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Identification and characterization of phenolic compounds in hydromethanolic extracts of sorghum wholegrains by LC-ESI-MS^n

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ABSTRACT

Hydromethanolic extracts of brown, red, and white sorghum whole grains were analysed by LC-MS^n in negative ESI mode within the range *m/z* 150 – 550 amu. Besides the flavonoids already reported in sorghum, a number of flavonoids were also identified in the sorghum grain for the first time, including flavanones, flavonols and flavanonols, and flavan-3-ol derivatives. Various phenylpropane glycerides were also found in the sorghum grain, the majority of them are reported here for the first time, and a few of them were detected with abundant peaks in the extracts, indicating they are another important class of phenolic compounds in sorghum. In addition, phenolamides were also found in sorghum grain, which have not been reported before, and dicaffeoyl spermidine was detected in high abundance in the extracts of all three type sorghum grains. These results confirmed that sorghum is a rich source of various phenolic compounds.

KEY WORDS

Phenolic compounds, Sorghum, HPLC-ESI-MS^n

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops in the world. Its grain is used primarily as a food in many parts of Africa, Asia and the semi-arid tropics world-wide (Ragaee, Abdel-Aal, & Noaman, 2006). More recently sorghum grain foods are gaining popularity for their potential health benefits against chronic diseases related to over-nutrition (Stefoska-Needham, Beck, Johnson, & Tapsell, 2015). Compared with wheat, barley, millet, or rye, sorghum has the highest content of phenolic compounds (Ragaee, et al., 2006; Svensson, Sekwati-Monang, Lutz, Schieber, & Ganzle, 2010). Phenolic compounds are generally considered to be desirable components of human

food for their antioxidant activity. Epidemiological evidence and animal studies suggest sorghum in the diet promotes cardiovascular health better than other cereals, and it may also have beneficial effects for weight control (Awika & Rooney, 2004). Biochemical analyses demonstrated that food contain sorghum whole grain flour enhanced antioxidant status and this effect may in part be due to the polyphenolic antioxidants (Khan, Yousif, Johnson, & Gamlath, 2015). All sorghum varieties contain phenolic compounds. The types and levels present are related to pericarp colour and the presence of pigmented testa (Dykes & Rooney, 2006). Two major categories of phenolic compounds in sorghum are phenolic acids and flavonoids (Awika, et al., 2004). Phenolic acids found in sorghum grains include hydroxybenzoic acids and hydroxycinnamic acids, whilst the flavonoids isolated from sorghum grains include the flavan-3-ol catechin, the flavones apigenin and luteolin, which are predominant in tan-pigmented plant sorghums. In addition, other flavonoids such as flavanones naringenin and eriodictyol, flavonol kaempferol and flavanonol taxifolin have also been identified (Dykes, et al., 2006; Svensson, et al., 2010), and in red sorghum four phenylpropane glycerides were reported (Svensson, et al., 2010).

While sorghum grain is a rich source of phenolic compounds, the plant genotype and the environment in which it is grown influence their levels (Awika, et al., 2004; Dykes, et al., 2006). However, detailed and extended profiling of the phenolic compounds in sorghums remains so far incompletely investigated. HPLC–ESI-MS^n has been increasingly used in the structural characterisation of complex matrices, and elucidation of unknown structures by comparing on-line detected chromatograms and multiple-stage mass spectra with those of authentic compounds and other evidence. With a high resolution and characterization of a wide range of polar compounds HPLC–ESI-MS^n has proved to be a powerful tool to identify phenolic compounds (Abu-Reidah, Ali-Shtayeh, Jamous, Arráez-Román, & Segura-Carretero, 2015; Spínola, Pinto, & Castilho, 2015). The objective of this current work was to investigate the phenolic composition of hydromethanolic extracts of different color sorghum whole grains cultivated in Australia.

2. Materials and methods

2.1. Chemicals and solvents

Standards of the following phenolic acids and flavonoids are available commercially and used in the present work: protocatechuic acid, caffeic acid, ferulic acid, (+) catechin, luteolin, and eriodictyol were purchased from Sigma-Aldrich (Sydney, Australia); apigenin, naringenin, naringenin-7-*O*-glucoside, quercetin dihydrate, isorhamnetin, and taxifolin were purchased from Extrasynthese (Genay Cedex, France). Organic solvents acetonitrile and methanol (HPLC grade) were supplied by Crown Scientific (Sydney, Australia), and Milli-Q water (Milli-Q plus 185, Sydney, Australia) was used to make all aqueous solutions and HPLC mobile phase.

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69 *2.2. Extract preparation from sorghum whole grains*

70

71 White sorghum grain, variety Liberty (a commercial hybrid), and red sorghum grain, variety Alpha (an in-bred line),
72 were grown and supplied by Lochabar Enterprises Pty Ltd (Tara, QLD, Australia) using organic conditions specifically
73 for human food use. Brown sorghum grain, variety IS1311C (an in-bred line), was supplied by the Queensland
74 Government Department of Agriculture, Fisheries and Forestry and grown at the Hermitage Research Station (Warwick,
75 QLD, Australia). This white sorghum grain variety has previously been described as tannin free by simple chemical assay
76 (Licata, Chu, Wang, Coorey, James, Zhao, et al., 2014), and the red variety been reported to have low levels of tannins
77 and has a pigmented testa, whilst the brown variety has both a pigmented testa and high levels of tannins (Wu, Johnson,
78 Bornman, Bennett, Singh, Simic, et al., 2016). The un-processed whole grains were milled to flour using a rotor mill (ZM
79 200, Retsch GmbH, Haan, Germany) to pass 100% though a 500 µm sieve, and all milled whole grain flours were
80 vacuum packed and stored at below 15 °C in the dark prior to use. For sample extraction, 5 g of each milled whole grain
81 flour was extracted with 30 mL of methanol:water (80:20, v/v) by shaking at room temperature for 2h. The extract was
82 centrifuged at 2358 × g, at 20^{0}C, for 10 min. The supernatant was then decanted and the residue was extracted one more
83 time as above. The two supernatants were combined and evaporated to near dryness under vacuum at 50^{0}C using a rotary
84 evaporator. The residue was then reconstituted with 15 mL methanol and stored in a freezer below -5^{0}C prior to analysis.

85

86 *2.3. Liquid chromatography- mass spectrometry analyses*

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88 The profile of the phenolic compounds in the hydromethanolic extracts of sorghum whole grains were firstly
89 analysed by HPLC-MS in a LCMS-2020 system (Shimadzu, Kyoto, Japan). Phenolic compounds were separated on a
90 Phenomenex Kinetex™ 2.6 µm C18 Column (100×3.0 mm). The injection volume was 5 µL. The mobile phase consisted
91 of 0.1% formic acid in water (v/ v) (Solvent A) and acetonitrile (Solvent B). The mobile phase flow rate was 0.5 mL/min.
92 The HPLC gradient was: 0–5 min, 5% B; 5–40 min, 5–40% B; 40–60 min, 40–90% B; 60–65 min, 90% B. Mass
93 spectrometry was operated in ESI negative mode under the following conditions: nebulizing gas (N_2) 1.5 L/min; drying
94 gas (N_2) 5 L/min; desolvation line temperature 250ºC; interface temperature 350 ºC; interface voltage -4.5 kV; detector
95 voltage 1.1 kV. Phenolic compounds were detected by a full scan mode ranged *m/z* 150 – 550 amu.
96 Further identification and characterization of the phenolic compounds was performed by HPLC-MS^n on a LTQ ion
97 trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA). HPLC separation was conducted using the same column
98 and mobile phase as above, the mobile phase gradient used same program as above to locate the selected compounds in

HPLC chromatograms, and also used reduced time program: 0–5 min, 5% B; 5–20 min, 5–40% B; 20–23 min, 40–90% B; 23–30 min, 90% B in the repeating runs. The selected phenolic compounds were analyzed by MS^2 and MS^3, sometimes even MS^4 in total ion scan mode. Electrospray ionization was conducted in the negative mode under the following conditions: sheath gas (N_2) 29 units/min; auxiliary gas (N_2) 3 units/min; spray voltage 3.61 kV; capillary voltage -11.83V and; capillary temperature 274 ºC. Helium was used as the damping and collision gas at 0.1 Pa, the normalized collision energies ranged from 30% to 45% for each selected compound.

3. Result and discussion

The phenolic profile of the three hydromethanolic extracts of sorghum grain varieties was scanned by LC-MS (at ESI negative mode) in the range of *m/z* 150 – 550 amu, the base peak chromatograms (BPC) are shown in **Fig.1**. Initial analysis of the three hydromethanolic extracts indicated that brown sorghum whole grain contained much higher amounts and a greater variety of phenolic compounds than red and white, whereas the white sorghum whole grain exhibited more abundant peaks of hydroxy fatty acids than the other two. Further identification and characterization of the compounds was carried out by HPLC-MS^n fragmentation data and by comparison with authentic standards and published literature. **Table.1** reports all of the compounds with their chromatographic retention times, MS^n fragmentation ions, assigned identities, and the sample sources respectively. Compounds were numbered by their elution order. Some seventy five phenolic compounds were found, the majority of them were detected and characterized from sorghum whole grains and reported here for the first time.

Table 1

Phytochemical compounds detected and characterised in hydromethanolic extracts of brown, red and white sorghum whole grains.

