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# IDENTIFICATION OF OIL PALM (*Elaeis guineensis*) SPEAR LEAF METABOLITES USING MASS SPECTROMETRY AND NEUTRAL LOSS ANALYSIS

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## ABSTRACT

*Plant metabolites characterisation is an arduous challenge due to their structural diversity as a result of complicated biosynthetic pathways. These metabolites are not only important for metabolic events description but are also harnessed as valuable nutraceuticals. The detection and description of important plant tissue metabolome such as oil palm spear leaf can be carried out using a broad-range, non-targeted analytical tool such as mass spectrometry (MS). Identification of oil palm spear leaf constituents such as carboxyl group- and sugar- containing metabolites was facilitated by tandem mass spectrometry (MS/MS) with neutral loss information of 44 and 162 amu. A total of 13 metabolites ranging from carboxylic acids, catechins, phenolic acid glycosides and a stilbenoid were characterised in this manner and the results presented here demonstrated the usefulness of MS in characterising metabolites in a complex sample such as oil palm spear leaf tissue.*

**Keywords:** metabolites, oil palm spear leaf, mass spectrometry (MS), tandem mass spectrometry (MS/MS), neutral loss.

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## INTRODUCTION

Plants synthesise metabolites for a multitude of functions known and unknown to man. The production and accumulation of these metabolites involve complicated biosynthetic pathways containing intricate arrays of catalytic enzymes (Lewinsohn and Gijzen, 2009). Metabolite characterisation enables the description of metabolic events and facilitates chemotaxonomy of plants as demonstrated by Williams (1975) for monocots leaf tissue constituents. More than 350 000 plant species have been identified (Wolfender *et al.,* 2003) yet the characterisation and utilisation of their valuable and beneficial metabolites (Balandrin *et al*., 1985; Fang *et al*., 2002; Llorach *et al*., 2003; Rimando *et al*., 2004; Urpi-Sarda *et al*., 2009) is still a difficult challenge due to structural diversity.

Information rich, non-selective but specific analytical approaches are required to investigate complex processes such as disease progression, toxic reactions and genetic manipulation in plants. The employment of liquid chromatography (LC) has become a common routine in chemical analysis as it is non-destructive and applicable to thermally sensitive compounds (Flanagan *et al*., 2007) and over the years, separation has been paired with mass spectrometry (MS) to obtain more information such as the molecular weight of the analyte. This combined or 'hyphenated' platform allows a systematic separation of complex samples, characterisation and identification of phytochemicals for numerous purposes such as quality control (Kite *et al*., 2003) and description of intra- and interspecific variations among species (Greenham *et al*., 2007). Metabolite classification and identification through pattern recognition

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of mass spectrometric data is one of several high-throughput, rapid strategies employed for complex samples (Garcìa-Pérez *et al*., 2008). The ability in separating and detecting small molecules using present technology permits the decoding of gene-protein-metabolite correlation and plant biochemical functions and as well as discovering novel metabolites.

Consecutive stages of mass analyses that involve changes in ion mass or charge by dissociation process or chemical reaction is termed tandem mass spectrometry, often abbreviated as MS/MS analysis (De Hoffman and Stroobant, 2001). For collision-induced dissociation (CID) MS/MS in a quadrupole mass analyser, fragmentation of molecular ions is performed by the application of collision energy at a gas phase of noble or inert gas such as argon or nitrogen (Sobott *et al.,* 2002). The collision of ions with the gas molecules generates kinetic energy that is then converted into internal energy which contributes to dissociation of the molecular ions into product ions or fragment ions (March *et al*., 2007). During MS/MS events, some fragments retain charges from its precursor form while fragments such as terminal unit of sugars which do not receive charge are not detected and are termed as neutrals (Martin *et al*., 2005). By examining the losses of the neutrals and fragmentation pattern, functional groups of compounds and structural information in complex mixtures can be deduced (Dron *et al*., 2007; Qu *et al*., 2004; Ma *et al*., 2000). The MS/MS allows the provision of global molecular weight information of detected compounds, unlike other spectroscopy techniques such as nuclear magnetic resonance (NMR) or Fourier transform infrared (FTIR) analysis (Dron *et al*., 2007).

Oil palm (*Elaeis guineensis* Jacq.) fruits are harvested for their oil-rich mesocarp (Basiron, 2007) and palm oil is an important commodity of Malaysia (Basiron, 2002). Leaves have long been employed as test specimens in oil palm research and have traditionally been used for the ease of sampling (Corley and Thinker, 2003). Their analyses are effective in gauging the effects of soil chemical and physical properties on oil palm (Paramananthan, 2003; Breure, 2003). Oil palm yield is highly correlated with leaf nutrient status as demonstrated in field trials, in view of the fact that leaves are the photosynthesis sites, the tissue where carbohydrates are manufactured for the production of fruit bunch (Foster, 2003; Lapointe, 1998). There is currently very little information available for the oil palm metabolome. This study provides a systematic technique for identification of oil palm spear leaf metabolites using reversed-phase high performance liquid chromatography (HPLC)-electrospray (ESI)-MS and MS/MS. Findings from this work will present another facet of advanced understanding of this species.

