M - H) - 144 - 90]^-$ and 443, $[(M - H) - 144 - 120]^-$. Peak **15** was submitted to acid hydrolysis, and its hydrolysis products were analyzed by LC–MS (Figure 7). The hydrolysis product of peak **15** contains m/z 563 and 161 in which m/z 161 is further decarboxylated into m/z 99 during MS detection.

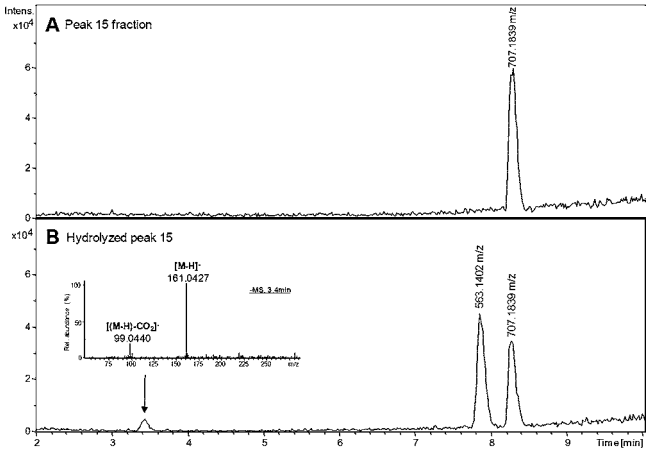


Figure 7. Ion chromatogram of peak **15** fraction (m/z 707.18) (A) and its hydrolysis product (B).

The molecular formula generated from the isotopic pattern for m/z 161 and 99 insinuates that the compound is composed of a hydroxymethylglutaric acid (HMG acid; $C_6H_{10}O_5$) as reported previously in bitter orange (*Citrus aurantinum*) juice,[47] Roman chamomile (*Chamaemelum nobile* L.)[52] and Spanish moss (*Tillandsia usneoides*).[53] Peaks **15**, **16**, **17** and **21** are proposed as apigenin-*C*-hexose-*C*-pentose-*O*-hydroxymethyl glutaric acid while peaks **12** and **14** (m/z 737) are identified as apigenin-di-*C*-hexose-*O*-hydroxymethyl glutaric acid. Using relative abundance of their fragment ions, an attempt was made to assign the positions of their sugar moieties. Peaks **15** and **16** are established as apigenin-6-*C*-hexose-8-*C*-pentose-*O*-hydroxymethyl glutaric acid with HMG acid *O*-conjugated at the 6-*C*-hexose based on the abundance of m/z 443, $[M - H) - 144 - 120]^-$. Due to significant presence of m/z 647, $[(M - H) - 60]^-$ and 617, $[(M - H) - 90]^-$ and lower abundance of m/z 443 ions, the identities of peaks **17** and **21** are suggested as apigenin-6-*C*-pentose-8-*C*-hexose-*O*-hydroxymethyl glutaric acid with HMG acid *O*-conjugated at the 8-*C*-hexose. Apigenin-6-*C*-pentose-8-*C*-hexose-6″-*O*-hydroxymethyl glutaric acid was also isolated from fenugreek (*Trigonella foenum-graecum*) seeds.[54]

Peak **18** (m/z = 577.1581, $[M - H]^-$, t_R = 47.6 min) exhibited m/z 269.0455 fragment ion, and from its UV spectrum, the identity of peak **18** is established as apigenin-*O*-hexose-*O*-deoxyhexose. The compound lost both its sugars simultaneously during CID MS/MS, and other fragments besides the aglycon could not be observed. Under CID fragmentation, peaks **19** (m/z = 575.1443, $[M - H]^-$, t_R = 48.7 min) and **22** (m/z = 575.1392, $[M - H]^-$, t_R = 52.2 min) break into m/z 473, $[(M - H) - 102]^-$; 431, $[(M - H) - 144]^-$; 413, $[(M - H) - 144 - H_2O]^-$; 353.0677, $[(M - H) - 144 - 60 - H_2O]^-$; 341, $[Agly + 71]^-$; 311, $[Agly + 41]^-$; 161, $[C_6H_{10}O_5 - H]^-$ and 99, $[C_6H_{10}O_5 - CO_2 - H]^-$. These two compounds are identified as isomers of apigenin-*C*-hexose-*O*-hydroxymethyl glutaric acid.