Peak No.	Rt (min)	$[M-H]^-$ *m/z*	HPLC-ESI-MS^n *m/z* (intensity,%)	Identification	Type of sorghum*
1	3.1	153	MS^2 [153]: 109(100)	Protocatechuic acid [a]	B, R, W
2	4.0	197	MS^2 [197]: 179(15), 153(100), 109(15); MS^3[197 → 153]: 138(100), 121(15), 109(95), 107(20), 83(10)	*β*,3,4-Trihydroxy benzenepropanoic acid [c]	B, R, W
3	4.5	451	MS^2 [451]: 289(100); MS^3 [451 → 289]: 245(100), 179(20), 167(10)	Catechin hexoside [b]	B, R
4	6.9	341	MS^2 [341]:179(100), 135(5)	Caffeic acid hexose [b]	B, R, W
5	7.1	465	MS^2 [465]: 447(10), 303(70), 285(100), 259(5), 177(10),	Taxifolin hexoside I [b]	B
6	7.6	253	MS^2 [253]: 179(30), 161(100), 135(50); MS^3 [253 → 179]: 135(100); MS^3 [161]: 133(100)	2-*O*-Caffeoylglycerol [b]	B, R
7	7.9	289	MS^2 [289]: 245(100), 231(20), 205(90), 179(15), 125(5)	Catechin [a]	B, R, W
8	8.6	179	MS^2 [179]: 135(100)	Caffeic acid [a]	B, R, W
9	8.9	415	MS^2 [415]: 253(100), 179(2), 161(2), 135(2); MS^3 [415 → 253]: 179(30), 161(40), 135(100)	1-*O*-Caffeoyl-2-*O*- glucosylglycerol [b]	B, R, W
10	9.0	353	MS^2 [353]: 263(8), 219(3), 191(100), 179(10); MS^3 [353 → 191]: 173(80), 153(15), 127(100), 111(30), 93(10), 85(50)	Caffeoylquinic acid [b]	B, R, W
11	9.5	465	MS^2 [465]: 447(40), 303(40), 285(100), 259(60), 217(10)	Taxifolin hexoside II [b]	B
12	9.9	255	MS^2 [253]: 181(50), 180(5), 179(10), 163(35), 162(10), 161(25), 137(70), 136(30), 135(100)	1-*O*-Dihydrocaffeoylglycerol [b]	B, R, W
13	10.0	253	MS2 [253]: 179(40), 161(50), 135(100); MS^3 [253 → 179]: 135(100)	1-*O*-Caffeoylglycerol [b]	B, R, W

			MS^3 [253 → 161]: 133(100)		
14	10.2	371	MS^2 [371]: 249(100), 231(10), 121(5), 113(3); MS^3 [371 → 249]: 231(100), 189(10), 175(20), 113(90), 95(10), 85(20), 75(10	Saccharide [b]	B, R, W
15	12.1	399	MS^2 [399]: 237(20), 219(15), 163(60), 145(100), 117(10)	1-*O*-Coumaroyl-2-*O*-glucosylglycerol [b]	B, R, W
16	12.8	237	MS^2 [237]: 163(50), 145(90), 119(100)	2-*O*-Coumaroylglycerol [b]	B, R, W
17	13.6	237	MS^2 [237]: 163(60), 145(40), 119(100)	1-*O*-Coumaroylglycerol [b]	B, R, W
18	13.8	468	MS^2 [468]: 397(2), 332(100), 306(20), 276(3),161(5); MS^3 [468 → 332]: 289(100), 133(3), 108(1); MS^3 [468 → 306]: 246(50), 135(100)	N^1,N^4 -Dicaffeoyl spermidine [b]	B, R, W
19	14.2	449	MS^2 [449]: 405(10), 329(10), 287(100), 269(5); MS^3 [499 → 287]: 259(100), 243(30), 199(10), 167(50), 125(20)	Dihydrokaempferol hexside [b]	B
20	14.3	193	MS^2 [193]: 178(70), 149(100), 134(30)	Ferulic acid [a]	B, R, W
21	14.5	335	MS^2 [335]: 179(50), 161(50), 135(100)	Caffeoylshikimic acid [b]	B
22	14.6	468	MS^2 [468]: 332(100), 306(20), 161(5), 135(2); MS^3 [468 → 332]: 289(100), 135(2), 109(1); MS^3 [468 → 306]: 246(50), 135(100)	N^1,N^8 -Dicaffeoyl spermidine [b]	B, R, W
23	15.0	187	MS2[187]: 169(5), 125(100)	Gallic acid monohydrate [b]	B, R, W
24	15.3	269	MS^2 [269]: 251(5), 241(50), 225(100), 201(30), 197(50), 133(3)	7,3',4'-Trihydroxyflavone [c]	B
25	15.4	335	MS^2 [335]: 291(10), 273(15), 247(5), 229(20), 193(100), 179(15), 151(5), 107(2), 97(1); MS^3[335 → 193]: 175(20), 165(100), 137(70), 121(50), 93(15)	6-Hydroxy ampelopsin [c]	B
26	15.7	303	MS^2 [303]: 285(100), 275(20), 259(10), 217(10), 177(15), 125(8)	Taxifolin [a]	B
27	16.0	301	MS^2 [301]: 283(3), 273(100), 257(10), 233(5), 191(3), 163(2), 151(2)	Unknown flavonoid or polyphenol	B, R, W
28	16.1	303	MS^2 [303]: 285(15), 275(100), 259(10), 257(20), 217(30), 215(15), 177(20), 151(5), 149(10), 143(5), 127(5)	Isomer of Taxifolin [b]	B, R, W
29	16.4	433	MS^2 [433]: 313(10), 271(100); MS^3 [433 → 271]: 177(10), 151(100)	Naringenin hexoside I [b]	B, R, W
30	16.5	431	MS^2 [431]: 341(8), 311(100), 269(2); MS^3 [431 → 311]: 283(100), 267(30), 243(20), 225(25), 191(10), 149(8), 117(5)	Apigenin-6-*C*-glucoside [b]	B
31	16.6	287	MS^2 [287]: 269(10), 243(5), 207(20),177(5), 161(90), 151(100), 135(5), 125(50), 107(5), 95(8)	7,3',4',5'-Tetrahydroxy flavanone [c]	B
32	16.7	449	MS^2 [449]: 287(100); MS^3[449 → 287]: 259(30), 245(100), 243(15), 219(15), 201(20), 175(10)	Unknown flavonoid hexoside	B, R
33	16.9	482	MS^2 [482]: 467(2), 346(70), 332(100), 320(5), 306(40), 175(5), 161(5); MS^3 [482 → 332]: 289(100); MS^3 [482 → 306]: 331 (100), 303(95), 161(35)	N^1,N^8 -Caffeoyl-feruloyl spermidine [b]	B, R, W
34	17.3	253	MS^2 [253]: 225(20), 209(100), 185(15), 181(8), 133(2), 117(5)	Dihydroxyflavone [b]	B, R, W
35	17.6	499	MS^2 [499]: 337(100); MS^3 [499 → 337]: 277(100), 161(40), 113(10)	Caffeoyl derivative hexose [c]	B, R
36	17.9	463	MS^2 [463]: 301(100); MS^3 [463 → 301]: 273(10), 257(10),179(100), 151(60)	Quecertin hexoside [b]	B
37	18.6	431	MS^2 [431]: 311(5), 269(100); MS^2 [431 → 269]: 225(100), 201(20), 197(20),149(40)	Apigenin-7-*O*-hexoside [b]	B, R, W
38	18.9	447	MS^2 [447]: 285(100); MS^3 [447 → 285]: 267(10), 257(5), 243(20), 241(90), 217(100), 198(90), 185(15), 177(20), 151(5)	Luteolin hexoside [b]	B, R, W
39	19.3	433	MS^2 [433]: 271(100), 269(10), MS^3 [433 → 271]: 177(10), 151(100)	Naringenin-7-*O*-glucoside [a]	B, R, W
40	19.4	271	MS^2 [271]: 227(2), 191(10), 177(20), 151(100)	Isomer of Naringenin [b]	B
41	19.5	461	MS^2 [461]: 341(5), 323(2), 299(100), 284(3); MS^3 [461 → 299]: 284(100), 255(10), 240(5), 149(2)	Chrysoeriol hexoside I [b]	B, R, W
42	20.3	477	MS^2 [477]: 357(20), 315(30), 314(100); MS^3 [447 → 315]: 300(100)	Isorharmnetin hexoside [b]	B
43	20.6	431	MS^2 [431]: 269(100); MS^3 [431 → 269]: 227(10), 225(100), 197(20), 151(8), 149(30), 123(5)	Apigenin-4'-*O*-hexoside [b]	B
44	20.7	301	MS^2 [301]: 283(2), 273(20), 257(15), 229(15), 215(5), 177(30), 165(5), 151(100), 137(15), 107(2)	Tricetin [c]	B, R, W
45	21.1	301	MS^2 [301]: 283(10), 191(20), 161(100), 151(5), 109(3); MS^3[301 → 161]: 133(60), 117(100)	5,6,7,3',4'-Pentahydroxy flavone [c]	B, R, W
46	21.2	303	MS^2 [303]: 285(2), 259(2), 193(100), 163(10), 151(5), 139(3), 124(2), 109(20); MS^3[303 → 193]: 178(100), 163(5), 151(20), 139(5); MS^4[303 → 193 → 178]: 163(100); MS^4[303 → 193 → 151]: 136(100)	7-*O*-Methyl catechin [c]	B, R
47	21.4	461	MS^2 [461]: 341(2), 323(1), 299(100), 284(5); MS^3 [461 → 299]: 284(100), 255(8), 240(5)); MS^4[461 → 299 → 284]: 267(5), 256(100), 240(10), 227(20), 212(10), 195(5), 134(5)	Chrysoeriol hexoside II [b]	B, R, W
48	21.9	491	MS^2 [491]: 343(8), 329(100), 314(10); MS^3 [491 → 329]: 314(100), 299(2); MS^4[491 → 329 → 314]: 299(100), 285(3)	Tricin hexoside [b]	B, R, W
49	22.6	287	MS^2 [287]: 269(8), 259(100), 243(20), 201(10), 125(5)	Dihydrokaempferol [b]	B
50	23.2	433	MS^2 [433]: 415(5), 313(8), 271(100); MS^3 [433 → 271]: 151(100)	Naringenin hexoside II [b]	B, R
51	23.7	285	MS^2 [285]: 270(2), 257(2), 241(3), 215(1) 213(1), 191(100), 176(1), 175(1),171(3), 165(30), 150(5), 93(8); MS^3 [285 → 191]: 176(100), 163(40), 147(20)	5-Methoxy-7,4'-dihydroxy flav-2-en-3-ol [c]	B, R, W
52	23.7	395	MS^2 [395]: 375(20), 351(40), 335(20), 327(15), 321(100), 295(25), 275(20)	Unknown	B, R, W

