*Determination of Phytochemicals by UPLC-Q/TOF-MS/MS*

Peak **4** with the formula C13H16O8 was identified as salicylic acid 2-*O*-*β*-D-glucoside due to the molecular ions at m/z 299 [M-H]-, accompanied by a characteristic fragment at m/z 137 [M-H-162]- from the loss of a glucose reside as previously reported [[1](#_ENREF_1)]. Compared to peak **5** (1-*O*-caffeoyl-*β*-D-glucoside) that absorbed at 330 nm, peak **6** and peak **7** had similar absorbance spectra with λmax at 314 nm. Based on the MS/MS analysis in negative mode, compound **6** and compound **7** exhibited same molecular ion at m/z 325 and produced fragment ions at m/z 163 (loss of a hexose), 145, 117, and 59, which is characteristic for 1-*O*-(4-Coumaroyl)-*β*-D-Glucose and its isomer. The identification of the molecule was also found to be consistent with published data [[2](#_ENREF_2)].

Flavonoids, especially flavone *C*-glycosides, are the major active constituents of *D. officinale*. Flavone *C*-glycoside, which was different from flavone *O*-glycoside, usually produces neutral loss of characteristic fragments of 90 Da, 120 Da and 150 Da [[2](#_ENREF_2)]. Fifteen flavone *C*-glycosides were identified in this study (compounds **9**, **10**, **11**, **12**, **14**, **15**, **17**, **18**, **19**, **20**, **21**, **22**, **26**, **29,** and **30**). As shown in Table 1, though compounds **10** and **20** exhibited similar molecular ions at m/z 593 [M-H]-, compound **10** produced extra fragment ions at m/z 473 [(M-H)-120]- and 353 [(M-H)-120]-, indicating losses of glucose residues at the 6-position and 8-position [[2](#_ENREF_2)]. Thus compounds **10** was apigenin-6,8-di-*C*-glucoside, also known as vicenin-2, which was further confirmed with authentic standards. Compound **20** was characterised as glucosyl-vitexin, owing to the direct loss of 162 Da and an aglycone radical ion at m/z 431. Fragment ions at m/z 311([M-H-162-120]-) suggested a hexose residue was substituted in compound **20**, which was in accordance with reported data [[3](#_ENREF_3)]. The peaks **14**, **17**, **19,** and **21** exhibited the similar molecular ions at m/z 563 [M-H]−. Compounds **14**, **17**, **19,** and **21** generated the similar fragment ions at m/z 383 and 353, which were characteristic of apigenin-6, 8-di-*C*-glucosides. For the fragment ion m/z 503 [(M-H)-60]−,m/z 473 [(M-H)-90]− and m/z 443 [(M-H)-120]−, suggesting a glucose and pentose residue at the 6-position and 8-position optionally. By comparing to the fragmentation of the reference standards and the published data [[2](#_ENREF_2); [4](#_ENREF_4)], the compound corresponding to peaks **14**, **17**, **19,** and **21** were proposed as apigenin-6-*C*-*β*-D-xyloside-8-*C*-*β*-D-glucoside (also known as vicenin-1), apigenin 6-*C*-*β*-D-glucoside-8-*C*-*α*-L-arabinoside (also known as schaftoside), apigenin-6-*C*-*β*-D-glucoside-8-*C*-*β*-D-xyloside (also known as vicenin-3) and apigenin 6-*C*-*β*-D-glucoside-8-*C*-*β*-L-arabinoside (also known as neoschaftoside), respectively. A typical MS spectrogram fragmentation mechanism for flavonoid disaccharide *C*-glycoside such as schaftoside was shown as fig 6. Compound **22** produced the molecular ion at m/z 533 [M-H-]−, the fragment ion at m/z 443 [(M-H)-90]− and m/z 383 [(M-H)-90-90]− suggested a 6,8-di-*C*-pentose substitution pattern. Based on literature data [[5](#_ENREF_5)]and comparison to reference, compound **22** was confirmed to be apigenin-6-*C*-*β*-D-xyloside-8-*C*-*α*-L-arabinoside. Applying a similar method, compound **26** was tentatively assigned to be apigenin-6-*C*-*α*-L-arabinoside-8-*C*-*β*-D-xyloside by comparing with authentic standard and examining the known flavonoids in different parts of *D. officinale*. Both compounds **29** and **30** exhibited same molecular ion at m/z [M+H]+ 565, and possessed same characteristic fragment ion at m/z 385 [(M+H)-180]+, which implied the loss of a glucose reside. Thus, peaks **29** and **30** were deduced as apigenin-6-*C*-*α*-L-arabinosyl-(1→2)-*O*-*β*-D-glucoside and apigenin-8-*C*-glucosyl-(1→2)-*α*-L-arabinoside, respectively [[5](#_ENREF_5)]. Compounds **9**, **11**, **12**, **15,** and **18** were all preliminarily identified as luteolin *C*-glycosides. MS spectrum of **18** showed [M-H]- ion at m/z 447, and MS/MS fragment ions appeared at m/z 357 and 327, corresponding to [M-H-90]- and [M-H-120]- ions. By comparing with MS of a reference standard and previously reported data, compound **18** was identified as luteolin-6-*C*-*β*-D-glucoside [[6](#_ENREF_6); [7](#_ENREF_7)]. Compared with reference standards, compounds **9**, **11,** and **15** were assigned to be luteolin-6-*C*-*β*-D-glucoside-8-*C*-*β*-D-galactoside, luteolin-6-*C*-*β*-D-xyloside-8-*C*-*β*-D- glucoside and luteolin-6-*C*-*β*-D-glucoside-8-*C*-*β*-D-xyloside. Compound **12** has same [M-H]- ion at m/z 579 and similar MS/MS fragment ions with compounds **11** and **15**. Consequently, compound **12** can just be tentatively deduced as isomer of luteolin-6-*C*-xyloside-8-*C*-glucoside in the absence of a standard.