MS/MS fragmentation of peak **20** (m/z = 721.2030, $[M - H]^-$, t_R = 49.2 min) results in m/z 577.1575, $[(M - H) - 144]^-$; 559.1468, $[(M - H) - 144 - H_2O]^-$; 457.1090, $[(M - H) - 144 - 120]^-$ and fragments that are similar to MS/MS results of peak **19** and **22** such as m/z 413, 341, 311, 161 and 99. Loss of 144 amu suggests the presence of hydroxymethyl glutaric acid while loss of 120 amu indicates cleavage of a hexose. From the molecular formula of the precursor ion of peak **20**, $C_{33}H_{38}O_{18}$, it is suggested that a deoxyhexose is a substituent of the molecule. The key fragments to MS/MS products showed $[(M - H) - 144 - 120]^-$ as the next most abundant ion after $[(M - H) - 144]^-$, thus, the identity of the peak is assigned as apigenin-6-*C*-hexose-8-*C*-deoxyhexose-*O*-hydroxymethyl glutaric acid.

Peaks **25** and **26** found in oil palm frond sample are of similar m/z: 577. Both peaks ceded similar MS/MS fragment ions of m/z 457, $[(M - H) - 120]^-$; 413, $[(M - H) - 120 - 44]^-$ and 341, $[Agly + 71]^-$. Due to high abundance of m/z 413 fragment ions, both peaks are suggested to be apigenin-6-*C*-hexose-8-*C*-deoxyhexose. Peak **26** has a similar t_R to peak **14** in spear leaf (m/z 737), and m/z 737 is detected very faintly underneath peak **26**. Peak **18** (apigenin-*O*-hexose-*O*-deoxyhexose) and peaks **15**, **16**, **17**, **20** and **21** which contain HMG acid are also detected only at noise level and could not be quantitated using UV from the frond sample. It is interesting to observe different occurrence patterns of flavone derivatives between the young and mature leaves of oil palm, and this finding provided information for optimizing the usage of both tissues. Previous work suggests that HMGA possesses hypolipidemic activity[55] and administration of the HMG acid moiety significantly improved glucose tolerance in mice.[52] Apigenin and luteolin derivatives found in the oil palm leaves are warranted for further study considering their potential usefulness for human health and their growing utilization as livestock feed. Bioactive properties of these characterized compounds can benefit humans directly by developing them into nutraceutical products or indirectly by increasing the well-being of livestock.

AUTHOR INFORMATION

Corresponding Author

*E-mail: umi@mpob.gov.my.

Funding

The authors are grateful to the Director General of MPOB for the grant provided under the Board Approved Program (R005606000).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful to the Director General of MPOB for granting permission to publish this report. The authors thank the Breeding and Quantitative Genetics Group of Advanced Biotechnology and Breeding Centre (ABBC) for the oil palm samples.

REFERENCES

(1) Sharma, M.; Gupta, S. K.; Mondal, A. K. Production and Trade of Major World Oil Crops. In *Technological Innovations in Major World Oil Crops*; Gupta, S. K., Ed.; Springer Science+Business Media LLC: 2012, *1* (Breeding), pp 1−15.

(2) Astimar, A. A.; Anis, M.; Kamaruddin, H.; Rosnah, M. S. Hassan, W. H. W. Development in oil palm biomass utilization. In *Further Advances in Oil Palm Research (2001−2010)*; Malaysian Palm Oil Board: 2011; Vol. *2*, pp 896−929.

(3) Gapor, A.; Kato, A.; Ong, A. S. H. α-Tocopherol content in oil palm leaflet. *JAOCS, J. Am. Oil Chem. Soc.* **1986**, *63*, 330−331.

(4) Atil, O. Palm-based animal feed and MPOB's Energy and Protein Centre. *Palm Oil Dev.* **2004**, *40*, 1−4.

(5) Dahlan, I. Oil palm frond, a feed for herbivores. *Asian-Australas. J. Anim. Sci.* **2000**, *13*, 300−303.