53	23.8	287	MS^2 [287]: 269(5), 151(100), 125(5), 107(5)	Eriodictyol [a]	B
54	24.3	287	MS^2 [287]: 193(100), 151(5), 147(40), 139(20), 124(3), 93(5); MS^3 [287 → 193]: 178(100), 151(20), 139(2)	7-*O*-Methyl afzelechin [c]	B, R, W
55	24.6	415	MS^2 [415]: 253(100), 179(5), 161(20), 135(2); MS^3 [415 → 253]: 179(30), 161(40), 135(100)	1,3-*O*-Dicaffeoylglycerol [b]	B, R, W
56	24.7	417	MS^2 [417]: 255(100), 254(45), 253(40), 237(3), 236(2), 235(2), 181(2), 180(2), 179(2), 163(8), 162(10), 161(20), 135(2); MS^3[417 → 255]: 181(50), 179(60), 163(70), 161(50), 137(100), 135(50)	1,3-*O*-Caffeoyl-dihydrocaffeoylglycerol [b]	B, R, W
57	25.1	345	MS^2 [345]: 327(100), 309(20), 301(2), 283(5), 281(2), 265(10), 247(15), 215(1), 183(1)	Tetrahydroxy-octadecenoic acid [b]	B, R, W
58	25.3	301	MS^2 [301]: 273(15), 257(20), 193(15), 179(100), 151(85), 107(5)	Quercetin [a]	B
59	25.7	329	MS^2 [329]: 311(100), 309(10), 293(30), 265(2), 249(2), 247(1), 229(50), 211(30), 201(10), 171(20), 155(2), 139(2), 113(2), 99(1)	Trihydroxy-octadecenoic acid [b]	B, R, W
60	26.1	285	MS^2 [285]: 257(10), 243(70), 241(100), 217(50), 199(70), 175(60), 151(20), 133(5), 107(2)	Luteolin [a]	B, R, W
61	27.1	399	MS^2 [399]: 253(100), 235(20), 163(10); MS^3[399 → 253]: 179(40), 161(50), 135(100)	1,3-*O*-Coumaroyl-caffeoyl-glycerol [b]	B, R, W
62	27.3	431	MS^2 [431]: 295(30), 255(40), 254 (100), 253(80), 237(50), 236(70), 235(30), 193(50), 181(30), 175(50), 162(50), 160(25), 137(10)	1,3-*O*-Feruloyl-dihydrocaffeoylglycerol [b]	B, R, W
63	27.5	271	MS^2 [271]: 227(2), 177(20), 165(8), 151(100), 125(5), 107(3)	Naringenin [a]	B, R, W
64	27.8	429	MS^2 [429]: 253(100), 235(50), 193(30), 161(20)	1,3-*O*-Feruloyl-caffeoylglycerol [b]	B, R, W
65	27.9	329	MS^2 [329]: 314(100); MS^3 [329 → 314]: 299(100); MS^4 [329 → 314 → 299]: 271(100)	Tricin [b]	B, R, W
66	28.1	327	MS^2 [327]: 309(30), 291(100), 239(60), 221(10), 195(20)	Trihydroxy-octadecadienoic acid [b]	B, R, W
67	28.3	301	MS^2 [301]: 286(10), 273(5), 242(10), 199(3), 177(20), 165(5), 151(100), 149(2), 107(5)	Homoeriodictyol [c]	B, R, W
68	29.3	269	MS^2 [269]: 241(10), 225(100), 201(30), 151(20), 149(50), 107(5)	Apigenin [a]	B, R, W
69	29.8	315	MS^2 [315]: 300(100); MS^3 [315 → 300]: 283(40), 272(100), 271(70), 227(50), 188(10), 151(70), 107(10)	Isorhamnetin [a]	B
70	29.9	383	MS^2 [383]: 263(10), 237(30), 219(60), 163(100), 145(30), 119(30)	1,3-*O*-Dicoumaroylglycerol [b]	B, R, W
71	30.5	413	MS^2 [413]: 235(100), 177(30), 161(15), 135(10); MS^3 [413 → 235]: 173(20), 161(100), 121(5)	1,3-*O*-Coumaroyl-feruloylglycerol [b]	B, R, W
72	30.8	443	MS^2 [443]: 293(5), 267(10), 249(20), 235(100), 207(60), 193(30), 161(25), 135(5), 134(4)	1,3-*O*-Diferuloylglycerol [b]	B, R, W
73	34.8	311	MS^2 [311]: 293(100), 275(30), 253(10), 235(30), 223(90)	Dihydroxy-octadecadienoic acid [b]	B, R, W
74	37.2	313	MS^2 [313]: 295(100), 277(15), 249(10), 213(10), 195(15), 183(50); 169(5), 123(10)	Dihydroxy-octadecenoic acid I [b]	B, R, W
75	36.7	313	MS^2 [313]: 295(90), 277(40), 249(5), 233(2), 201(100), 171(40), 155(5), 139(2)	Dihydroxy-octadecenoic acid II [b]	B, R, W

*Compounds detected from the extract of: R = red sorghum whole grain; B = brown sorghum whole grain; W = white sorghum whole grain

[a] Identified based on MS^n data and retention time and comparison with authentic standard

[b] Identified based on MS^n data and retention time and their comparison with MS^n and other data from reference sources

[c] Tentatively identified based on MS^n data and retention time and other literature evidence

3.1. Free phenolic acids and derivatives

Compounds **1**, **8**, and **20** were identified as protocatechuic acid, caffeic acid, and ferulic acid respectively as their chromatographic retention times and MS^2 fragmentations were identical with their authentic standards. Compound **2** with a very early eluting time (Rt 4.0 min) exhibited $[M - H]^-$ ions at *m/z* 197. Its molecular mass and retention time show similarities to Danshensu (α,3,4-trihydroxy benzenepropanoic acid), but the MS^2 fragmentation pattern of compound **2** was quite distinct from that of Danshensu (Liu, Guo, Ye, Lin, Sun, Xu, et al., 2007). Danshensu has a base peak at *m/z* 179, and followed by a *m/z* 135 peak; whereas the compound **2** gave a base fragment peak $[M - H - CO_2]^-$ at *m/z* 153, and the *m/z* 179 peak with an abundance of only 15% of the base peak. Additionally it had another fragment peak at *m/z* 109 in the MS^2[197] fragmentation with 15% abundance of the base peak, and this *m/z* 109 peak also yield in the further

MS^3[197→153] fragmentation with 95% abundance of the base peak, which confirms the presence of a dihydroxyl benzene residue. The *m/z* 179 peak indicates a loss of H_2O from the propanoic chain, which shows similarity with Danshensu, confirms a hydroxyl group is linked to the propanoic chain. The MS^3[197→153] gave a base fragment peak at *m/z* 138, corresponding to loss of a methyl radical; which gives clue that the position of the hydroxyl group on the propionic chain is linked at the β-position, so it could lose a methyl radical after the compound lose a carboxyl group. On the basis of above analyses, compound **2** was tentatively characterized as *β*,3,4-trihydroxy benzenepropanoic acid.

Compound **4** showed $[M - H]^-$ ions at *m/z* 341, and its MS^2 fragmentation gave a base peak of *m/z* 179, a loss of 162 Da. Compared with its elution order and its MS^2 fragmentation pattern it was assigned as caffeic acid hexoside (Narváez-Cuenca, Vincken, & Gruppen, 2012). Compound **10** exhibited $[M - H]^-$ ions at *m/z* 353 and gave a base MS^2 fragmentation peak at *m/z* 191, followed by a peak *m/z* 179. Further MS^3[353 →191] yielded a base peak at *m/z* 127, which corresponded to a quinic acid residue enabling this compound to be assigned as caffeoylquinic acid (Alakolanga, Siriwardane, Savitri Kumar, Jayasinghe, Jaiswal, & Kuhnert, 2014). Compound **21** gave $[M - H]^-$ ions at *m/z* 335, while its MS^2 fragmentation gave a base peak of *m/z* 135, and other peaks at *m/z* 179 and *m/z* 161. Considering both its chromatographic retention time and MS^2 spectrum this compound could be assigned as caffeoylshikimic acid (Alakolanga, et al., 2014). Compound **23** yielded pseudo-molecular ions at $[M - H]^-$ *m/z* 187 whilst the MS^2 fragmentation only gave two peaks, one a base peak at *m/z* 125, and another a peak at *m/z* 169 with 5% of the base peak. This MS^2 fragmentation is characteristic of gallic acid (Khallouki, Haubner, Ricarte, Erben, Klika, Ulrich, et al., 2015), and allowed compound **23** to be identified as gallic acid monohydrate.

