Identification of chemical compounds from agarwood hydrosol (Aquilaria malaccensis) fruits via LC-QTOF-MS/MS analysis

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Abstract. Gaharu hydrosol is being considered as a by-product produced during the hydrodistillation of resinous wood part of Aquilaria spp. Agarwood hydrosol was reported to possess many bioactive compounds that are beneficial for health. However, current studies on the chemical composition of agarwood hydrosol from the fruit part are still lacking. This research presents the untargeted chemical compound of agarwood hydrosol from Aquilaria malaccensis fruit (AF) via liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS) and comparison the active functional groups with industrial hydrosol grade using Fourier transform infrared (FTIR). Qualitative tandem LC-QTOF-MS/MS was utilised to identify compounds in the extracted sample. The data processing revealed the presence of 128 known compounds in the hydrosol from A. malaccensis fruit in negative ionization mode and only one chemical profile detected after switched to positive ionization mode. This result contains the retention times value of m/z [M - H-], [M + HCOO-], [M + H+] and similar database search hit identities of the 129 compounds detected during the LC-QTOFMS/MS analysis in Table A1 and A2.

1. Introduction
Nowadays, Agarwood is one of the typical plants that are highly in demand in the world [1]. Agarwood is a resin-impregnated heartwood of Aquilaria species from Thymelaeaceae family that encompasses about 15 species in tropical Asia [2]. Aquilaria malaccensis is one of the species that was found and grows in Malaysia. Agarwood and its essential oil have been used and known a long time ago to have medicinal properties and used in traditional medicine, pharmaceutical; incense in religious practice and mostly in perfume. Hashim et al. (2019) discussed on agarwood used as medicine in traditional practices as well as their pharmacologic pieces of evidence in modern science [3]. Several researchers reported that this plant contained more than 60 chemical compounds. Therefore, the presence of the bioactive compounds in Agarwood trees such as mangiferin, genkwanin 5-O-β-primeveroside and iriflophenone 3,5-C-β-diglucoside can be used as herbal supplement [4]. Among all the compounds, mangiferin has a wide range of pharmacological effects such as anti-HIV, antioxidant, antidiabetic and anticancer activity [5].
The standard extraction method for the extraction of essential oil from agarwood wood parts was hydrodistillation. In this process, by-product is produced known as hydrosol (distillate fraction), that is used in traditional medicine and aromatherapy. Hydrosol is made up of water-soluble non-volatile organic compounds and may contain a small amount of essential oil. Agarwood hydrosol is sold as health supplement in Malaysia, claimed to be used in cancer treatment [6]. Unfortunately, the literature on chemical profile in agarwood hydrosols and the use of hydrosol in human health from the fruit parts are scarce.

Thus, this study aimed to provide information about the chemical profiles in agarwood hydrosol from the fruit parts obtained by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS). This data can help researchers in herbal medicinal plant to design effective drug discovery assays for the discovery of new therapeutic applications of the compounds.

2. Materials and method
All the materials and method used in this study will be explained further in this section.

2.1 Materials and equipments used
The fresh fruits of agarwood species of A. malaccensis were obtained from Pahang. The fruits’ species were identified based on the comparison with reference specimens from the Forest Research Institute Malaysia Herbarium. The equipment used for hydrodistillation extraction process is Soxhlet extraction apparatus and mini rotary evaporator. Prior to the analysis, all samples were ground using Retsh Ultra Centrifugal Mill ZM 200. To characterize the sample, Vion IMS QTof 1.0 was used to quantify chemical compounds, and Nicolet iS50 FTIR Spectrometer was used to identify functional groups.

2.2 Sample preparation
The fruits were dried in the oven at 60°C for one day and ground into powder using the Retsh Ultra Centrifugal Mill ZM 200 to 0.5 cm to 1 cm. Then, 40g of ground sample was weighed and put inside a beaker for further soaking with 1L of distilled water for 7 days, at room temperature. The purpose of this process is to break the parenchyma cells so that it will facilitate the oil glands rupture and consequently easier to extract the hydrosol.

2.3 Hydrodistillation extraction process
For the extraction process, Soxhlet extraction apparatus and mini rotary evaporator were set up as in Figure 1(a) and (b), respectively. The ground fruits were placed inside the cellulose thimble before it was inserted into the extraction chamber. The experiment took place for 6 hours (24 cycles) at 100°C. Then, it was left for an hour to cool before the extracted sample was collected. Then, the process was continued to separate the extracted sample using a mini rotary evaporator at 80°C. Hydrosol sample were collected in the receiving flask while concentrated agarwood extracts remained in the evaporating flask. The parameters was selected based on literature.

2.4 Liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS)
The hydrosol sample from the mini rotary evaporator was analyzed using liquid chromatography quadrupole time of flight mass spectrometry of Vion IMS QTof 1.0 series liquid chromatography. Negative electrospray ionization (ESI-) and Positive electrospray ionization were set up to identify the compounds in the hydrosol sample. The analytical run was set at 20 minutes, and the flow profile of the mobile phase is shown in Table 1. Other parameters of the system are summarized in Table 2. The identification of the compounds present in hydrosol samples was performed by comparing with MS/MS spectra from literature and records from the METLIN database. The tentative identification of some derivatives was based on the fragmentation patterns of known compounds.
Table 1. Isocratic and gradient flow profiles of the mobile phase.

| Time (min) | Flow Rate (mL/min) | Composition A [Water +0.1% Formic Acid (%)] | Composition B [Acetonitrile (%)] | Curve |
|------------|--------------------|---------------------------------------------|---------------------------------|-------|
| 0          | 0.5                | 99                                          | 1                               | Initial |
| 0.5        | 0.5                | 99                                          | 1                               | 6     |
| 16         | 0.5                | 65                                          | 35                              | 6     |
| 18         | 0.5                | 0                                           | 100                             | 1     |
| 20         | 0.5                | 99                                          | 1                               | 1     |