## MATERIALS AND METHODS

### Chemicals

HPLC grade methanol, acetonitrile and acetic acid were purchased from Merck (Darmstadt, Germany) while purified water was obtained from Milli-Q system (Millipore Lab, Bedford, MA, USA). Chelidonic acid (≥98%) was purchased from Fluka (Sigma-Aldrich, Buchs, Switzerland) while sucrose, citric acid, (+)-catechin (≥98%) and (-)-epicatechin (≥90%), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Plant Material

Oil palm spear leaf tissues of commercial *tenera* (*Dura* x *Pisifera*, DxP) variety were harvested from the Malaysian Palm Oil Board (MPOB) Kluang Research Station. The leaves were cut into small pieces of an inch (2.54 cm) in length and were shock-frozen in liquid nitrogen before they were pulverised in liquid nitrogen using mortar and pestle. The powder was then lyophilised using Labconco FreeZone® Freeze Dry System (MO, USA).

### Extraction of Metabolites

Lyophilised oil palm spear leaf tissue powder (0.1 g) was added with 5 ml 80% methanol, vortex-mixed for 20 s and sonicated for 30 min. After centrifugation at 4000 rpm, 25°C for 15 min, the supernatant was collected and dried under nitrogen stream before reconstituted in 1 ml water. The extract was filtered using cellulose acetate membrane syringe filter of 0.25 µm pore size from Sartorius AG, Goettingen, Germany for HPLC injection.

### Liquid Chromatography-Mass Spectrometry (LC-MS)

The oil palm spear leaf extract was separated using a C18 Reversed-Phase Acclaim® 120 column of 150 mm length, 4.6 mm internal diameter (ID) and 5 µm particle size (Dionex, Sunnyvale, CA, USA) at 35°C (thermostated column compartment) on Dionex Ultimate 3000 HPLC with a diode-array PDA-3000 detector. 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 59.5 min. The mobile phase for LC was composed of acetic acid as a common practice to enhance compound peak sharpness (De Moraes *et al.*, 2009). The injection volume was 1 µl with constant flow rate at 1.00 ml $min^{-1}$ and the flow was split to allow 200 µl $min^{-1}$ of eluent into MS.



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The MS analysis was performed on MicrOTOF-Q™ quadrupole-time-of-flight (QTOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The source conditions were: nebuliser gas ($N_2$) at 4.0 bar, dry gas (nitrogen, $N_2$) at 8.0 litres $min^{-1}$, dry temperature at 200°C, capillary voltage at -3500 V and end plate offset at -500 V. The MS acquisitions were performed in the negative ESI ionisation mode, in the mass range of 50 to 1000 *m/z*. For tandem MS (MS/MS) low-energy CID was carried out in the automatic mode at 20 eV collision energy. Nitrogen gas was used as collision gas, as argon was reported to produce higher CID due to larger cross-section (Gómez-Romero *et al*., 2011). 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.

## RESULTS AND DISCUSSION

### Chromatographic Separation

The LC-MS analysis of aqueous methanol extract of oil palm spear leaf revealed the complexity of metabolites contained in the tissue observable by both diode-array ultra violet (UV) and MS detections. The negative ion mode is visually more sensitive than positive ion mode for LC-MS detection of oil palm spear leaf constituents (data not shown) and as reported previously by Wang and Morris (2005). *Figure 1* shows the ion chromatogram from LC-MS and LC-UV chromatograms at wavelengths of 270 and 330 nm respectively. More than 13 molecular ions and UV peaks were detected in the chromatograms of the extract as labelled in A (*Figure 1*) according to their neutral losses. To identify and characterise the structure of these compounds, MS/MS was conducted via CID.

One of the techniques to extract information from LC-MS/MS data is by studying neutral loss incidence throughout the CID course by which the neutral losses pattern reveals characteristic functional groups of the individual component. Elevated energy during CID causes the loss of water (18 atomic mass unit, amu) as a neutral fragment and this has allowed the simplest and most common neutral loss to be observed for compounds containing hydroxyl (OH) group. Other neutral fragments that are regularly lost during CID are 44 and 162 amu; which usually but not always accounts for loss of $CO_2$ from carboxyl group and *O*-glycosylated hexose unit, respectively.