3.2. Flavonoids

3.2.1. Flavan-3-ol derivatives

Compound **7,** with its $[M - H]^-$ ions at *m/z* 289, gave a highly abundant peak in the extract of brown sorghum whole grain, this compound was identified as (+) catechin by comparison of its chromatographic retention time and MS^2 fragmentation pattern with those of its authentic standard. Compound **3,** eluted early in the chromatogram and gave $[M - H]^-$ ions at *m/z* 451; its MS^2 fragmentation yielded only one base peak at *m/z* 289 by loss of 162 Da, which indicated the loss of a hexose. MS^3[451→289] of Compound **3** gave the same fragmentation pattern as compound **7**, so compound **3** was identified as catechin hexoside (Amarowicz, Estrella, Hernández, Robredo, Troszyńska, Kosińska, et al., 2010).

Compounds **46** and **54** eluted at different times with their $[M - H]^-$ ions at *m/z* 303 and *m/z* 287 respectively. However, these two compounds displayed similar MS^2 fragmentation patterns with a base peak at *m/z* 193, which corresponds to $[M - H - 110]^-$ of compound **46** and $[M - H - 94]^-$ of compound **54**. In addition, compound **46** gave an

m/z 109 peak and compound **54** gave an *m/z* 93 peak; these fragmentation ions indicated a B-ring cleavage, which is more likely to occur in flavan-3-ols. The *m/z* 151 ions, corresponding to a typical $^{1,3}A^-$ fragment in flavonoids (de Rijke, Out, Niessen, Ariese, Gooijer, & Brinkman, 2006), were also observed in MS^2 fragmentation of both compounds. The other distinctive ions *m/z* 163 in compound **46** and *m/z* 147 in compound **54** were $^{1,4}B^-$ fragments of the two compounds respectively, and *m/z* 139 in MS^2 fragmentation of both compounds were their $^{1,4}A^-$ fragments. All above data indicated that the two compounds were from the same series of flavan-3-ols, with the same A-ring moiety but with different hydroxyl numbers in the B-ring. Further evidence from MS^3 [303→139] and MS^3 [287→139] carried out on the two compounds resulted in both yielding the same single base fragment peak at *m/z* 124. This confirmed the presence of a methoxyl group in the A- ring of both compounds. It is worth noting that the absence of a peak for the loss of a methyl radical in their MS^2 fragmentation spectra, which was in accordance with reported literature (Cren-Olivé, Déprez, Lebrun, Coddeville, & Rolando, 2000), was possibly due to a less conjugated A-ring in the structure of flavan-3-ols. Additionally, it was difficult to determine the exact position of the methoxyl group at 7- position or at 5- position based only on the MS^n fragmentations, however, their comparatively late chromatographic elution order supported 7- position (Cren-Olivé, et al., 2000), and also noting that 7-methylated anthocyanin compounds have been found in natural plants (Kirby, Wu, Tsao, & McCallum, 2013; Romeo, Ballistreri, Fabroni, Pangallo, Li Destri Nicosia, Schena, et al., 2015). Therefore compound **46** was tentatively characterized as 7-*O*-methyl catechin, and compound **54** was tentatively characterized as 7-*O*-methyl afzelechin. **Fig.2** illustrates their MS^2 and MS^3 spectra and the proposed fragmentation pathways.

Compound **51** exhibited $[M - H]^-$ ions at *m/z* 285, and yielded a base peak at *m/z* 191 in MS^2 fragmentation by loss of 94 Da, with a *m/z* 93 peak also being observed; this indicated a B-ring cleavage similar to compound **54.** Another distinctive fragment ion at *m/z* 165 corresponded to a $^{0,2}A^-$ fragment by loss of 120 Da. The *m/z* 150 ions were yielded from the *m/z* 165 ions by further loss of a methyl radical. There were also some less intensive ions in MS^2 fragmentation by small neutral loss: *m/z* 270 from the loss of *O*-linked methyl radical, *m/z* 257 and *m/z* 241 from loss of CO and CO_2 at the C-ring respectively, and *m/z* 215 from sequential loss of CO + C_2H_2O, and *m/z* 199 from consecutively loss of CO_2 + C_2H_2O. Further MS^3 [285→191] fragmentation gave a base peak at *m/z* 176 which confirmed the loss of a methyl radical at A-ring while *m/z* 163 and *m/z* 147 ions were accounted for the loss of CO or CO_2 at C-ring. Comparing its structure and earlier chromatographic elution order with compound **54**, compound **51** was tentatively assigned as 5-methoxy-7,4'-dihydroxy flav-2-en-3-ol. Flav-2-en-3-ols have been reported as an intermediate between flavan-3-ols and cyanidins (Wellmann, Griesser, Schwab, Martens, Eisenreich, Matern, et al., 2006) and have been found in plant materials of soybeans (Fukami, Yano, & Iwashita, 2013).

199 *3.2.2. Flavone and flavanone derivatives*

200 Compounds **26**, **39**, **53**, **58**, **60**, **63**, **68**, and **69** were identified as taxifolin, naringenin -7-*O*-gucoside, eriodictyol,
201 quercetin, luteolin, naringenin, apigenin, and isorhamnetin respectively, by comparison of their chromatographic
202 retention times and MS^2 fragmentation spectra with those of authentic standards. In addition, some of their isomers were
203 also found in the extracts. Compound **28** gave $[M - H]^-$ ions at *m/z* 303 and produced the similar MS^2 fragmentation
204 pattern as compound **26**. It was therefore assigned as an isomer of taxifolin. Compounds **29** and **50** eluted at different
205 retention times, both gave $[M - H]^-$ ions at *m/z* 433 and displayed the same fragmentation pattern on MS^2 and MS^3 as
206 compound **39**, they were consequently assigned to naringenin hexoside I and naringenin hexoside II respectively.
207 Compound **40** yielded almost same MS^2 spectra with compound **63**, but with different chromatographic retention time,
208 and was assigned as an isomer of naringenin.

209 Compound **24** exhibited $[M - H]^-$ ions at *m/z* 269, its MS^2 fragmentation pattern was similar with that of apigenin
210 but much earlier in its eluting order, this compound did not give the *m/z* 149 fragment of apigenin, but gave a *m/z* 133
211 fragment peak, which corresponds to same $^{0,2}A^-$ fragmentation ions as apigenin with a difference of 16 Da due to one less
212 hydroxyl group on A-ring. Considering the fragmentation pattern and its early chromatographic elution, compound **24**
213 was tentatively assigned to 7,3',4'-trihydroxyflavone.

214 Compound **25** gave $[M - H]^-$ ions at *m/z* 335 and eluted earlier in the chromatogram; this compound was tentatively
215 characterized as 6-hydroxy ampelopsin based on its MS^2 and MS^3 fragmentation behaviors and chromatographic
216 retention time. This compound possessed high degree of hydroxylation on its flavanonol structural frame, its MS^2
217 fragmentation yielded the same predominant $^{1,4}B^-$ fragmentation ions as previously reported compound ampelopsin
218 (Abu-Reidah, et al., 2015), because they have same C-ring structure and same high degree of hydroxylation on the B-ring,
219 which led to the similar fragmentation pattern for both compounds. MS^3 fragmentation further supported this
220 identification. **Fig.3** shows the MS^2 and MS^3 spectra of compound **25** and their fragmentation pathways.

221 Compounds **30**, **37**, and **43** were observed at different retention times in the chromatogram but all gave $[M - H]^-$
222 ions at *m/z* 431; MS^2 fragmentation of **30** yielded a base peak of *m/z* 311 and less intensive fragment ions at *m/z* 341 (8%
223 of the base peak), which corresponded to the loss of moieties from the C-linked glucose (Cuyckens & Claeys, 2004)
224 whilst MS^2 fragmentation of both compounds **37** and **43** yielded a single base peak at *m/z* 269 , corresponding to the loss
225 of moiety of *O*-linked glucose. Further MS^3 [431→269] spectra showed similar fragmentation patterns to apigenin and
226 based on their chromatographic elution orders, further MS^3 fragmentation patterns and reported literature these three
227 compounds were identified as isomers of apigenin glucosides, with compound **30** being identified as apigenin-6-C-
228 glucoside, i.e. isovitexin (Ye, Yang, Liu, Qiao, Li, Cheng, et al., 2012), compound **37** to apigenin-7-*O*-gluciside (O. R.
229 Pereira, Silva, Domingues, & Cardoso, 2012), and compound **43** identified as apigenin-4'-*O*-gluciside (Jaiswal, Müller,

Müller, Karar, & Kuhnert, 2014), **Fig.4** depicts a selective MS^2[431] screening chromatogram and the MS^2 and MS^3 spectra for these three compounds.