Table 2. Parameters of the LC-QTOF-MS/MS system.

| Acquisition Parameter                  | Source type          | Scan          | Collision energy | Set capillary | Source temperature | Desolvation temperature | Desolvation gas | Cone gas |
|----------------------------------------|----------------------|---------------|------------------|---------------|--------------------|------------------------|-----------------|----------|
| **Source type**                        | Electrospray ionization | 100-1000 m/z  | 4.00-45.00 eV    | 2.5kV         | 120 °C             | 550 °C                 | 800 L/h         | 50 L/h   |
| **Ion polarity**                       | Negative             |               |                  |               |                    |                        |                 |          |
| **Positive**                           |                      | 50-1000 m/z   | 4.00-40.00 eV    |               |                    |                        |                 |          |

2.5 Functional Group Analysis
All spectra were obtained using a Fourier Transform Infrared Spectroscopy (FTIR) with attenuated total reflectance on crystal and knob of the Nicole i50. Sample analysis was carried out in the spectral range 4000 to 400 cm⁻¹, and the signal was subjected to 32 scans at a resolution of 4.0 cm⁻¹.

3. Result and discussion

3.1 Chemical profiling of hydrosol from *A. malaccensis* fruit via LC-QTOF-MS/MS
Based on table A1 and table A2, negative ionization mode shows more detection of chemical compounds in Agarwood hydrosol compared to positive mode. These findings were in agreement with Steckel & Schlosser (2019), as negative ion mode (ES-) were importantly used for the characterisation of...
flavonoids (polyphenol), oligosaccharides, carboxylic acids, sulphonamides, oligonucleotides, and rarely peptides [7]. There are 128 active chemical compounds founds in negative ionization mode. In comparison, only one chemical compound can be found when the mode was switched to positive ionization mode.

Referring to the study by Kruve et al. (2014), ions in negative ionization mode were generated via deprotonation, adduct formation with anions or via simultaneous deprotonation and adduct formation with cations [8]. Based on figure 2, the results obtained revealed some important biomarkers such as Decaffeoylacteoside (m/z=461.1667; Rt=11.08 min), Moracin C (m/z=355.1180; Rt= 12.29 min), Eugenol (m/z= 209.0817 ; Rt= 13.81 min), Dendrocandin B (m/z= 527.1940; Rt=15.53 min) and Xanthohumol (m/z= 353.1384 ; Rt= 16.61 min). The results from the mass-to-charge ratio revealed that Moracin C (C_{19}H_{18}O_{4}) was one of the most abundant phenolics compounds with inherent cancer capacity and potent antibacterial activity. Khyade & Lonkar (2013) investigated the inhibitory effect of Moracin C on cell mouse skin tumorigenesis model. It was found that Moracin can be used in cancer treatment when double dosage of 12-O-tetradecanoylphorbol 13-acetate (TPA) has been applied [9]. Hence, the utilisation of Moracin C may open a new avenue in the treatment of tumorigenesis.

Moreover, the second most abundant phenolics constituent was Decaffeoylacteoside (C_{20}H_{30}O_{12}) with antioxidant capacity. Decaffeoylacteoside has been utilised in traditional Chinese medicine to reduce heat from blood and disintegrate agglomerate [10]. Eugenol (C_{10}H_{12}O_{2}) known as clove oil was widely used as a flavouring for foods, and as a herbal oil used topically to treat toothache. Eugenol is one of the phenolic compounds found in essential oils or hydrosol [11].

Another phenolic compound that was found in this study is Dendrocandin B (C_{27}H_{30}O_{8}). Mittraphab et al., (2016) reported that Dendrocandin B acts as a cell-killing agent against three human cancer cell lines, including MDA-231(Breast cancer cell line), HepG2 and HT-29 (Colorectal tumour cells) [12]. Hence, the cumulative of bioactivities of chemical profile found in these studies are primarily responsible for the numerous therapeutic functions. The result described from this investigation can be used for further studies into other nutraceutical or food applications of agarwood hydrosol from *Aquilaria malaccensis* fruits.

### 3.2 Functional Group Analysis

Result obtained via Fourier transform infrared spectroscopy illustrated that the hydrosol sample from the experiment has a similar peak with commercial agarwood hydrosol. The result also showed both sample present similar active functional groups; alcohol/phenol, alkyne, and amide I. Commonly, alcohol/phenol band group was characterised in the frequency range of 3600 cm⁻¹ to 3200 cm⁻¹ [13]. From the broad spectrum in figure 3, O-H bond which indicates the presence of alcohol/phenol band group was found in the concentrated experimental agarwood hydrosol and industrial-grade agarwood hydrosol, at a wavenumber of 3270.59 cm⁻¹ and 3272.60 cm⁻¹, respectively. Since both samples has strong H-bond, therefore the frequency becomes lower. The presence of the alcohol/phenols functional groups are significant to prove the existence of a phenolic compound inside the experimental hydrosol sample. This finding is in agreement with Khalil et al. (2013), where the phenolic compound in *Aquilaria malaccensis* leaves with the frequency of 3388 cm⁻¹ was detected. The C=O band of alkyne group frequency (2260 cm⁻¹ to 2100 cm⁻¹) was present in both commercial hydrosol sample and experimental hydrosol extract with the frequency of 2136.97 cm⁻¹ and 2137.04 cm⁻¹, respectively [14]. Amides bands were also found with N-H bending at frequency of 1650 cm⁻¹ -1560 cm⁻¹, identified in both leaves and hydrosol extracts with the frequency of 1635.08 cm⁻¹ and 1635.05 cm⁻¹, respectively. The presence of amide indicated the existence of protein inside the agarwood hydrosol sample.
Figure 2. LC-QTOF chromatogram (negative ionization mode) of hydrosol from agarwood fruit extracts (a) BPI plot (b) Confirmed phenolic compound.
Figure 3. Overlaid of FTIR spectra (a) commercialise agarwood hydrosol (b) experiment agarwood hydrosol (temperature 80°C).