### Neutral Loss Scanning Post-MS/MS

*Table 1* shows the neutral loss scan chromatograms for 44 and 162 amu from the LC-MS analysis of aqueous methanol oil palm spear leaf extract. The compounds detected having

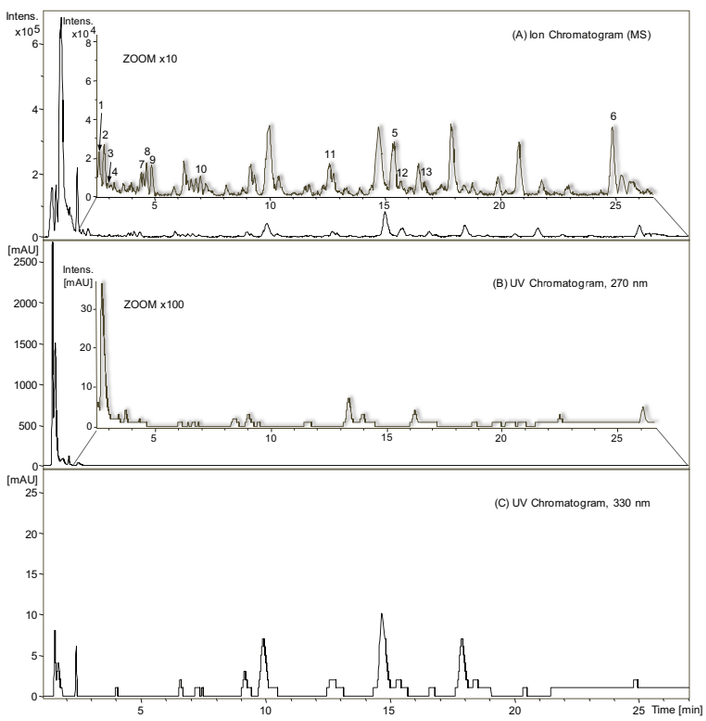


*Figure 1. Aqueous methanol oil palm spear leaf extract chromatograms (A: LC-MS ion chromatogram, B: LC-UV chromatogram at 270 and C: LC-UV chromatogram at 330 nm).*



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**TABLE 1. 44 AND 162 AMU NEUTRAL LOSSES CHROMATOGRAMS**

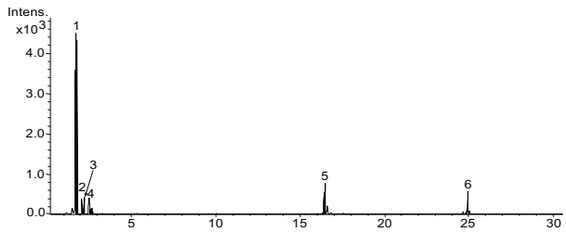
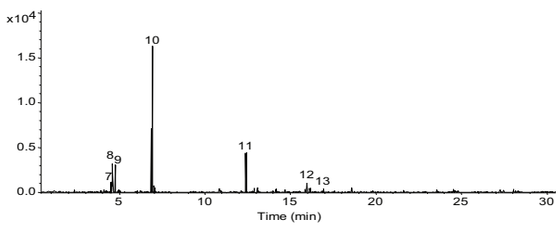
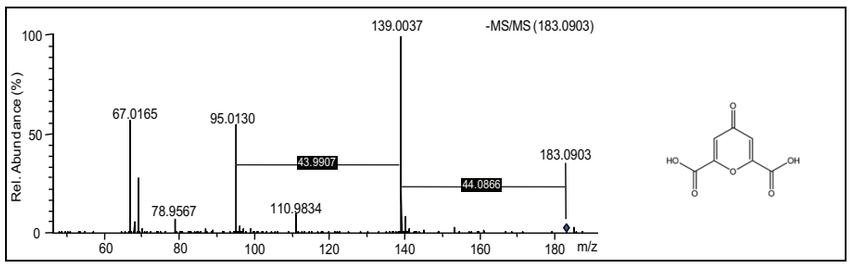
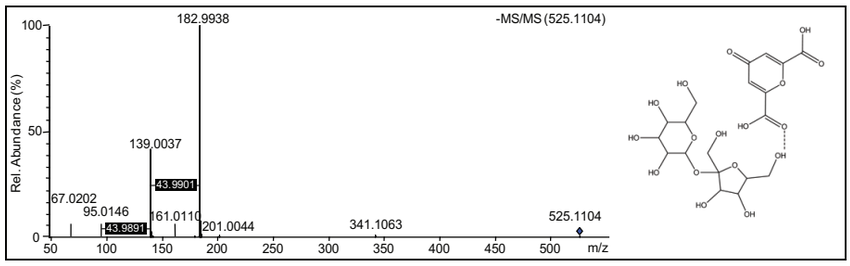
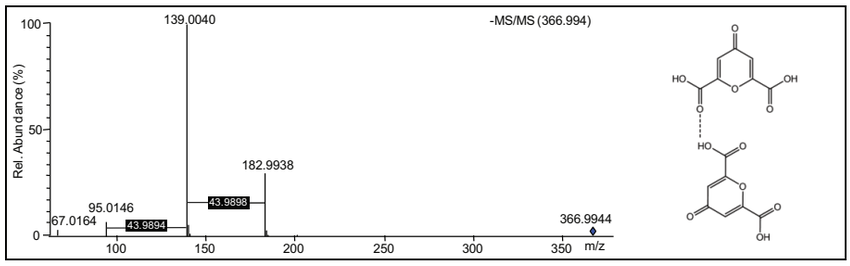
| Neutral loss | Neutral loss chromatograms |
|---|---|
| 44 amu |  |
| 162 amu |  |