Based on comparison of their chromatographic elution orders and MS^2 spectra with reported literature, compounds **5**, **11**, **19**, **34**, **36**, **38**, **41**,**42**, **47**, **49**, and **65** were assigned to taxifolin hexoside I (Svensson, et al., 2010),taxifolin hexoside II (Svensson, et al., 2010), dihydrokaempferol hexside (Mena, Calani, Dall'Asta, Galaverna, García-Viguera, Bruni, et al., 2012), dihydroxyflavone (O. R. Pereira, et al., 2012), quercetin hexoside (Regos, Urbanella, & Treutter, 2009), luteolin hexoside (Jaiswal, et al., 2014), chrysoeriol hexoside I (Olívia R. Pereira, Peres, Silva, Domingues, & Cardoso, 2013), isorharmnetin hexoside (Abu-Reidah, et al., 2015), chrysoeriol hexoside II (Olívia R. Pereira, et al., 2013), dihydrokaempferol (Fischer, Carle, & Kammerer, 2011) and tricin (Duarte-Almeida, Negri, Salatino, de Carvalho, & Lajolo, 2007), respectively. Compounds **31**,**44**, **45**, and **67** were tentatively identified as 7,3',4',5'-tetrahydroxy flavanone, tricetin, 5,6,7,3',4'-pentahydroxy flavone, and homoeriodictyol, respectively, based on their MS^2 fragmentation behavior, chromatographic retention times, and comparison with the similar known compounds and other reference evidence (Medina, Domínguez-Perles, García-Viguera, Cejuela-Anta, Martínez-Sanz, Ferreres, et al., 2012; Skoula, Grayer, Kite, & Veitch, 2008; Ye, et al., 2012).

3.3. Phenylpropane glycerides

Compounds **6** and **13** both showed $[M - H]^-$ at *m/z* 253, and gave the same MS^2 fragmentation ions: *m/z* 179, *m/z* 161, and *m/z* 135 only varying with some differences in peak abundance; these three fragmentation ions indicated the presence of a caffeoyl moiety, and the fragmentation ions m/z 179 $[M - H - 74]^-$ and *m/z* 161 $[M - H - 92]^-$ were characteristic of a loss of glycerol residue, so both compounds were isomers of caffeoylglycerol. Based on literature (Ma, Xiao, Li, Wang, & Du, 2007), compound **6** was identified as 2-*O*-caffeoylglycerol, and compound **13** was identified as 1-*O*-caffeoylglycerol. Compound **12**, eluting little earlier than compound **13**, exhibited $[M - H]^-$ ions at *m/z* 255, MS^2 fragmentation showed similar pattern with compound **13** but yielded fragmentation ions *m/z* 181$[M - H - 74]^-$ and *m/z* 163 $[M - H - 92]^-$, so compound**12** was identified as 1-*O*-dihydrocaffeoylglycerol. Compounds **16** and **17** both exhibited $[M - H]^-$ ions at *m/z* 237, both compounds produced base peak of *m/z* 119 and other two peaks at *m/z* 163 and *m/z* 145 with a difference in peak abundance; these three ions indicated a coumaroyl moiety, and *m/z* 163 $[M - H - 74]^-$ and *m/z* 145 $[M - H - 92]^-$ further indicated the loss of glycerol residues; based on literature (Ma, et al., 2007), compound 1**6** was assigned to 2-*O*-coumaroylglycerol, and compound **17** was assigned to 1-*O*-coumaroylglycerol.

Compounds **9** and **55** both exhibited $[M - H]^-$ ions at *m/z* 415, and with substantial difference in their chromatographic retention times. Although they displayed the same MS^2 fragmentation pattern with a base peak at *m/z*

253, and further MS^3[415→253] fragmentation of both compounds gave identical fragmentation pattern of caffeoylglycerol, but actually the *m/z* 253 in compound **9** was produced by loss of a glucose moiety (162 Da), whereas *m/z* 253 in compound **55** was by loss of a caffeoyl moiety (also 162 Da). Compound **9** was identified as 1-*O*-caffeoyl-2-*O*- glucosylglycerol (Munafo & Gianfagna, 2015), while compound **55** was identified as 1,3-*O*-dicaffeoylglycerol (Svensson, et al., 2010). Similarly, compounds **15** and **61** both gave $[M - H]^-$ ions at *m/z* 399 but with much difference in chromatographic retention times, however, MS^2 fragmentation ions of compounds **15** (*m/z* 145, *m/z* 163, and *m/z* 237) showed a characteristic of coumaroylglycerol, whereas MS^2 fragmentation ions of compound **61** showed a characteristic of caffeoylglycerol. The *m/z* 253 peak also indicated a loss of a coumaroyl group (146 Da), so, compound **15** was identified as 1-*O*-coumaroyl-2-*O*- glucosylglycerol (Munafo, et al., 2015), and compound **61** was characterized as1,3-*O*-coumaroyl-caffeoylglycerol (Svensson, et al., 2010). Compound **56** gave $[M - H]^-$ ions at *m/z* 417 and its MS^2 fragmentation gave a mixture of ions from dihydocaffeoyl and caffeoyl moieties with *m/z* 255, m/z181, *m/z* 163 and *m/z* 137 corresponding to dihydrocaffeioyl moieties, whereas *m/z* 253, *m/z* 179, *m/z* 161, and *m/z* 135 corresponded to a caffeoyl moiety. Therefore compound **56** was identified as 1,3-*O*-caffeoyl-dihydrocaffeoylglycerol. Compound **62** with $[M - H]^-$ at *m/z* 431 displayed a complex mixture of fragmentation ions from both feruoyl and dihydrocaffeoyl residues, and this compound was identified as 1,3-*O*-feruloyl-dihydrocaffeoylglycerol (**Fig.4** illustrates its chromatographic elution and proposed fragmentation pathways). Based on their characteristics of phenylpropane glycerides in MS^n fragmentations, compounds **64**, **70**, **71**, **72** were identified as 1,3-*O*-feruloyl-caffeoylglycerol (Ma, et al., 2007), 1,3-*O*-dicoumaroylglycerol, 1,3-*O*-coumaroyl-feruloylglycerol (Ma, et al., 2007), and 1,3-*O*-diferuloylglycerol respectively.

3.4. Phenolamides

A number of phenolamides were found in the sorghum extracts. Compound **22,** with its $[M - H]^-$ ions at *m/z* 468, displayed an abundant peak in all the three sorghum whole grain hydomethanolic extracts. This compound gave a base peak at *m/z* 332 in its MS^2 fragmentation by loss of 136 Da and the second abundant peak at *m/z* 306 by loss of 162 Da. Other peaks included *m/z* 161 and *m/z* 289. All these fragmentation ions indicated the presence of a caffeoyl group and amide moiety in the structure. Further MS^3[468→332] gave one base fragment peak *m/z* 289 by loss of a HN=C=O moiety (43 Da), which confirmed its caffeoylamide structure. MS^3[468→306] also produced a base fragment peak *m/z* 135 ions of caffeoyl group residue. Based on these MS^n fragmentation ions and published references (Gancel, Alter, Dhuique-Mayer, Ruales, & Vaillant, 2008; Helmja, Vaher, Püssa, & Kaljurand, 2009), compound **22** was characterised as N^1, N^8 –dicaffeoyl spermidine. The MS spectra and principal cleavage pathways are shown in **Fig.5**. Compound **18,** with a little earlier chromatographic retention time, also exhibited $[M - H]^-$ ions at *m/z* 468 and its MS^2 fragmentation

produced almost the same pattern as compound **22** with only some differences in peak abundance. MS^3[468→306] and MS^3[468→332] also gave identical fragmentation ions as compound **22**, indicating that this compound is clearly an isomer of compound **22**. Based on this evidence and its retention time and comparison with published literature (Gancel, et al., 2008),this compound was assigned as N^1, N^4 -dicaffeoyl spermidine.

Compound **33** gave $[M - H]^-$ ions at *m/z* 482 whilst its MS^2fragmentation gave a base peak at *m/z* 332, followed by peaks at *m/z* 346 and *m/z* 306 and other less intensive fragment peaks at *m/z* 161 and *m/z* 175. This compound exhibited a similar fragmentation pattern to compound **22** but with an extra mass (14 Da) in its deprotonated molecular ions. The *m/z* 332 and *m/z* 306 corresponded to loss of 150 and 176 Da from a furoyl group; and the *m/z* 346 and *m/z* 320 corresponded to loss of 136 and 162 Da from the caffeoyl group. Similar to compound **22**, MS^3 [482→332] of compound **33** gave one base fragment peak at *m/z* 289. In addition, MS^3[482→346] gave base fragment peaks at *m/z* 331 by loss of a methyl radical (15 Da), *m/z* 303 through loss of HN=C=O (43 Da), and *m/z* 161 ions which was formed by a rearrangement after losing methyl radical. Based on the above MS^n fragmentation information and published literature (Handrick, Vogt, & Frolov, 2010), compound **33** was identified as N^1, N^8 –caffeoyl-feruloyl spermidine. This also is summarized in **Fig.5**.

3.5. Other compounds

In addition to the phenolic compounds described above, hydroxy fatty acids were also detected. Compounds **57**, **59**, **66**, **73**, **74** and **75** were identified as hydroxy fatty acids, a few of them showed abundant peaks, especially in the white sorghum extract; all detected hydroxy fatty acids identified possessed 18 carbon atoms, but varied in the number and position of hydroxyl groups and double bonds; their MS^2 spectra showed consecutive losses of water molecules and aliphatic residues (see **Table**. **1**), which were in accordance with reported literature (Farag, Sakna, El-fiky, Shabana, & Wessjohann, 2015; Llorent-Martinez, Spinola, Gouveia, & Castilho, 2015; Martin-Arjol, Bassas-Galia, Bermudo, Garcia, & Manresa, 2010). The hydroxy fatty acids have been reported to perform a variety of biological functions such as anti-inflammatory or cytotoxic activity (Martin-Arjol, et al., 2010).

Compound **14** was identified as a saccharide based on its MS^2 fragmentation pattern (Spínola, et al., 2015).