4. Conclusion
The data obtained from liquid chromatography quadrupole time of flight mass spectrometry found 128 untargeted compounds (known compound) in negative ionization mode and only one chemical profile detected after switching to positive ionization mode. From this data, we conclude that agarwood hydrosols from A. malaccensis fruit possesses many bioactive compounds useful for health. Agarwood hydrosol and fruits could be new resources for bioactive compounds and can stand as a potential halal and safe ingredients for the development of food, nutraceutical and pharmaceutical as well as cosmeceutical products. This study can help researchers in designing fractionation and insulation for effective drug discovery assays for new therapeutic application from agarwood hydrosol.
### Table A1. Chemical compounds detected in Agarwood hydrosol at temperature 80°C via negative mode LC-QTOF MS.

| No | Compound                                      | Formula | Observed neutral mass (Da) | Observed m/z | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts | Observed CCS (Å²) | Total Fragments Found |
|----|-----------------------------------------------|---------|----------------------------|--------------|------------------|-------------------------------|----------|---------|-------------------|------------------------|
| 1  | Polydatin                                     | C_{20}H_{22}O_{8} | 390.1333                   | 435.1315     | 1.8              | 8.89                          | 188      | +HCOO              | 199.06                 | 0                      |
| 2  | Decaffeoylacteoside                           | C_{20}H_{30}O_{12} | 462.1739                   | 461.1667     | 0.2              | 11.08                         | 472      | -H                 | 198.79                 | 0                      |
| 3  | 2-Hydroxy-5-methylhypnone                    | C_{9}H_{16}O_{2} | 150.0684                   | 195.0666     | 0.3              | 11.36                         | 305      | +HCOO              | 139.81                 | 0                      |
| 4  | Yakuchinone A                                 | C_{20}H_{24}O_{3} | 312.1735                   | 357.1717     | 1.0              | 12.10                         | 299      | +HCOO              | 195.49                 | 0                      |
| 5  | Moracin C                                     | C_{19}H_{18}O_{4} | 310.1198                   | 355.1180     | -0.7             | 12.29                         | 177      | +HCOO              | 192.31                 | 0                      |
| 6  | 2,7-Dihydroxy-3,5-dimethoxy-9,10-dihydrophenanthrene | C_{16}H_{16}O_{4} | 272.1043                   | 271.0970     | -0.6             | 12.63                         | 151      | -H                 | 166.47                 | 0                      |
| 7  | Gingerone                                     | C_{11}H_{14}O_{3} | 194.0941                   | 193.0868     | -0.2             | 13.78                         | 258      | -H                 | 144.76                 | 0                      |
| 8  | Dihydroeugenol                                | C_{10}H_{14}O_{2} | 166.0993                   | 165.0921     | 0.0              | 13.80                         | 1585     | -H                 | 145.09                 | 0                      |
| 9  | Eugenol                                       | C_{10}H_{12}O_{2} | 164.0835                   | 209.0817     | -0.2             | 13.81                         | 4130     | +HCOO              | 146.20                 | 0                      |
| 10 | Obtustyrene                                   | C_{16}H_{16}O_{2} | 240.1146                   | 285.1128     | -0.4             | 13.93                         | 172      | +HCOO              | 172.42                 | 0                      |
| 11 | Moscatilin                                    | C_{17}H_{20}O_{5} | 304.1302                   | 349.1284     | -0.9             | 14.63                         | 115      | +HCOO              | 181.48                 | 0                      |
| 12 | Eugenol                                       | C_{10}H_{12}O_{2} | 164.0836                   | 209.0818     | -0.2             | 14.67                         | 210      | +HCOO              | 146.85                 | 0                      |
| No | Compound                  | Formula  | Observed neutral mass (Da) | Observed m/z  | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts | Observed CCS (Å²) | Total Fragments Found |
|----|---------------------------|----------|----------------------------|---------------|-----------------|-----------------------------|----------|---------|------------------|-----------------------|
| 13 | Brazilin                 | C_{16}H_{14}O_{5} | 286.0843                  | 285.0770     | -0.2            | 14.82                       | 164      | -H      | 171.39           | 1                     |
| 14 | Blestriarene C           | C_{30}H_{22}O_{6} | 478.1408                  | 523.1390     | -0.8            | 15.50                       | 196      | +HCOO   | 216.71           | 0                     |
| 15 | Dendrocandin B           | C_{27}H_{30}O_{8} | 482.1958                  | 527.1940     | 1.7             | 15.53                       | 445.29   | +HCOO   | 219.77           | 11                    |
| 16 | Dendrocandin B           | C_{27}H_{30}O_{8} | 482.1936                  | 527.1918     | -0.5            | 15.53                       | 414.10   | +HCOO   | 221.15           | 11                    |
| 17 | Cistanoside H            | C_{22}H_{32}O_{13} | 504.1837                 | 503.1764     | -0.6            | 15.53                       | 308      | -H      | 212.43           | 10                    |
| 18 | 2-Hydroxy-5-methylhypnone | C_{9}H_{10}O_{2} | 150.0681                  | 149.0608     | 0.0             | 15.53                       | 358      | -H      | 135.22           | 0                     |
| 19 | Blestrianol D            | C_{29}H_{32}O_{5} | 452.1632                  | 497.1614     | 0.8             | 15.54                       | 770      | +HCOO   | 223.66           | 2                     |
| 20 | 2-Ethyl-4,5-dimethylphenol | C_{10}H_{14}O | 150.1046                  | 149.0974     | 0.2             | 15.54                       | 1981     | -H      | 137.54           | 0                     |
| 21 | Eugenol                  | C_{10}H_{12}O_{2} | 164.0837                  | 163.0764     | 0.0             | 15.54                       | 173      | -H      | 139.24           | 1                     |
| 22 | 2-Ethyl-4,5-dimethylphenol | C_{10}H_{14}O | 150.1047                  | 149.0974     | 0.2             | 15.57                       | 1019     | -H      | 163.02           | 0                     |
| 23 | Tran-Ferulaldehyde       | C_{10}H_{10}O_{3} | 178.0629                  | 177.0556     | -0.1            | 15.62                       | 1383     | -H      | 134.07           | 0                     |
| 24 | Gingerone                | C_{11}H_{10}O_{3} | 194.0942                  | 193.0869     | -0.1            | 15.64                       | 162.48   | -H      | 143.49           | 1                     |
| 25 | Aspidinol                | C_{12}H_{16}O_{4} | 224.1048                  | 223.0975     | -0.1            | 15.68                       | 386      | -H      | 154.25           | 0                     |