(6) Hassan, O. A.; Ishida, M.; Shukri, M. I.; Tajuddin, Z. A. Oil palm fronds as a roughage feed source for ruminants in Malaysia. *FFTC Asian Pac. Reg.* **1994**, 1−8.
(7) Hassan, O. A.; Ishida, M. Status of utilization of selected fibrous crop residues and animal performance with emphasis on processing of oil palm fronds (OPF) for ruminant feed in Malaysia. Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan. *Trop. Agric. Res. Center TARS* **1992**, *25*, 134−143.
(8) Latif, J.; Mamat, M. N. A financial study of cattle integration in oil palm plantations. *Oil Palm Ind. Econ. J.* **2002**, *2*, 34−44.
(9) Zahari, M. A. K. M.; Zakaria, M. R.; Ariffin, H.; Mokhtar, M. N.; Salihon, J.; Shirai, Y.; Hassan, M. A. Renewable sugars from oil palm frond juice as an alternative novel fermentation feedstock for value-added products. *Bioresour. Technol.* **2012**, *110*, 566−571.
(10) Corley, R. H. V. Thinker, P. B. The products of the oil palm and their extraction. In *The Oil Palm*; Blackwell Science: 2003; Vol. *13*, pp 445−466.
(11) Sarmidi, M. R.; El Enshasy, H.; Mariani, A. H. Oil palm: the rich mine for pharma, food, feed and fuel industries. *Am.-Eurasian J. Agric. Environ. Sci.* **2009**, *5*, 767−776.
(12) Dit, M. Palm kernel shell (PKS) is more than biomass for alternative fuel after 2005. In *Proceedings of the PIPOC 2007 International Palm Oil Congress (Chemistry & Technology)*; PORIM International Palm Oil Congress, Kuala Lumpur, Malaysia, 2007; Vol. *C23*, pp 275−287.
(13) Jaffri, J. M.; Mohamed, S.; Rohimi, N.; Ahmad, I. N.; Noordin, M. M.; Manap, Y. A. Antihypertensive and cardiovascular effects of catechin-rich oil palm (*Elaeis guineensis*) leaf extract in nitric oxide-deficient rats. *J. Med. Food* **2011**, *14*, 775−783.
(14) Han, N. M.; May, C. Y. Determination of antioxidants in oil palm leaves (*Elaeis guineensis*). *Am. J. Appl. Sci.* **2010**, *7*, 1243−1247.
(15) Rosalina Tan, R. T.; Mohamed, S.; Samaneh, G. F.; Noordin, M. M.; Goh, Y. M.; Manap, M. Y. A. Polyphenol rich oil palm leaves extract reduce hyperglycaemia and lipid oxidation in STZ-rats. *Int. Food Res. J.* **2011**, *18*, 179−188.
(16) Jaffri, J. M.; Mohamed, S.; Ahmad, I. N.; Mustapha, N. M; Manap, Y. A.; Rohimi, N. Effects of catechin-rich oil palm leaf extract on normal and hypertensive rats' kidney and liver. *Food Chem.* **2011**, *128*, 433−441.
(17) Abeywardena, M.; Runnie, I.; Nizar, M.; Mohamed, S.; Head, R. Polyphenol-enriched extract of oil palm fronds (*Elaeis guineensis*) promotes vascular relaxation via endothelium-dependent mechanisms. *Asia Pac. J. Clin. Nutr.* **2002**, *11*, S467−S472.
(18) Hassim, H. A.; Lourenço, M.; Goel, G.; Vlaeminck, B.; Goh, Y. M.; Fievez, V. Effect of different inclusion levels of oil palm fronds on *in vitro* rumen fermentation pattern, fatty acid metabolism and apparent biohydrogenation of linoleic and linolenic acid. *Anim. Feed Sci. Technol.* **2010**, *162*, 155−158.
(19) Hollman, P. C. H.; Katan, M. B. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* **1997**, *51*, 305−310.
(20) Omar, M. H.; Mullen, W.; Crozier, A. Identification of proanthocyanidin dimmers and trimers, flavones C-glycosides, and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. *J. Agric. Food Chem.* **2011**, *59*, 1363−1369.
(21) Martens, S.; Mithöfer, A. Flavones and flavone synthases. Molecules of Interest. *Phytochemistry* **2005**, *66*, 2399−2407.
(22) Gripenberg, J. Flavones. In *The chemistry of flavonoid compounds*; Geissman, T. A., Ed.; Pergamon Press Inc.: 1962; Vol. *13*, pp 406−440.
(23) Wu, W.; Liu, Z.; Song, F.; Liu, S. Structural analysis of selected characteristic flavones by electrospray tandem mass spectrometry. *Anal. Sci.* **2004**, *20*, 1103−1105.
(24) Williams, C. A.; Harborne, J. B.; Clifford, H. T. Negatively charged flavones and tricin as chemosystematic markers in the Palmae. *Phytochemistry* **1973**, *12*, 2417−2430.
(25) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolics acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.