4. Conclusion

HPLC-ESI-MS^n analysis was successfully applied to identify a wide range of phenolic compounds in sorghum whole grain hydromethanolic extracts. Except for anthrocyandins which required acidic extraction, insoluble-bound phenolic acids which required alkaline hydrolysis and polyflavanes which possess molecular weight beyond our scanning

range, the present work investigated all neutral phenolic compounds with small and medium molecular weight in the selected species of sorghum whole grains. A variety of phenolic compounds were found in their extracts, including free phenolic acids, flavonoids, phenylpropane glycerides, and phenolamides. The majority of these compounds are reported for the first time in sorghum whole grains. These results confirmed sorghum grains are rich in phenolic compounds. The brown sorghum extract exhibited greater diversity and abundance of phenolic compounds than either red or white sorghum. Phenylpropane glycerides and phenolamides were found with abundant peaks in all the extracts of the three sorghum species, indicating they are another two important classes of phenolic compounds in sorghum.

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References

Abu-Reidah, I. M., Ali-Shtayeh, M. S., Jamous, R. M., Arráez-Román, D., & Segura-Carretero, A. (2015). HPLC–DAD–ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. *Food Chemistry, 166*, 179-191.

Alakolanga, A. G. A. W., Siriwardane, A. M. D. A., Savitri Kumar, N., Jayasinghe, L., Jaiswal, R., & Kuhnert, N. (2014). LC-MS^n identification and characterization of the phenolic compounds from the fruits of *Flacourtia indica* (Burm. F.) Merr. and *Flacourtia inermis* Roxb. *Food Research International, 62*, 388-396.

Amarowicz, R., Estrella, I., Hernández, T., Robredo, S., Troszyńska, A., Kosińska, A., & Pegg, R. B. (2010). Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). *Food Chemistry, 121*(3), 705-711.

Awika, J. M., & Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry, 65*(9), 1199-1221.

Cren-Olivé, C., Déprez, S., Lebrun, S., Coddeville, B., & Rolando, C. (2000). Characterization of methylation site of monomethylflavan-3-ols by liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry, 14*(23), 2312-2319.

Cuyckens, F., & Claeys, M. (2004). Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass Spectrometry, 39*(1), 1-15.

de Rijke, E., Out, P., Niessen, W. M. A., Ariese, F., Gooijer, C., & Brinkman, U. A. T. (2006). Analytical separation and detection methods for flavonoids. *Journal of Chromatography A, 1112*(1–2), 31-63.

Duarte-Almeida, J. M., Negri, G., Salatino, A., de Carvalho, J. E., & Lajolo, F. M. (2007). Antiproliferative and antioxidant activities of a tricin acylated glycoside from sugarcane (*Saccharum officinarum*) juice. *Phytochemistry, 68*(8), 1165-1171.

Dykes, L., & Rooney, L. W. (2006). Sorghum and millet phenols and antioxidants. *Journal of Cereal Science, 44*(3), 236-251.

Farag, M. A., Sakna, S. T., El-fiky, N. M., Shabana, M. M., & Wessjohann, L. A. (2015). Phytochemical, antioxidant and antidiabetic evaluation of eight *Bauhinia* L. species from Egypt using UHPLC–PDA–qTOF-MS and chemometrics. *Phytochemistry, 119*, 41-50.

Fischer, U. A., Carle, R., & Kammerer, D. R. (2011). Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MS^n. *Food Chemistry, 127*(2), 807-821.

Fukami, H., Yano, Y., & Iwashita, T. (2013). Isolation of a reduced form of cyanidin 3-O-β-D-glucoside from immature black soybean (*Glycine max* (L.) Merr.) and its reducing properties. *Journal of Oleo Science, 62*(8), 623-629.

Gancel, A. L., Alter, P., Dhuique-Mayer, C., Ruales, J., & Vaillant, F. (2008). Identifying carotenoids and phenolic compounds in naranjilla (*Solanum quitoense* Lam. var. puyo hybrid), an andean fruit. *Journal of Agricultural and Food Chemistry, 56*(24), 11890-11899.

Handrick, V., Vogt, T., & Frolov, A. (2010). Profiling of hydroxycinnamic acid amides in Arabidopsis thaliana pollen by tandem mass spectrometry. *Analytical and Bioanalytical Chemistry, 398*(7-8), 2789-2801.

Helmja, K., Vaher, M., Püssa, T., & Kaljurand, M. (2009). Analysis of the stable free radical scavenging capability of artificial polyphenol mixtures and plant extracts by capillary electrophoresis and liquid chromatography-diode array detection-tandem mass spectrometry. *Journal of Chromatography A, 1216*(12), 2417-2423.

Jaiswal, R., Müller, H., Müller, A., Karar, M. G. E., & Kuhnert, N. (2014). Identification and characterization of chlorogenic acids, chlorogenic acid glycosides and flavonoids from *Lonicera henryi* L. (Caprifoliaceae) leaves by LC-MS^n. *Phytochemistry, 108*, 252-263.

Khallouki, F., Haubner, R., Ricarte, I., Erben, G., Klika, K., Ulrich, C. M., & Owen, R. W. (2015). Identification of polyphenolic compounds in the flesh of Argan (Morocco) fruits. *Food Chemistry, 179*, 191-198.

Khan, I., Yousif, A. M., Johnson, S. K., & Gamlath, S. (2015). Acute effect of sorghum flour-containing pasta on plasma total polyphenols, antioxidant capacity and oxidative stress markers in healthy subjects: A randomised controlled trial. *Clinical Nutrition, 34*(3), 415-421.

Kirby, C. W., Wu, T., Tsao, R., & McCallum, J. L. (2013). Isolation and structural characterization of unusual pyranoanthocyanins and related anthocyanins from Staghorn sumac (*Rhus typhina* L.) via UPLC-ESI-MS, ^{1}H, ^{13}C, and 2D NMR spectroscopy. *Phytochemistry, 94*, 284-293.

Licata, R., Chu, J., Wang, S., Coorey, R., James, A., Zhao, Y., & Johnson, S. (2014). Determination of formulation and processing factors affecting slowly digestible starch, protein digestibility and antioxidant capacity of extruded sorghum-maize composite flour. *International Journal of Food Science and Technology, 49*(5), 1408-1419.

Liu, A.-H., Guo, H., Ye, M., Lin, Y.-H., Sun, J.-H., Xu, M., & Guo, D.-A. (2007). Detection, characterization and identification of phenolic acids in Danshen using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. *Journal of Chromatography A, 1161*(1–2), 170-182.

Llorent-Martinez, E. J., Spinola, V., Gouveia, S., & Castilho, P. C. (2015). HPLC-ESI-MS^n characterization of phenolic compounds, terpenoid saponins, and other minor compounds in *Bituminaria bituminosa*. *Industrial Crops and Products, 69*, 80-90.

Ma, C., Xiao, S.-y., Li, Z.-g., Wang, W., & Du, L.-j. (2007). Characterization of active phenolic components in the ethanolic extract of *Ananas comosus* L. leaves using high-performance liquid chromatography with diode array detection and tandem mass spectrometry. *Journal of Chromatography A, 1165*(1–2), 39-44.

Martin-Arjol, I., Bassas-Galia, M., Bermudo, E., Garcia, F., & Manresa, A. (2010). Identification of oxylipins with antifungal activity by LC–MS/MS from the supernatant of *Pseudomonas* 42A2. *Chemistry and Physics of Lipids, 163*(4–5), 341-346.

Medina, S., Domínguez-Perles, R., García-Viguera, C., Cejuela-Anta, R., Martínez-Sanz, J. M., Ferreres, F., & Gil-Izquierdo, A. (2012). Physical activity increases the bioavailability of flavanones after dietary aronia-citrus juice intake in triathletes. *Food Chemistry, 135*(4), 2133-2137.

Mena, P., Calani, L., Dall'Asta, C., Galaverna, G., García-Viguera, C., Bruni, R., Crozier, A., & Del Rio, D. (2012). Rapid and comprehensive evaluation of (Poly)phenolic compounds in pomegranate (*Punica granatum L.*) Juice by UHPLC-MS^n. *Molecules, 17*(12), 14821-14840.

Munafo, J. P., Jr., & Gianfagna, T. J. (2015). Quantitative Analysis of Phenylpropanoid Glycerol Glucosides in Different Organs of Easter Lily (*Lilium longiflorum* Thunb.). *Journal of Agricultural and Food Chemistry, 63*(19), 4836-4842.

Narváez-Cuenca, C.-E., Vincken, J.-P., & Gruppen, H. (2012). Identification and quantification of (dihydro) hydroxycinnamic acids and their conjugates in potato by UHPLC–DAD–ESI-MS^n. *Food Chemistry, 130*(3), 730-738.

Pereira, O. R., Peres, A. M., Silva, A. M. S., Domingues, M. R. M., & Cardoso, S. M. (2013). Simultaneous characterization and quantification of phenolic compounds in *Thymus x citriodorus* using a validated HPLC–UV and ESI–MS combined method. *Food Research International, 54*(2), 1773-1780.

Pereira, O. R., Silva, A. M. S., Domingues, M. R. M., & Cardoso, S. M. (2012). Identification of phenolic constituents of *Cytisus multiflorus*. *Food Chemistry, 131*(2), 652-659.

Ragaee, S., Abdel-Aal, E.-S. M., & Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry, 98*(1), 32-38.

Regos, I., Urbanella, A., & Treutter, D. (2009). Identification and quantification of phenolic compounds from the forage legume sainfoin (*Onobrychis viciifolia*). *Journal of Agricultural and Food Chemistry, 57*(13), 5843-5852.

Romeo, F. V., Ballistreri, G., Fabroni, S., Pangallo, S., Li Destri Nicosia, M. G., Schena, L., & Rapisarda, P. (2015). Chemical characterization of different sumac and pomegranate extracts effective against *Botrytis cinerea* rots. *Molecules, 20*(7), 11941-11958.