| 26 | Stibostemin B            | C_{15}H_{16}O_{2} | 228.1157                  | 273.1139     | 0.6             | 15.70                       | 232      | +HCOO   | 174.22           | 0                     |
| 27 | Eugenol                  | C_{10}H_{12}O_{2} | 164.0831                  | 209.0813     | -0.6            | 16.05                       | 172      | +HCOO   | 147.43           | 0                     |
| 28 | Moracin C                | C_{19}H_{16}O_{4} | 310.1201                  | 355.1183     | -0.5            | 16.33                       | 183      | +HCOO   | 192.36           | 0                     |
| 29 | Isomucronustyrene         | C_{17}H_{16}O_{3} | 270.1262                  | 269.1189     | 0.6             | 16.45                       | 104      | -H      | 169.27           | 0                     |
| 30 | Dendrocandin C           | C_{16}H_{10}O_{5} | 290.1141                  | 289.1068     | -1.4            | 16.47                       | 153      | -H      | 179.29           | 0                     |
| 31 | 2-Ethyl-4,5-dimethylphenol | C_{10}H_{14}O | 150.1045                  | 149.0973     | 0.1             | 16.52                       | 526      | -H      | 162.71           | 0                     |
| 32 | 2-Ethyl-4,5-dimethylphenol | C_{10}H_{14}O | 150.1045                  | 149.0972     | 0.0             | 16.53                       | 717      | -H      | 137.33           | 0                     |
| 33 | Cishinokiresinol         | C_{17}H_{16}O_{2} | 252.1145                  | 251.1072     | -0.5            | 16.55                       | 211      | -H      | 162.78           | 0                     |
| No | Compound                                             | Formula     | Observed neutral mass (Da) | Observed m/z     | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts          | Observed CCS (Å²) | Total Fragments Found |
|----|------------------------------------------------------|-------------|----------------------------|------------------|------------------|-----------------------------|----------|------------------|------------------|-----------------------|
| 34 | Flavanthrinin                                        | C15H12O3    | 240.0784                   | 239.0711         | -0.2             | 16.56                       | 1363     | -H               | 153.47            | 2                     |
| 35 | 2-Octylphenol                                        | C14H22O     | 206.1671                   | 251.1653         | 0.0              | 16.57                       | 106      | +HCOO           | 165.18            | 2                     |
| 36 | Efluosol                                             | C17H16O2    | 252.1142                   | 251.1069         | -0.8             | 16.57                       | 161      | -H, +HCOO       | 154.92            | 2                     |
| 37 | Moracin O                                            | C19H14O5    | 326.1145                   | 325.1072         | -1.0             | 16.57                       | 499      | -H               | 180.15            | 3                     |
| 38 | Eugenol                                              | C10H12O2    | 164.0830                   | 209.0812         | -0.7             | 16.58                       | 241      | +HCOO           | 143.34            | 0                     |
| 39 | 3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone  | C10H12O4    | 196.0728                   | 195.0655         | -0.7             | 16.58                       | 88       | -H               | 137.67            | 1                     |
| 40 | Oxyphyllacinol                                       | C20H26O3    | 314.1864                   | 359.1846         | -1.8             | 16.58                       | 345      | +HCOO           | 191.88            | 14                    |
| 41 | Aspidinol                                            | C12H16O4    | 224.1033                   | 223.0960         | -1.6             | 16.58                       | 139      | -H               | 149.26            | 3                     |
| 42 | 4'-Methylninosylvan                                  | C15H14O2    | 226.0986                   | 225.0914         | -0.7             | 16.58                       | 211      | -H, +HCOO       | 152.83            | 0                     |
| 43 | Shogaol                                              | C17H24O3    | 276.1715                   | 321.1697         | -1.0             | 16.58                       | 252      | +HCOO           | 181.50            | 1                     |
| 44 | Agrimol E                                            | C33H38O12   | 626.2360                   | 671.2342         | -0.4             | 16.58                       | 878      | +HCOO           | 246.01            | 0                     |
| 45 | Feralolide                                           | C18H16O7    | 344.0899                   | 343.0826         | 0.3              | 16.58                       | 277      | -H               | 177.72            | 2                     |
| 46 | Yakuchinone A                                        | C20H24O1    | 312.1745                   | 311.1672         | 2.0              | 16.58                       | 232      | -H               | 175.74            | 1                     |
| 47 | 1-O-Methyl-3,5-O-dicaffeyquinic acid methyl ester    | C27H28O12   | 544.1576                   | 543.1503         | -0.5             | 16.59                       | 313      | -H               | 219.20            | 4                     |
| 48 | Obtustyrene                                          | C16H16O2    | 240.1151                   | 285.1133         | 0.1              | 16.59                       | 241      | +HCOO           | 170.52            | 0                     |
| 49 | 4-(4'-Hydroxy-3',5'-dimethoxyphenyl)-3-buten-2-one   | C12H14O4    | 222.0894                   | 221.0821         | 0.2              | 16.59                       | 125      | -H               | 147.45            | 1                     |
| 50 | Cyclocurcumin                                        | C21H20O6    | 368.1252                   | 367.1179         | -0.8             | 16.59                       | 433      | -H               | 190.15            | 9                     |
| No | Compound                                                                 | Formula   | Observed neutral mass (Da) | Observed m/z     | Mass error (mDa) | Observed retention time (min) | Response | Adducts          | Observed CCS (Å²) | Total Fragments Found |
|----|--------------------------------------------------------------------------|-----------|---------------------------|------------------|-----------------|------------------------------|----------|------------------|-------------------|---------------------|
| 51 | Erianin                                                                  | C_{18}H_{22}O_{5} | 318.1457                 | 317.1384         | -1.1            | 16.59                        | 640      | -H               | 181.57            | 3                   |
| 52 | Isoscoparone                                                             | C_{17}H_{18}O_{4} | 286.1194                 | 331.1176         | -1.1            | 16.59                        | 254      | +HCOO, -H        | 187.29            | 6                   |
| 53 | (±)-Vestitol                                                             | C_{16}H_{16}O_{3} | 272.1051                 | 271.0978         | 0.2             | 16.59                        | 135      | -H               | 161.81            | 3                   |