(26) Way, T.; Kao, M.; Lin, J. Apigenin induces apoptosis through proteosomal degradation of HER2/*neu* in HER2/*neu*-overexpressing breast cancer cells via the phosphotidylinositol 3-Kinase/Akt-dependent pathway. *J. Biol. Chem.* **2004**, *6*, 4479−4489.
(27) Horinaka, M.; Yoshida, T.; Shiraisi, T.; Nakata, S.; Wakada, M.; Sakai, T. The dietary flavonoid apigenin sensitizes malignant tumor cells to tumor necrosis factor-related apoptosis-inducing ligand. *Mol. Cancer Ther.* **2006**, *5*, 945−951.
(28) Chowdhury, A. R.; Sharma, S.; Mandal, S.; Goswami, A.; Mukhopadhyay, S.; Majumder, H. K Luteolin, an emerging anti-cancer flavonoid, poisons eukaryotic DNA topoisomerase I. *Biochem. J.* **2002**, *366*, 653−661.
(29) Shi, R.; Ong, C.; Shen, H. Luteolin sensitizes tumor necrosis factor-α-induced apoptosis in human tumor cells. *Oncogene* **2004**, *23*, 7712−7721.
(30) Sato, Y.; Suzaki, S.; Nishikawa, T.; Kihara, M.; Shibata, H.; Higuti, T. Phytochemical flavones isolated from *Scutellaria barbata* and antibacterial activity against methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol.* **2000**, *72*, 483−488.
(31) Hwang, Y. P.; Oh, K. Y.; Yun, H. J.; Jeong, H. G. The flavonoids apigenin and luteolin suppress ultraviolet A-induced matrix metalloproteinase-1 expression via MAPKs and AP-1-dependent signaling in HaCaT cells. *J. Dermatol. Sci.* **2011**, *61*, 23−31.
(32) Williams, C. A.; Harborne, J. B.; Clifford, H. T. Flavonoid patterns in the monocotyledons. Flavonols and flavones in some families associated with the Poaceae. *Phytochemistry* **1971**, *10*, 1059−1063.
(33) Miean, K. H.; Mohamed, S. Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *J. Agric. Food Chem.* **2001**, *49*, 3106−3112.
(34) Stochmal, A.; Piacente, S.; Pizza, C.; De Riccardis, F.; Leitz, R.; Oleszek, W. Alfalfa (*Medicago sativa* L.) Flavonoids. 1. Apigenin and Luteolin Glycosides from Aerial Parts. *J. Agric. Food Chem.* **2001**, *49*, 753−758.
(35) Proestos, C.; Chorianopoulos, N.; Nychas, G. E.; Komaitis, M. RP-HPLC analysis of the phenolic compounds of plant extracts. Investigation of their antioxidant capacity and antimicrobial activity. *J. Agric. Food Chem.* **2005**, *53*, 1190−1195.
(36) Stobiecki, M. Application of mass spectrometry for identification and structural studies of flavonoid glycosides (Review). *Phytochemistry* **2000**, *54*, 237−256.
(37) March, R. E.; Lewars, E. G.; Stadey, C. J.; Miao, X.; Zhao, X.; Metcalfe, C. D. A comparison of flavonoid glycosides by electrospray tandem mass spectrometry. *Int. J. Mass Spectrom.* **2006**, *248*, 61−85.
(38) Bovy, A.; Schijlen, E.; Hall, R. D. Metabolic engineering of flavonoids in tomato (*Solanum lycopersicum*): the potential for metabolomics. *Metabolomics* **2007**, *3*, 399−412.
(39) Treutter, D. Significance of flavonoids in plant resistance: a review. *Environ. Chem. Lett.* **2006**, *4*, 147−157.
(40) Darus, A. Major diseases of oil palm. In *Advances in oil palm research*; Basiron, Y., Jalani, B. S., Chan, K. W., Eds.; Malaysian Palm Oil Board: 2000; Vol. *1*, pp 596−622.
(41) Naczk, M.; Amarowicz, R.; Zadernowski, R.; Pegg, R. B.; Shahidi, F. Antioxidant activity of crude phenolic extracts from wild blueberry leaves. *Pol. J. Food Nutr. Sci.* **2003**, *12*, 166−169.
(42) Steed, L. E.; Truong, V. D. Anthocyanin content, antioxidant activity and selected physical properties of flowable purple-fleshed sweetpotato purees. *J. Food Sci.* **2008**, *73*, S215−S2221.
(43) Domon, B.; Costello, C. E. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconjugate J.* **1988**, *5*, 397−409.
(44) Wu, W.; Yan, C; Li, L.; Liu, Z.; Liu, S. Studies on the flavones using liquid chromatography-electrospray ionization tandem mass spectrometry. *J. Chromatogr. A* **2004**, *1047*, 213−220.
(45) Kite, G. C.; Porter, E. A.; Denison, F. C.; Grayer, R. J.; Veitch, N. C.; Butler, I.; Simmonds, M. S. J. Data-directed scan sequence for the general assignment of *C*-glycosylflavone *O*-glycosides in plant extracts by liquid chromatography-ion trap mass spectrometry. *J. Chromatogr. A* **2006**, *1104*, 123−131.