**TABLE 2. MS/MS SPECTRA OF COMPOUNDS 1, 2 AND 3**

| Compound | MS/MS spectrum | Proposed structure |
|---|---|---|
| 1 |  |  |
| 2 |  |  |
| 3 |  |  |





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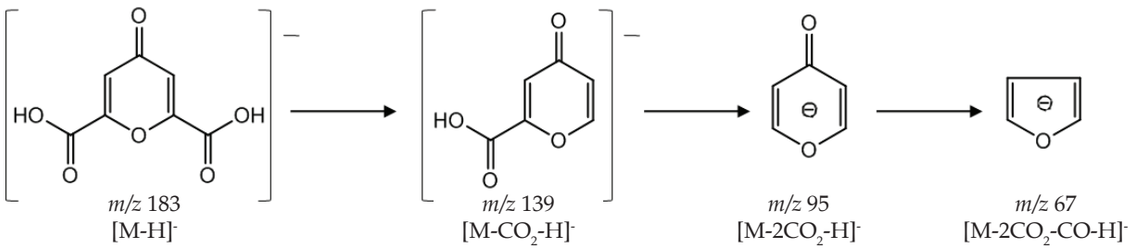


*Figure 2. Proposed fragmentation pathway of* m/z *183.*

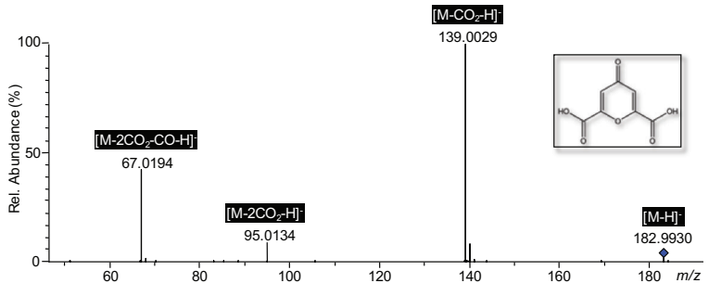


*Figure 3. MS/MS spectrum of chelidonic acid commercial standard.*

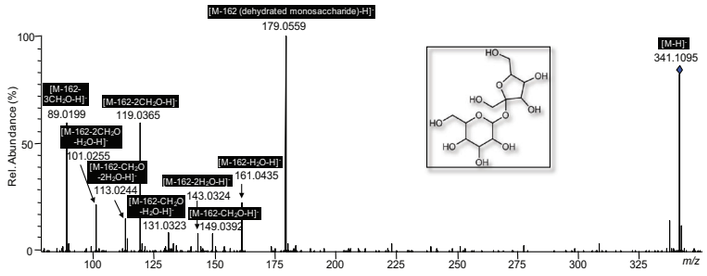


*Figure 4. Annotated MS/MS spectrum of sucrose* (m/z *341) in aqueous methanol oil palm spear leaf extract.*

neutral losses of 44 amu were numbered as **1** to **6**, while those detected having neutral losses of 162 amu were numbered as **7** to **13**. The identity of each compound was then further analysed by their respective MS/MS spectrum.

***Compounds with 44 amu neutral loss.*** For compounds with terminal carboxyl groups, CID promotes the loss of $CO_2$ fragment as a neutral resulting in formation of $[M-H-44]^-$ ion (Gómez-Romero *et al*., 2011; Dron *et al*., 2007). If the original compound bears oxygen- or nitrogen-functional groups attached to the $CO_2$ moiety, lower signal intensity will be observed for the neutral loss (Dron *et al*., 2007). At least six compounds were detected to display neutral losses of 44 amu. Further inspection of the MS/MS results showed that compounds **1**, **2** and **3** all exhibited analogous fragment ions of *m/z* 67, 95 and 139, indicating that they share a common sub-structure. Their respective ion spectrum with annotation of 44 amu neutral losses is shown in *Table 2*. Compounds **1**, **2** and **3** are highly polar compounds eluting closely together as early as 1.8 to 2.2 min retention time.

Two incidents of 44 amu losses were discernible in the MS/MS spectra of compounds **1**, **2** and **3** starting from the ion *m/z* 183. From the precursor ion *m/z* 183, a formula of $C_7H_3O_6$, $[M-H]^-$ was calculated using its isotopic pattern and accurate molecular ion mass. Meanwhile fragment ions of *m/z* 139 and 95 which arose from two consecutive losses of neutral $CO_2$ were assigned as $C_6H_3O_4$, $[M-CO_2-H]^-$ and $C_5H_3O_2$, $[M-2CO_2-H]^-$ respectively. Molecular formula of $C_7H_3O_6$ with molecular weight of 184 g $mol^{-1}$ was described for chelidonic acid, a dicarboxylic acid found in the Lily of the Valley (*Convallaria majalis* L.) (Bohm, 1966) and grain sorghum (*Sorghum vulgare* Pers.) (Bough and Gander, 1972). It follows from this deduction that the smallest fragment ion observed in the



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**TABLE 3. MS/MS SPECTRA OF CITRIC ACID COMMERCIAL STANDARD AND COMPOUND 4 (m/z 191.1111)**