Flavone *O*-glycoside was also a prominent flavonoid in DOF extract. Compounds **8**, **16**, **23**, **24**, **25**, **27,** and **33** were assigned to be quercetin glycosides by their product ions at m/z 300 and m/z 301 in negative MS2 spectra. The kinds of sugars conjugated with flavonoid aglycone are easily assigned by their fragment ion information in MS2 spectrum, which result from the losses of 162 Da for a hexose (a glucose or a galactose), 146 Da for a rhamnose, and 132 Da for a pentose (an apiose, an arabinose, or a xylose) or 324 Da for a sophoroside residue. Compared with the standards, compounds **16**, **23,** and **24** were determined to be quercetin-3-*O*-sophoroside, quercetin-3-*O*-rutinoside (rutin) and quercetin 3-*O*-*β*-D-glucoside (isoquercetrin), respectively. Compound **8** gave a molecular ion [M-H]- (m/z = 771), and fragments of 609, 463, and 301was attributed to the loss of [M-H-162], [M-H-308] and [M-H-162-308]- units, fitting the loss of glucose, rutinose, and both, respectively. MS spectrum of compound **23** showed the [M-H]- ion at m/z 609, which was less 162 Da than that of compound **8**. Compound **23** possessed the similar MS/MS fragments at m/z 463 and 301. As a result, compound **8** and **23** was tentatively assigned to be quercetin 3-*O*-glucosyl-rutinoside and quercetin-7-*O*-rutinoside. Compound **27** produced [M-H-]− at m/z 549 and fragment ions at m/z 505, 463, 301, 300, 271, and 255 which was consistent with previous literature [[8](#_ENREF_8)].Thus, compound **27** was tentatively proposed as quercetin-3-*O*-(6''-malonyl-glucoside). The ion at 505 indicated loss of CO2. Ion at 463 was the result of loss of a neutral fragment malondiacyl group with 86 Da. Molecular ion 549 lost the neutral malonyl-glucose fragment of 248 Da to form quercetin ion fragment m/z 301. Fragment ion 255 was produced by the loss of H2O and CO from 301 fragment ion. Similar to compound **27**, compound **33** displayed the [M-H]- ion at m/z 506, which was less 44 Da than that of compound **27**. Compound **33** was then tentatively deduced as quercetin 3-*O*-(6''-acetyl-glucoside). Compounds **28** and **31** was identified as kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside (also known as astragalin). Thus, compound **34** may be malonyl-glucoside of kaempferol, which presented similar fragmentation pathway to that of compound **27**. So, compound **34** was tentatively identified as kaempferol 3-*O*-(6′′-malonyl-glucoside). Compared with the standard, compound **32** deprotonated molecule [M-H]- at m/z 477 and was identified as isorhamnetin-3-*O*-glucoside. Similarly, compound **13** lost C12H20O9 to yield the ion of aglycone at m/z 315. Additionally, the product ion at m/z 357 was generated by the cleavage of glycosyl [[9](#_ENREF_9)]. Compounds **13** was tentatively assigned to be isorhamnetin-3-*O*-neohesperidoside.



**Supplementary Figure 1**. MS fragmentation mechanism proposed for apigenin 8-C-α-L-arabinoside 6-C-β-D-glucoside (schaftoside).



**Supplementary Figure 2**. UPLC-PDA-Qda traces at 254 nm and online antioxidant detection at 734 nm basic component of DOF aqueous extract. Compounds 5, 10, 11, 16, 24, 25, and 27 was 1-O-caffeoyl-β-D-glucoside, vicenin-2, luteolin-6-C-β-D-xyloside-8-C-β-D-glucoside, quercetin-3-O-sophoroside, rutin, isoquercitrin, and quercetin 3-O-(6’’-O-malonyl)-β-D-glucoside, respectively.

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