Skoula, M., Grayer, R. J., Kite, G. C., & Veitch, N. C. (2008). Exudate flavones and flavanones in *Origanum* species and their interspecific variation. *Biochemical Systematics and Ecology, 36*(8), 646-654.

Spínola, V., Pinto, J., & Castilho, P. C. (2015). Identification and quantification of phenolic compounds of selected fruits from Madeira Island by HPLC-DAD–ESI-MS^n and screening for their antioxidant activity. *Food Chemistry, 173*, 14-30.

Stefoska-Needham, A., Beck, E. J., Johnson, S. K., & Tapsell, L. C. (2015). Sorghum: an underutilized cereal whole grain with the potential to assist in the prevention of chronic disease. *Food Reviews International, 31*(4), 401-437.

Svensson, L., Sekwati-Monang, B., Lutz, D. L., Schieber, A., & Ganzle, M. G. (2010). Phenolic acids and flavonoids in nonfermented and fermented red sorghum (*Sorghum bicolor* (L.) Moench). *Journal of Agricultural and Food Chemistry, 58*(16), 9214-9220.

Wellmann, F., Griesser, M., Schwab, W., Martens, S., Eisenreich, W., Matern, U., & Lukačin, R. (2006). Anthocyanidin synthase from *Gerbera hybrida* catalyzes the conversion of (+)-catechin to cyanidin and a novel procyanidin. *FEBS Letters, 580*(6), 1642-1648.

Wu, G., Johnson, S. K., Bornman, J. F., Bennett, S. J., Singh, V., Simic, A., & Fang, Z. (2016). Effects of genotype and growth temperature on the contents of tannin, phytate and in vitro iron availability of sorghum grains. *PLoS One, 11*(2).

Ye, M., Yang, W. Z., Liu, K. D., Qiao, X., Li, B. J., Cheng, J., Feng, J., Guo, D. A., & Zhao, Y. Y. (2012). Characterization of flavonoids in *Millettia nitida* var. *hirsutissima* by HPLC/DAD/ESI-MS^n. *Journal of Pharmaceutical Analysis, 2*(1), 35-42.

List of Figure Captions:

Fig.1. HPLC-ESI-MS base peak chromatograms (BPC) of the three hydromethanolic extracts of (A) brown sorghum whole grains, (B) red sorghum whole grains, and (C) white sorghum whole grains.

Fig.2. MS^n spectra of compounds **46** and **54**, and their proposed principal fragmentation pathways.

Fig.3. MS^n spectra of compound **25** and the proposed principal fragmentation pathways.

Fig.4. Selective MS^2[431] scan chromatogram and MS^n spectra of (a) compounds **30**, (b) compound **37**, (c) compound **43** and (d) compound **62**, and proposed fragmentation pathways for compound **62**.

Fig.5. MS^n spectra of compounds **22** and **33**, and proposed principal cleavage pathways for these two compounds.