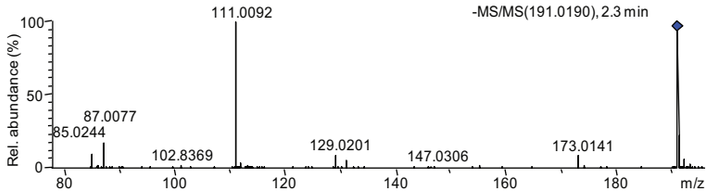
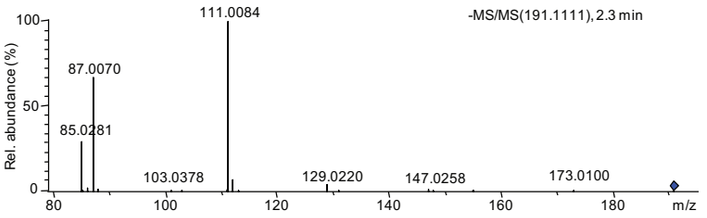
| | MS/MS spectrum |
|---|---|
| Citric acid commercial standard ($C_6H_8O_7$, MW 192) |   |
| Compound 4; *m/z* 191.1111 ($C_6H_8O_7$, MW 192) |   |

**TABLE 4. MS/MS SPECTRA AND MOLECULAR STRUCTURES OF COMPOUNDS 5 AND 6**

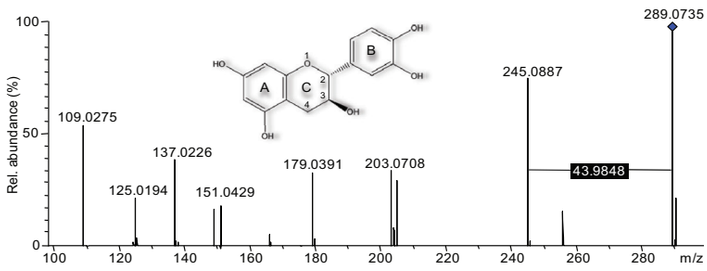
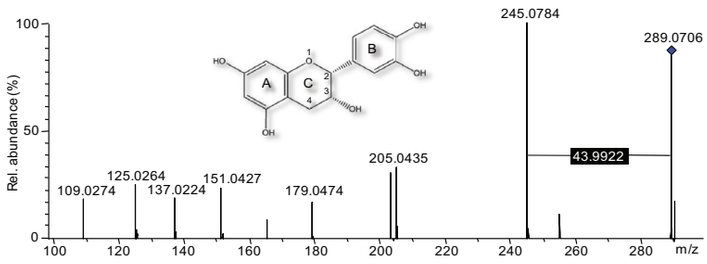
| | |
|---|---|
| Compound 5 |   |
| Compound 6 |   |

**TABLE 5. PEAK ASSIGNMENT OF COMPOUNDS WITH 44 amu NEUTRAL LOSSES**

| Peak | $t_R$ (min) | $[M-H]^-$ (m/z) | Formula | Key MS/MS fragments (*m/z*) | Compound |
|---|---|---|---|---|---|
| 1 | 1.9 | 183.0903 | $C_7H_4O_6$ | 139.0037, 95.0130, 67.0165 | Chelidonic acid |
| 2 | 2.0 | 525.1104 | $C_{19}H_{26}O_{17}$ | 341.1063, 182.9938, 161.0110, 139.0037, 95.0146 | Chelidonic acid-sucrose conjugate |
| 3 | 2.1 | 366.9944 | $C_{14}H_8O_{12}$ | 182.9938, 139.0040, 95.0146, 67.0164 | Chelidonic acid dimer |
| 4 | 2.3 | 191.1111 | $C_6H_8O_7$ | 147.0258, 111.0084, 103.0378, 87.0070, 85.0281 | Citric acid |
| 5 | 16.5 | 289.0735 | $C_{15}H_{14}O_6$ | 245.0887, 179.0391, 151.0429, 137.0226, 125.0194 | Catechin |
| 6 | 24.8 | 289.0706 | $C_{15}H_{14}O_6$ | 245.0784, 179.0474, 151.0427, 137.0224, 125.0264 | Epicatechin |

spectra, *m/z* 67 is the results of carbon monoxide (CO; 28 amu) elimination from deprotonated pyrone ion structure of *m/z* 95 forming a furan ion (*m/z* 67) during MS/MS as described by Nakata and Tatematsu (1967), and as reported by Chen *et al*. (2010) in switchgrass (*Panicum virgatum* L.) compositional analysis. The proposed schematic diagram for fragmentation of *m/z* 183 that corresponds to MS/MS fragments of compounds **1**, **2** and **3** is given in *Figure 2*. The retention time and MS/MS spectrum of compound **1** were further compared and found to be of similar properties to that of chelidonic acid commercial standard (*Figure 3*). Compound **1** is thus established as chelidonic



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acid ($C_7H_3O_6$, MW 184) whilst compounds **2** and **3** are derivatives of chelidonic acid on the basis of their fragmentation pattern.