| 54 | Agrimol D                                                                | C_{35}H_{42}O_{12} | 654.2670                 | 699.2652         | -0.7            | 16.59                        | 574      | +HCOO            | 253.10            | 1                   |
| 55 | Isoarundinin II                                                          | C_{22}H_{22}O_{4} | 350.1499                 | 349.1426         | -2.0            | 16.60                        | 687      | -H               | 193.86            | 5                   |
| 56 | tran-Ferulaldehyde                                                       | C_{10}H_{10}O_{3} | 178.0632                 | 177.0559         | 0.2             | 16.60                        | 253      | -H               | 135.58            | 0                   |
| 57 | Obovatol                                                                 | C_{18}H_{18}O_{3} | 282.1242                 | 327.1224         | -1.4            | 16.60                        | 502      | +HCOO, -H        | 180.66            | 2                   |
| 58 | Neosappanone A                                                           | C_{33}H_{28}O_{11} | 600.1625                 | 599.1552         | 0.0             | 16.60                        | 615      | -H               | 234.15            | 3                   |
| 59 | 6-Gingerol                                                               | C_{17}H_{16}O_{4} | 294.1830                 | 293.1757         | 0.1             | 16.60                        | 472      | -H               | 304.72            | 3                   |
| 60 | Dendrobin A                                                              | C_{16}H_{18}O_{4} | 274.1196                 | 273.1123         | -1.0            | 16.60                        | 109      | -H               | 165.45            | 4                   |
| 61 | 2,7-Dihydroxy-1-(p-hydroxybenzoyl)-4-methoxy-9,10-dihydrophenanthrene    | C_{22}H_{18}O_{5} | 362.1159                 | 361.1086         | 0.5             | 16.60                        | 420      | -H               | 191.68            | 6                   |
| 62 | Moscatilin                                                               | C_{17}H_{20}O_{3} | 304.1322                 | 303.1249         | 1.1             | 16.60                        | 289      | -H               | 175.16            | 4                   |
| 63 | 7-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4E,6E-heptadecan-3-one | C_{20}H_{20}O_{4} | 324.1349                 | 323.1276         | 1.2             | 16.60                        | 605      | -H               | 183.65            | 3                   |
| 64 | Euparin                                                                  | C_{13}H_{12}O_{3} | 216.0777                 | 215.0704         | -1.0            | 16.60                        | 427      | -H, +HCOO        | 147.65            | 0                   |
| 65 | Dihydrosveratrol                                                         | C_{14}H_{14}O_{3} | 230.0957                 | 229.0884         | 1.4             | 16.61                        | 174      | -H               | 153.01            | 1                   |
| 66 | Moracin C                                                                | C_{19}H_{18}O_{4} | 310.1213                 | 355.1195         | 0.8             | 16.61                        | 1106     | +HCOO            | 188.47            | 5                   |
| 67 | Dendrocandin D                                                           | C_{17}H_{20}O_{3} | 304.1299                 | 303.1227         | -1.1            | 16.61                        | 129      | -H               | 191.73            | 8                   |
| No | Compound                                                                 | Formula         | Observed neutral mass (Da) | Observed m/z | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts       | Observed CCS (Å²) | Total Fragments Found |
|----|--------------------------------------------------------------------------|-----------------|----------------------------|--------------|------------------|-------------------------------|----------|---------------|-------------------|-----------------------|
| 68 | Agrimol C                                                                | C_{36}H_{44}O_{12} | 668.2842                   | 713.2825     | 1.0              | 16.61                         | 662      | +HCOO         | 256.46            | 1                     |
| 69 | 4,7-Dihydroxy-1-(p-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene    | C_{22}H_{20}O_{4} | 348.1361                   | 347.1288     | 0.0              | 16.61                         | 854      | -H            | 187.27            | 9                     |
| 70 | Xanthohumol                                                              | C_{21}H_{22}O_{5} | 354.1457                   | 353.1384     | -1.0             | 16.61                         | 1324     | -H            | 191.03            | 17                    |
| 71 | Bletilol C                                                               | C_{27}H_{26}O_{7} | 462.1686                   | 461.1613     | 0.7              | 16.62                         | 1013     | -H            | 210.33            | 18                    |
| 72 | 4,7-Dihydroxy-1-(p-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene    | C_{22}H_{20}O_{4} | 348.1361                   | 347.1288     | -0.1             | 16.62                         | 1494     | -H, +HCOO     | 193.27            | 7                     |
| 73 | 2-Hydroxyphenylpropanol                                                  | C_{9}H_{12}O_{2} | 152.0837                   | 151.0764     | -0.1             | 16.62                         | 222      | -H            | 139.22            | 0                     |
| 74 | 2,4-Dihydroxyacetophenone                                                | C_{8}H_{8}O_{3}  | 152.0482                   | 151.0409     | 0.9              | 16.62                         | 87       | -H            | 158.41            | 0                     |
| 75 | 6-Gingerdione                                                            | C_{17}H_{12}O_{4} | 292.1682                   | 291.1609     | 0.7              | 16.62                         | 1144     | -H            | 168.20            | 8                     |
| 76 | 2,6-Di-tert-butyl-4-hydroxytoluene                                       | C_{15}H_{24}O_{3} | 220.1830                   | 265.1812     | 0.3              | 16.62                         | 2754     | +HCOO         | 165.19            | 1                     |
| 77 | Polygoacetophenoside                                                     | C_{14}H_{18}O_{10} | 346.0884                   | 391.0866     | -1.6             | 16.62                         | 1867     | +HCOO         | 186.75            | 3                     |
| 78 | Isoarundinin II                                                          | C_{22}H_{22}O_{4} | 350.1510                   | 395.1492     | -0.8             | 16.63                         | 1092     | +HCOO, -H    | 199.59            | 6                     |
| 79 | Moracin H                                                                | C_{20}H_{18}O_{5} | 338.1153                   | 383.1135     | -0.1             | 16.63                         | 419      | +HCOO, -H    | 191.22            | 22                    |