(46) Ferreres, F.; Gil-Izquierdo, A.; Andrade, P. B.; Valentão, P.; Tomãs-Barberãn, F. A. Characterization of *C*-glycosyl flavones *O*-glycosylated by liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2007**, *1161*, 214−223.
(47) Barreca, D.; Bellocco, E.; Caristi, C.; Leuzzi, U.; Gattuso, G. Distribution of *C*- and *O*-glycosyl flavonoids, (3-hydroxy-3-methylglutaryl)glycosyl flavanones and furocoumarins in *Citrus auranticum* L. juice. *Food Chem.* **2011**, *124*, 576−582.
(48) Figueirinha, A.; Paranhos, A.; Pérez-Alonso, J. J.; Santos-Buelga, C.; Batista, M. T. *Cymbopogon citrates* leaves: Characterization of flavonoids by HPLC-PDA-ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols. *Food Chem.* **2008**, *110*, 718−728.
(49) Niessen, W. M. A. LC-MS analysis of plant phenols. In *Liquid Chromatography-Mass Spectrometry, 3*; CRC Press: 2007; Vol. *15*, pp 413−437.
(50) Ferreres, F.; Silva, B. M.; Andrade, P. B.; Seabra, R. M.; Ferreira, M. A. Approach to the study of C-glycosyl flavones by ion trap HPLC-PAD-ESI/MS/MS: application to seeds of quince (*Cydonia oblonga*). *Phytochem. Anal.* **2003**, *14*, 352−359.
(51) Qimin, L.; Heuvel, V. D.; Delorenzo, O.; Corthout, J.; Pieters, L. A. C.; Vlietinck, A. J.; Claeys, M. Mass spectral characterization of *C*-glycosidic flavonoids isolated from a medicinal plant (*Passiflora incarnata*). *J. Chromatogr.* **1991**, *562*, 435−446.
(52) König, G. M.; Wright, A. D.; Keller, W. J.; Judd, R. L.; Bates, S.; Day, C. Hypoglycaemic activity of an HMG-containing flavonoid glucoside, chamaemeloside, from *Chamaemelum nobile*. *Planta Med.* **1998**, *64*, 612−614.
(53) Witherup, K. M.; McLaughlin, J. L.; Judd, R. L.; Ziegler, M. H.; Medon, P. J.; Keller, W. J. Identification of 3-hydroxy-3-methylglutaric acid (HMG) as a hypoglycemic principle of Spanish Moss (*Tillandsia usneoides*). *J. Nat. Prod.* **1995**, *58*, 1285−1290.
(54) Rayyan, S.; Fossen, T.; Andersen, Ø. M. Flavone *C*-glycosides from seeds of fenugreek, *Trigonella foenum-graecum* L. *J. Agric. Food Chem.* **2010**, *58*, 7211−7217.
(55) Prasad, K. Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. *Atherosclerosis* **2005**, *179*, 269−275.