Compound **2** (*m/z* 525.1104) is proposed as sucrose-chelidonic acid complex due to the fact that a peak corresponding to sucrose appeared at 1.8 min retention time before the elution of compounds **1** and **2**. Their proximity in all probability allowed their existence as a complex and permitted single proton removal in the negative mode gas phase ionization. Sucrose is a glucose-fructose conjugate and *m/z* 161 as observed in MS/MS spectrum of compound **2** can be explained as dehydrated monosaccharide fragment. The disaccharide presence was confirmed with sucrose commercial standard LC-MS and MS/MS analysis (*Figure 4*) in which the MS/MS fragments in the spectra are annotated according to the works of Taylor *et al*. (2005). Another chelidonic acid derivative; compound **3**, is established as chelidonic acid dimer with occurrence of no other fragments save that of chelidonic acid in addition to its generated molecular formula ($C_{14}H_8O_{12}$) equivalent to two units of chelidonic acids.

The molecular formula, fragmentation pattern and retention time of compound **4** (*m/z* 191.1111) were similar to citric acid, an organic acid important for the transport of micronutrients in plants (López-Bucio *et al*., 2000) and has important functions in energy production of plant cell (Rivasseau *et al*., 2006). Comparison of the MS/MS spectrum of compound **4** to that of citric acid commercial standard is listed in *Table 3*. Annotation of their major fragments can be found in *Table 5* and the identity of compound **4** is established as citric acid.

Similar *m/z* ratios of 289.07 were observed for compounds **5** and **6**. Based on their calculated molecular formulae of $C_{15}H_{14}O_6$ and MS/MS information, the identities of these compounds correspond to catechin isomers. Using similar LC-MS parameters of analysing the oil palm spear leaf extract, the retention times of commercial standards of catechin and epicatechin were compared to compounds **5** and **6** (*Figure 5*).

Catechin and epicatechin are stereoisomeric polyphenols found in food and beverages such as green and black tea (Vuong *et al*., 2012; Wei *et al*., 2011). The isomers are differentiated by

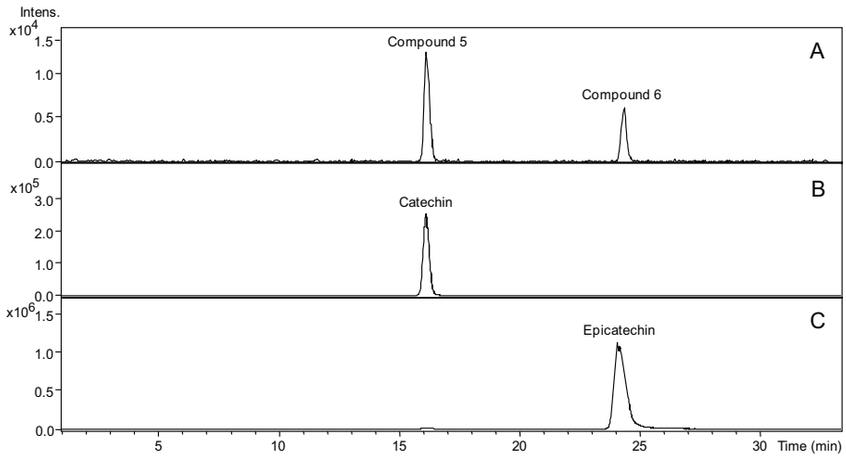


*Figure 5. LC-MS comparison of retention times ($t_R$) of compounds 5 and 6 to catechins commercial standards (A: LC-MS extracted ion chromatogram of m/z 289 from aqueous methanol oil palm spear leaf extract, B: LC-MS chromatogram of catechin and C: LC-MS chromatogram of epicatechin).*