| 80 | 1,7-Bis(4-hydroxyphenyl)-hepta-4E,6E-dien-3-one                           | C_{19}H_{18}O_{3} | 294.1239                   | 293.1166     | -1.7             | 16.63                         | 135      | -H            | 175.00            | 0                     |
| No | Compound                        | Formula | Observed neutral mass (Da) | Observed m/z | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts | Observed CCS (Å²) | Observed Total Fragments Found |
|----|--------------------------------|---------|----------------------------|--------------|-----------------|-------------------------------|----------|---------|--------------------|---------------------------------|
| 81 | Gigantol                       | C15H16O4 | 260.1041                   | 259.0968     | -0.8            | 16.64                         | 230      | -H      | 157.62             | 1                              |
| 82 | Pseudoaspidin                  | C25H32O8 | 460.2079                   | 459.2006     | -1.8            | 16.64                         | 599      | -H      | 216.24             | 10                             |
| 83 | 6-Gingerol                     | C17H26O4 | 294.1830                   | 293.1757     | -0.1            | 16.65                         | 367      | -H      | 175.77             | 5                              |
| 84 | Dendrocandin E                 | C15H16O5 | 276.0999                   | 275.0926     | 0.1             | 16.65                         | 386      | -H      | 209.73             | 1                              |
| 85 | 2-Octylphenol                  | C14H22O  | 206.1672                   | 205.1599     | 0.1             | 16.67                         | 4473     | -H, +HCOO | 155.29             | 0                              |
| 86 | Dihydrocurcumin                | C21H22O6 | 370.1411                   | 369.1338     | -0.5            | 16.67                         | 560      | -H      | 192.01             | 15                             |
| 87 | Yakuchinone B                  | C20H22O3 | 310.1566                   | 355.1548     | -0.3            | 16.67                         | 2733     | +HCOO   | 187.44             | 1                              |
| 88 | 2-Ethyl-4,5-dimethylphenol     | C10H14O  | 150.1043                   | 149.0970     | -0.2            | 16.68                         | 455      | -H      | 147.32             | 0                              |
| 89 | Dendrobina A                   | C16H18O4 | 274.1187                   | 273.1114     | -1.8            | 16.68                         | 190      | -H      | 168.70             | 1                              |
| 90 | (3R)-Duartin                   | C18H20O6 | 332.1246                   | 331.1173     | -1.4            | 16.68                         | 348      | -H      | 178.51             | 1                              |
| 91 | Shogaol                        | C17H24O3 | 276.1731                   | 321.1713     | 0.5             | 16.69                         | 576      | +HCOO, -H | 185.95             | 3                              |
| 92 | Dihydroeugenol                | C10H14O2 | 166.0989                   | 165.0916     | -0.5            | 16.69                         | 83       | -H      | 138.82             | 0                              |
| 93 | Obovatol                       | C18H18O3 | 282.1255                   | 327.1237     | -0.1            | 16.70                         | 461      | +HCOO, -H | 180.61             | 1                              |
| 94 | Oxyphyllacinol                 | C20H26O3 | 314.1876                   | 313.1803     | -0.6            | 16.70                         | 414      | -H      | 199.99             | 7                              |
| 95 | 2-Octylphenol                  | C14H22O  | 206.1672                   | 205.1599     | 0.1             | 16.70                         | 96       | -H      | 210.54             | 3                              |
| 96 | (3R,4R)-3,4-trans-7,2',3'-tri-  | C32H30O9 | 558.1877                   | 603.1859     | -1.3            | 16.70                         | 993      | +HCOO   | 237.57             | 4                              |
|    | hydroxy-4'-methoxy-4-(3R)-2',7'- |          |                            |              |                 |                               |          |         |                    |                                |
|    | dihydroxy-4'-methoxy-isoflavan |          |                            |              |                 |                               |          |         |                    |                                |
|    | -5'y-yl]-isoflavan             |          |                            |              |                 |                               |          |         |                    |                                |
| 97 | Octahydrocurcumin              | C21H28O6 | 376.1879                   | 375.1807     | -0.7            | 16.70                         | 239      | -H      | 313.90             | 2                              |
| No  | Compound                                                                 | Formula     | Observed neutral mass (Da) | Observed m/z       | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts | Observed CCS (Å²) | Total Fragments Found |
|-----|--------------------------------------------------------------------------|-------------|---------------------------|--------------------|------------------|-----------------------------|----------|---------|-------------------|-----------------------|
| 98  | Dendrocandin C                                                          | C_{16}H_{18}O_{5} | 290.1138                  | 289.1065           | -1.6             | 16.70                       | 112      | -H      | 193.63            | 1                     |
| 99  | (3R,4R)-3,4-trans-7,2',3'-Trihydroxy-4'-methoxy-4-[(3R)-2',7-dihydroxy-4'-methoxyisoflavan-5'-yl]-isoflavan | C_{32}H_{30}O_{8} | 542.1930                  | 587.1912           | -1.1             | 16.70                       | 951      | +HCOO   | 243.24            | 3                     |
| 100 | Blestriairene B                                                          | C_{30}H_{24}O_{6} | 480.1557                  | 525.1539           | -1.6             | 16.71                       | 1053     | +HCOO   | 221.97            | 8                     |
| 101 | Mulberrofuran N                                                          | C_{25}H_{28}O_{4} | 392.1972                  | 437.1954           | -1.9             | 16.71                       | 1816     | +HCOO   | 209.88            | 11                    |
| 102 | 5-O-Methylshanciguol                                                     | C_{29}H_{28}O_{5} | 456.1920                  | 501.1902           | -1.7             | 16.71                       | 560      | +HCOO   | 217.58            | 3                     |
| 103 | 3'-O-Methylbrazilin                                                      | C_{17}H_{16}O_{5} | 300.1015                  | 299.0942           | 1.7              | 16.71                       | 220      | -H      | 164.66            | 5                     |
| 104 | Blestrianol D                                                            | C_{29}H_{24}O_{5} | 452.1641                  | 497.1623           | 1.7              | 16.71                       | 820      | +HCOO   | 222.35            | 1                     |
| 105 | 2,7-Dihydroxy-1-{p-hydroxybenzoyl}4-methoxy-9,10-dihydrophenanthrene    | C_{22}H_{18}O_{5} | 362.1136                  | 361.1063           | -1.9             | 16.72                       | 844      | -H      | 185.95            | 5                     |
| 106 | Decaffeoylacteoside                                                      | C_{20}H_{30}O_{12} | 462.1724                  | 507.1706           | -1.3             | 16.72                       | 372      | +HCOO   | 215.63            | 15                    |
| 107 | Kuwanon P                                                                | C_{34}H_{30}O_{9} | 582.1879                  | 627.1861           | -1.1             | 16.73                       | 698      | +HCOO   | 244.10            | 4                     |
| 108 | Mulberrofuran O                                                          | C_{39}H_{34}O_{9} | 646.2192                  | 691.2174           | -1.1             | 16.73                       | 383      | +HCOO   | 260.97            | 0                     |
| 109 | 2-Octylphenol                                                            | C_{14}H_{22}O  | 206.1672                  | 205.1600           | 0.2              | 16.74                       | 627      | -H      | 180.40            | 0                     |
| 110 | 1-Galloyl-β-D-glucose                                                   | C_{13}H_{16}O_{10} | 332.0727                  | 377.0709           | -1.7             | 16.74                       | 139      | +HCOO   | 322.64            | 0                     |
| 111 | Dihydrocurcumin                                                          | C_{21}H_{12}O_{2} | 370.1426                  | 369.1354           | 1.0              | 16.76                       | 783      | -H      | 188.93            | 3                     |
| 112 | 1-Galloyl-β-D-glucose                                                   | C_{13}H_{16}O_{10} | 332.0726                  | 377.0708           | -1.8             | 16.76                       | 1971     | +HCOO   | 184.31            | 0                     |
| 113 | Dihydroyreveratrol                                                       | C_{14}H_{14}O_{3} | 230.0941                  | 275.0923           | -0.2             | 16.76                       | 257      | +HCOO   | 224.54            | 0                     |
| 114 | Dihydrooxyresveratrol                                                    | C_{14}H_{14}O_{4} | 246.0878                  | 291.0860           | -1.4             | 16.76                       | 103      | +HCOO   | 219.72            | 0                     |
| 115 | 6-Gingerol                                                              | C_{17}H_{26}O_{4} | 294.1840                  | 293.1767           | 0.9              | 16.77                       | 134      | -H      | 179.67            | 2                     |
| No  | Compound                                                                 | Formula       | Observed neutral mass (Da) | Observed m/z   | Mass error (mDa) | Observed Retention Time (min) | Response | Adducts        | Observed CCS (Å²) | Total Fragments Found |
|-----|---------------------------------------------------------------------------|---------------|----------------------------|----------------|-----------------|-------------------------------|----------|----------------|-------------------|----------------------|
| 116 | 4,7-Dihydroxy-1-(p-hydroxybenzyl)-2-methoxy-9,10-dihydrophenanthrene      | C22H20O4      | 348.1374                   | 393.1356       | 1.3             | 16.79                         | 392      | +HCOO          | 194.88             | 1                    |
| 117 | Xanthohumol                                                              | C21H22O5      | 354.1465                   | 353.1392       | -0.3            | 16.80                         | 838      | -H             | 189.00             | 1                    |
| 118 | 4-(4’-Hydroxy-3,5’-dimethoxyphenyl)-3-buten-2-one                         | C12H14O4      | 222.0891                   | 221.0818       | -0.1            | 16.95                         | 110      | -H             | 149.91             | 0                    |
| 119 | 3,7-Dihydroxy-2,4-dimethoxyphenanthrene-3-O-glucoside                    | C22H24O9      | 432.1418                   | -431.1345      | -0.3            | 17.07                         | 267      | -H             | 206.25             | 0                    |
| 120 | 7-(4-Hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4E,6E-heptadien-3-one  | C20H20O4      | 324.1362                   | 369.1344       | 0.0             | 17.22                         | 366      | +HCOO          | 201.51             | 0                    |
| 121 | Isoarundinin II                                                          | C22H22O4      | 350.1505                   | 349.1433       | -1.3            | 17.32                         | 93       | -H             | 205.73             | 0                    |
| 122 | Cyclocurcumin                                                             | C21H20O6      | 368.1261                   | 367.1188       | 0.1             | 17.54                         | 259      | -H             | 196.17             | 0                    |
| 123 | Chrysoptoxine                                                            | C18H22O5      | 318.1466                   | 317.1393       | -0.2            | 17.68                         | 185      | -H             | 183.91             | 0                    |
| 124 | 2-Octylphenol                                                            | C14H22O      | 206.1672                   | 205.1600       | 0.2             | 17.83                         | 165      | -H             | 158.76             | 0                    |
| 125 | 2-Octylphenol                                                            | C14H22O      | 206.1668                   | 205.1595       | -0.3            | 18.62                         | 287      | -H             | 156.36             | 0                    |
| 126 | Parvifloroside B                                                         | C29H36O15     | 624.2036                   | 623.1963       | -1.8            | 18.62                         | 125      | -H             | 258.61             | 0                    |
| 127 | Cyclocurcumin                                                             | C21H20O6      | 368.1245                   | 367.1172       | -1.5            | 18.62                         | 82       | -H             | 199.66             | 1                    |
| 128 | Octahydrocurcumin                                                        | C21H28O6      | 376.1888                   | 375.1816       | 0.3             | 18.64                         | 87       | -H             | 196.86             | 7                    |
| No. | Compound       | Formula | Observed neutral mass (Da) | Observed m/z | Mass error (mDa) | Observed Retention Time (min) | Response | Observed Adducts | Total Fragments Found | Observed CCS (Å²) | Adducts | Found Fragments |
|-----|----------------|---------|----------------------------|--------------|-----------------|---------------------------|----------|-----------------|----------------------|------------------|---------|-----------------|
| 1   | Caffeate 1     | C9H8O4  | 180.0428                   | 181.0500     | 0.5             | 16.61                     | 149      | +H              | 135.63               | 0                |         |                 |
References