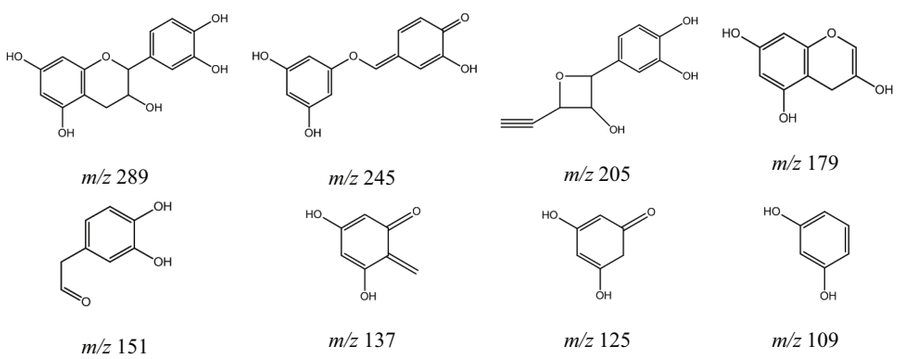


*Figure 6. MS/MS fragment ions of catechin isomers.*



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**TABLE 6. MS/MS SPECTRA AND ANNOTATIONS FOR COMPOUNDS 7 TO 13**

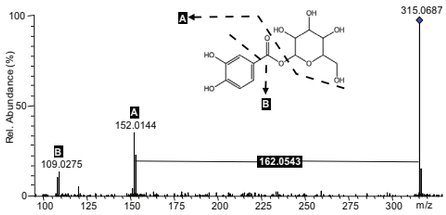
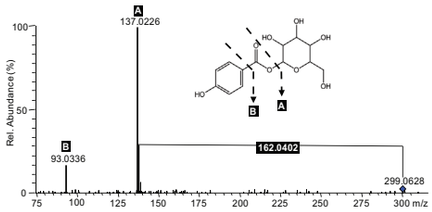
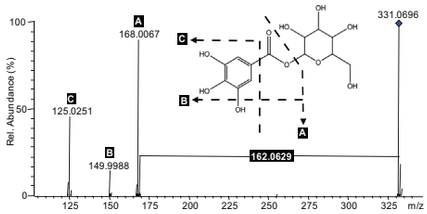
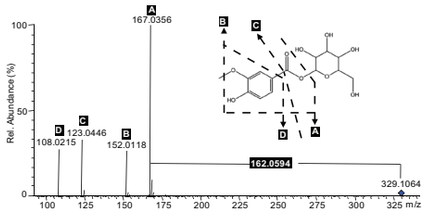
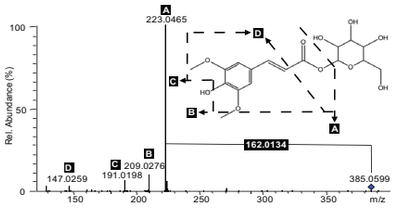
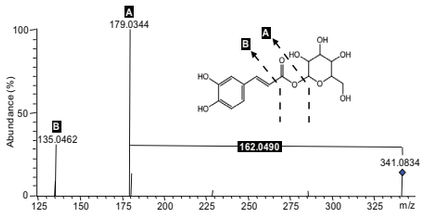
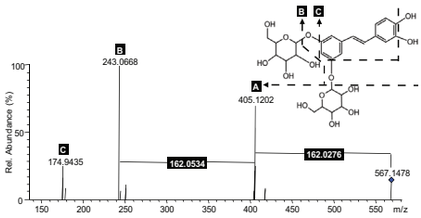
| Compound | MS/MS spectra and annotation |
|---|---|
| 7 |  |
| 8 |  |
| 9 |  |
| 10 |  |
| 11 |  |
| 12 |  |
| 13 |  |



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**TABLE 7. PEAK ASSIGNMENT OF COMPOUNDS WITH 162 amu NEUTRAL LOSSES**

| Peak | $t_R$ (min) | [M-H]$^-$ (m/z) | Formula | Key MS/MS fragments (*m/z*) | Compound |
|---|---|---|---|---|---|
| 7 | 4.6 | 315.0687 | $C_{13}H_{16}O_9$ | 152.0144 [M-162-H]$^{-\bullet}$, 109.0275 [M-162-$CO_2$-H]$^-$ | Dihydroxybenzoyl-*O*-hexoside |
| 8 | 4.7 | 299.0628 | $C_{13}H_{16}O_8$ | 137.0226 [M-162-H]$^-$, 93.0336 [M-162-$CO_2$-H]$^-$ | Hydroxybenzoyl-*O*-hexoside |
| 9 | 4.8 | 331.0696 | $C_{13}H_{16}O_{10}$ | 168.0067 [M-162-H]$^{-\bullet}$, 149.9988 [M-162-$H_2O$-H]$^{-\bullet}$, 125.0251 [M-162-$CO_2$-H]$^-$ | Galloyl-*O*-hexoside |
| 10 | 6.9 | 329.1064 | $C_{14}H_{18}O_9$ | 167.0356 [M-162-H]$^-$, 152.0118 [M-162-$CH_2$-H]$^{-\bullet}$, 123.0446 [M-162-$CO_2$-H]$^-$, 108.0215 [M-162-$CO_2$-$CH_2$-H]$^{-\bullet}$ | Vanilloyl-*O*-hexoside |
| 11 | 12.4 | 385.0599 | $C_{17}H_{22}O_{10}$ | 223.0465 [M-162-H]$^-$, 209.0276 [M-162-$CH_2$-H]$^-$, 191.0198 [M-162-$CH_2$-$H_2O$-H]$^-$, 147.0259 [M-162- $CH_2$-$H_2O$-$CO_2$-H]$^-$ | Sinapoyl-*O*-hexoside |
| 12 | 15.9 | 341.0834 | $C_{15}H_{18}O_9$ | 179.0344 [M-162-H]$^-$, 135.0462 [M-162-$CO_2$-H]$^-$ | Caffeoyl-*O*-hexoside |
| 13 | 16.9 | 567.1478 | $C_{26}H_{32}O_{14}$ | 405.1202 [M-162-H]$^-$, 243.0668 [M-2(162)-H]$^-$, 174.9435 [M-2(162)-2($H_2O$)-$O_2$-H]$^-$ | Piceatannol-*O*-dihexoside |

the configuration of the two hydrogens bound at positions 2 and 3 of the C-ring (Kajiya *et al*., 2001) in which catechin has a 2,3-*trans* (across) configuration while epicatechin has 2,3-*cis* (on the same side) arrangement (Huang *et al*., 2012). The MS/MS characteristic fragment ions and molecular structures of oil palm spear leaf catechins isomers (compounds **5** and **6**) are listed in *Table 4*. Looking at their molecular structures, neutral losses of 44 amu in compounds **5** and **6** could not be explained as loss of $CO_2$ as previously construed for compounds **1**, **2**, **3** and **4**.

According to Callemien and Collin (2008) and Li and Deinzer (2007), catechins MS/MS fragment ions can be explained via several fragmentation and rearrangement pathways such as retro-Diels-Alder (rDA) and heterocyclic ring fissions. Neutral losses of 44 amu in the MS/MS of compounds **5** and **6** were due to cleavage of $C_2H_4O$ molecule from the C-ring according to rDA rearrangement. Hypothetical structures of MS/MS fragment ions of catechins stereoisomers as reported by Callemien and Collin (2008) can be found in *Figure 6* and these fragments are consistent to fragments observed in the MS/MS products of compounds **5** and **6**. *Table 5* summarises the details of retention time ($t_R$), pseudomolecular mass to charge ratio (*m/z*), molecular formula, key MS/MS fragments and the identity of compounds **1**, **2**, **3**, **4**, **5** and **6** which were detected using neutral loss scan of 44 amu.

***Compounds with 162 amu neutral loss.*** Cleavage of a hexose that is *O*-linked to a compound is studied by observing loss of 162 amu (Qu *et al*., 2004). Extraction of 162 amu neutral loss information from MS data enabled finding of another seven compound peaks. Using similar approach, identities of the seven compounds were characterised using MS/MS information. *Table 6* shows the MS/MS spectra and proposed molecular structures with possible fragmentation pathways for compounds **7** to **13**, respectively. Alphabetical labels of **A**, **B**, **C** and **D** and dashed arrows on molecular structures of each compound attempt to explicate corresponding fragments observed in their respective MS/MS spectrum.

Compound **7** exhibited a molecular ion of *m/z* 315, [M-H]$^-$ and a loss of a hexose unit (162 amu). The presence of *m/z* 109.0275 fragment ion that is 44 amu less than the aglycone (molecule without glucose) fragment denotes loss of a neutral $CO_2$. The molecular formulae of the precursor (*m/z* 315) and the fragment ions (*m/z* 152 and 109) fit the formula of dihydroxybenzoic acid-hexoside; which are $C_{13}H_{15}O_9$, [*m/z* 315, M-H]$^-$; $C_7H_5O_4$, [*m/z* 152, M-162-H]$^{-\bullet}$, and $C_6H_5O_2$, [*m/z* 109, M-162-$CO_2$-H]$^-$. Similar fragmentation pattern, *i.e*. consecutive losses of 162 and 44 amu were observed for compounds **8**, **9**, **10**, **11** and **12** suggesting that these compounds are also of *O*-hexoside carboxylic acids. Based on the information of generated molecular formulae for these compounds, the possible fragmentation pathways for the major fragment ions as observed in *Table 6* are deduced.

The occurrence of even *m/z* values for MS/MS fragments observed for compounds **7**, **9** and **10** could be due to formation of radical anions which can arise during gas phase ionisation in the mass spectrometers (Cardozo *et al*., 2009; Wu *et al*., 2008; Sun *et al*., 2007). The existence of these unstable species can be attributed to resonance stabilisation and (or) homolytic bond cleavage that is more thermodynamic with a low reverse activation energy barrier (Cai *et al*., 2009). The occurrences of radical ions after deglycosylation (detachment of sugar moiety) may suggest different conjugation positions of sugars to flavonoids and phenolic acids (Ablajan, 2011).

Identities of compounds **8**, **9**, **10**, **11**, **12** and **13** are established as hydroxybenzoyl-*O*-hexoside, galloyl-*O*-hexoside, vanilloyl-*O*-hexoside, sinapoyl-*O*-hexoside, caffeoyl-*O*-hexoside and piceatannol-



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*O*-dihexoside respectively. *Table 7* provides list of the key fragments of compounds **7**, **8**, **9**, **10**, **11**, **12** and **13** with their possible fragmentation reactions and their established identities according to the information provided by these fragments.

## CONCLUSION

The oil palm spear leaf metabolites were indentified using MS/MS facilitated with neutral loss information extraction. The presence of specific plant compound classes such as carboxyl- and hexose-containing metabolites were detected in the tissue and this approach demonstrated to be a useful mass spectrometric data interpretation technique for mixture analysis. By systematically analysing the oil palm leaf metabolome, metabolites that to date have not been reported for oil palm spear leaf were identified. The application of state-of-the-art high-throughput mass spectrometry technology allows the identification of plant tissue components and will henceforward provide a platform for the description of oil palm tissue metabolic events and oil palm phenotyping for functional genomics.

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