[1] Yumi, Z-Y H, Erra, F R, Nur Aimi, A Z, & Nor Fadhillah, M A 2018 International Conference on Halal Innovation in Product and Services Songkhla, Thailand: International Institute for Halal research and Training (INHART) 61-63.

[2] Fazila, K N, & Halim, K K 2012 Journal of Tropical Forest Science 557-564.

[3] Hashim, Y Z-Y, Jamil, M A, Jamal, P, Zainurin, N A, & Azziz, S S 2019 Malaysian Journal of Fundamental and Applied Sciences, 15 842-846.

[4] Ito T, T, Kakino, M, Takawa, S, Watarai, T, Oyama, M, Maruyama, H, Inuma, M 2012 Journal of Nutritional Science and Vitaminology, 58 136-142.

[5] Hendra, R, Ahmad, S, Sukari, A, Shukor, M Y, & Oskoueian, E 2011 International journal of Molecular Sciences 12 3422-3431.

[6] Gameil, A H, Hashim, Y Z-Y, Zainurin, N A, Salleh, H M, & Abdullah, N S 2019 Malaysian Journal of Fundamental and Applied Sciences 15 761-766.

[7] Steckel, A, & Schlosser, G 2019 Molecules 24 1-11.

[8] Kruve, A, Kaupmees, K, Liigand, J, & Leito, I 2014 Analytical Chemistry 4822-4830.

[9] Khyade, V B, & Lonkar, U D 2013 Annals of Plant Sciences 02 (10) 412-419.

[10] Wagner, H, Bauer, R, Melchart, D, Xiao, P-G, & Staudinger, A 2004 Chromatographic Fingerprint Analysis of Herbal Medicines: Thin-layer and High Performance Liquid Chromatography of Chinese Drug. Germany: Springer Wien New York.

[11] Aldred, E M 2009 Phenols. In Pharmacology: A Handbook for Complementary Healthcare Professionals United Kingdom: Churchill Livingstone.

[12] Mittraphaba, A, Muangnoi, C, Likhitwitayahuwit, K, Rojsitthisak, P, & Sritularak, B 2016 Natural Product Communications 657-659.

[13] Jamahseri, N F, Rodhi, M N, Zulkarnain, N H, Husain, N C, & Masruddin, A F 2014 The Malaysian Journal of Analytical Sciences 18 (3) 683 - 689.

[14] Khalil, A S, Rahim, A A, Taha, K K, & Abdallah, K B 2013 Journal of Applied and Industrial Sciences, 1 (3) 78-88.

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