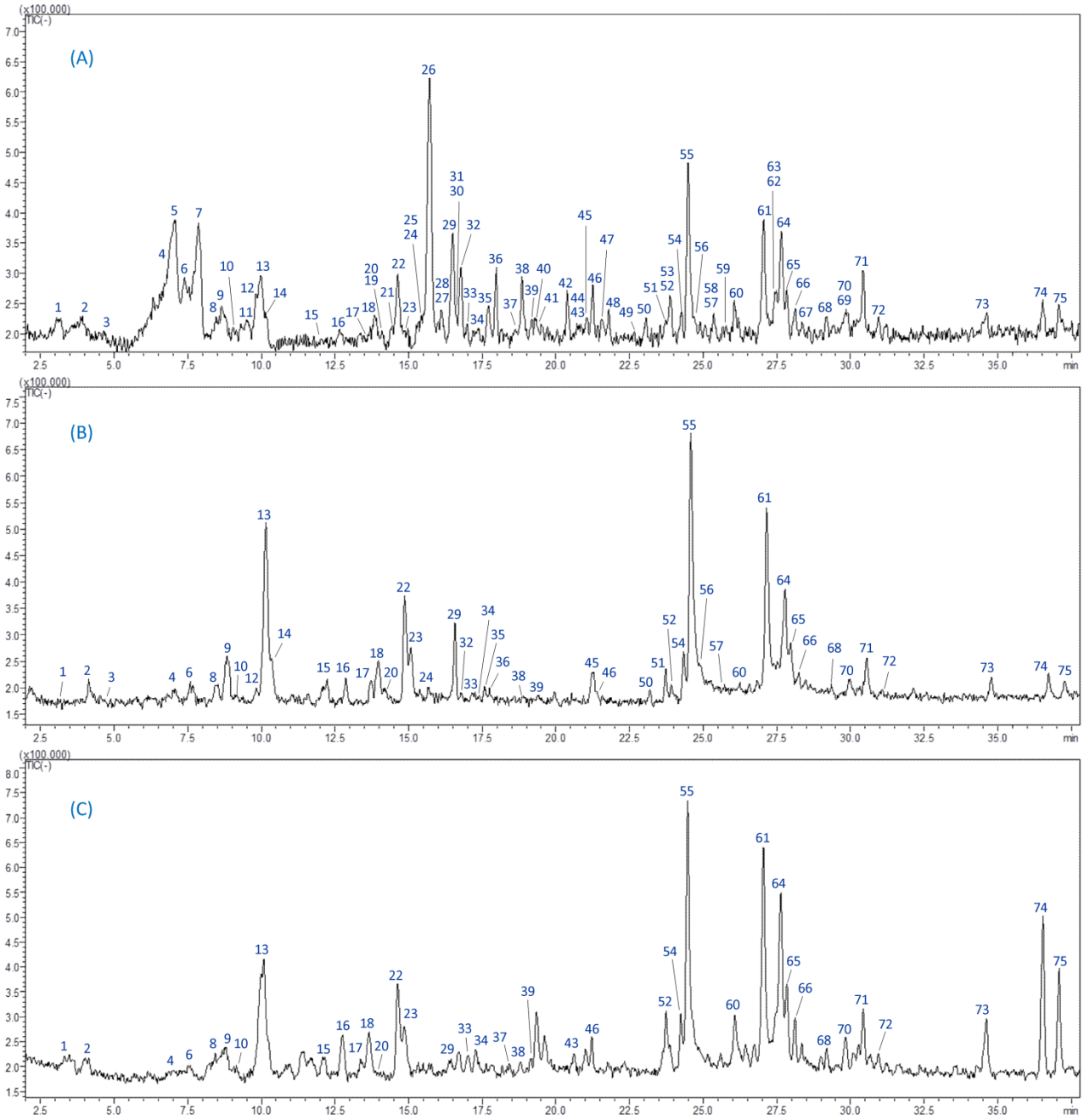


Figure 1

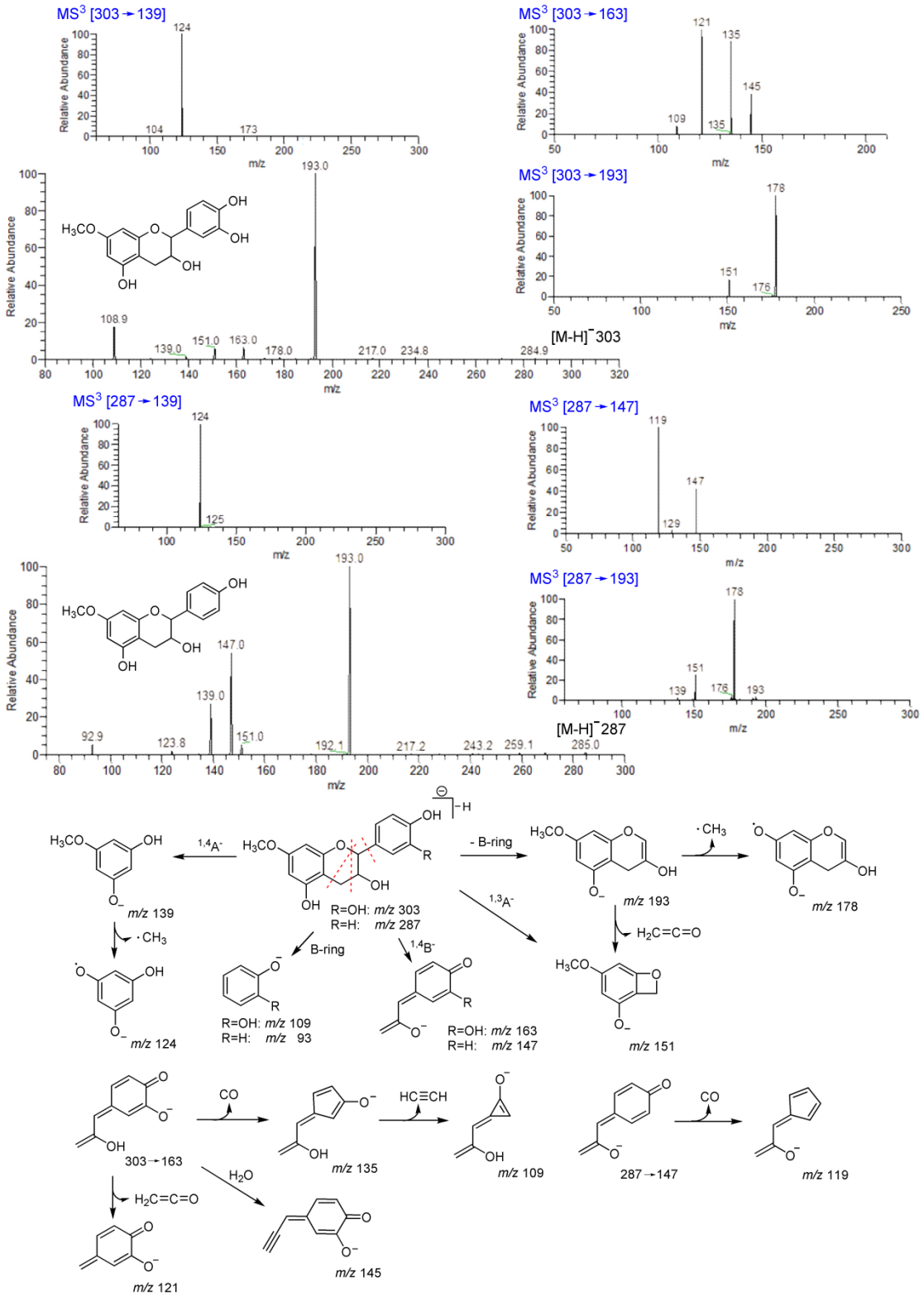

MS3 [303→139]
MS3 [303→163]
MS3 [303→193]
[M-H]¯303
MS3 [287→139]
MS3 [287→147]
MS3 [287→193]
[M-H]¯287
Relative Abundance
m/z
1,4A-
- B-ring
1,3A-
B-ring
1,4B-
R=OH: m/z 303
R=H: m/z 287
m/z 139
m/z 193
m/z 178
m/z 124
R=OH: m/z 109
R=H: m/z 93
R=OH: m/z 163
R=H: m/z 147
m/z 151
303→163
m/z 135
m/z 109
287→147
m/z 119
m/z 121
m/z 145
CO
HC≡CH
H2O
H2C=C=O
·CH3

Figure 2

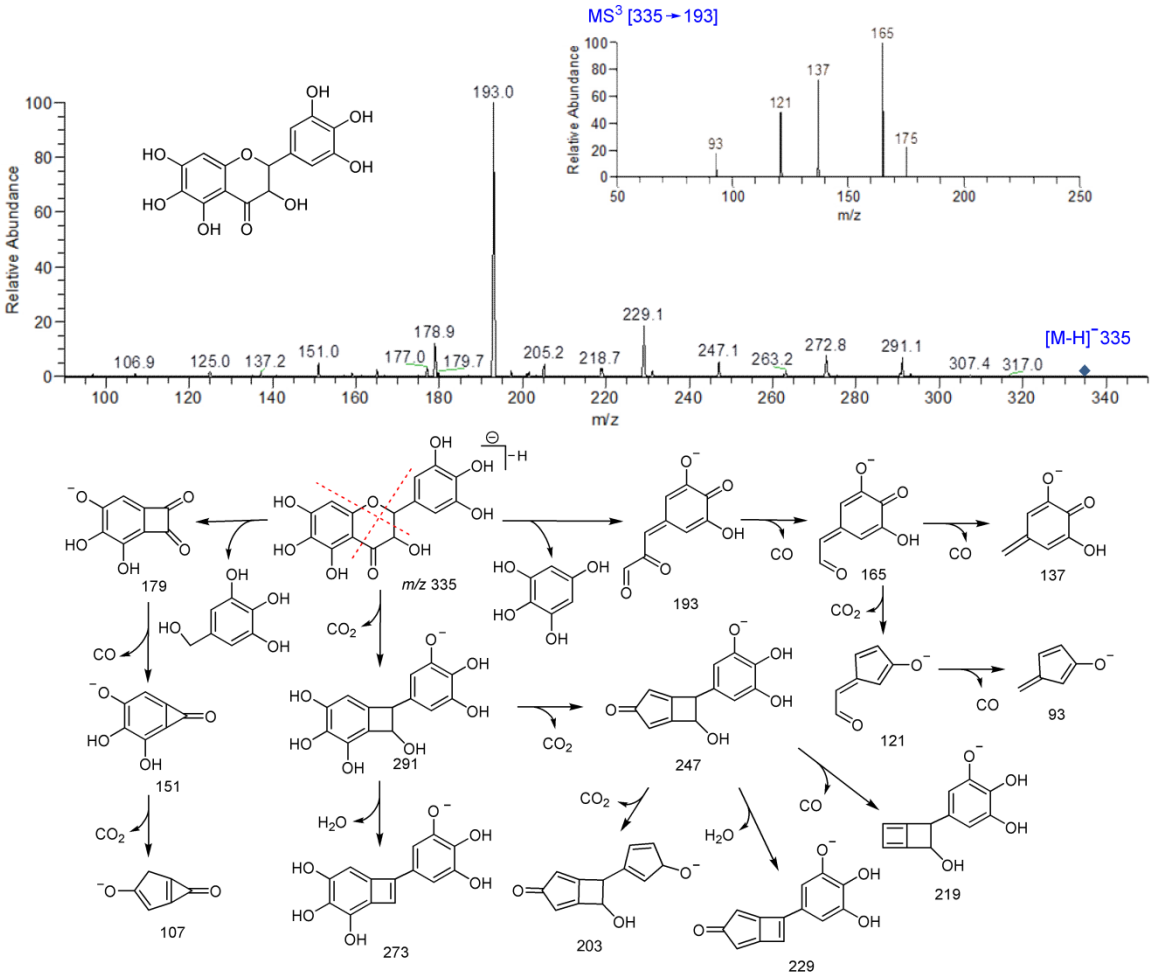

MS3 [335→193]
Relative Abundance
m/z
193.0
178.9
229.1
[M-H]- 335
m/z 335
179
193
165
137
151
291
247
121
93
107
273
203
229
219
CO
CO2
H2O

figure 3

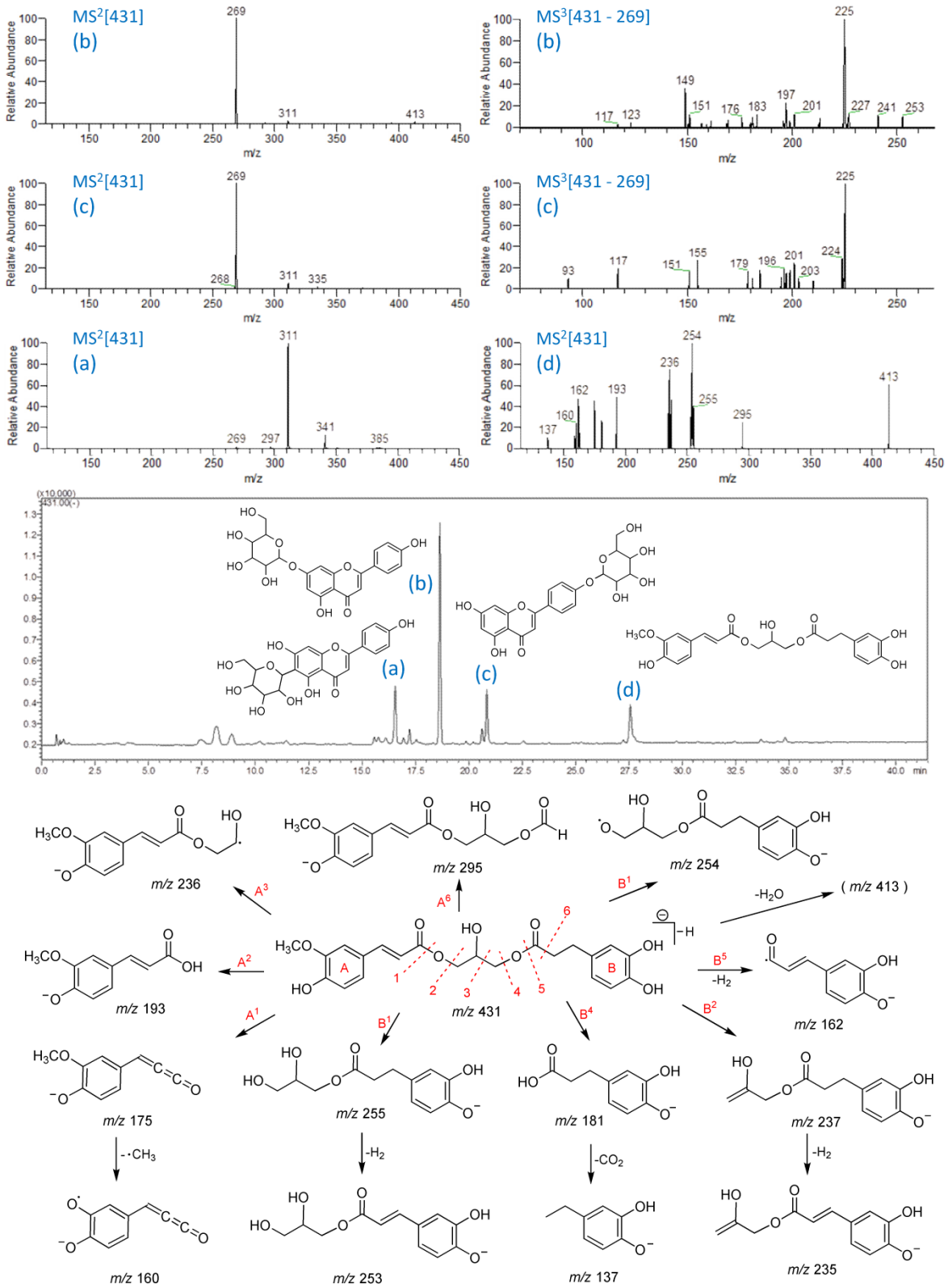
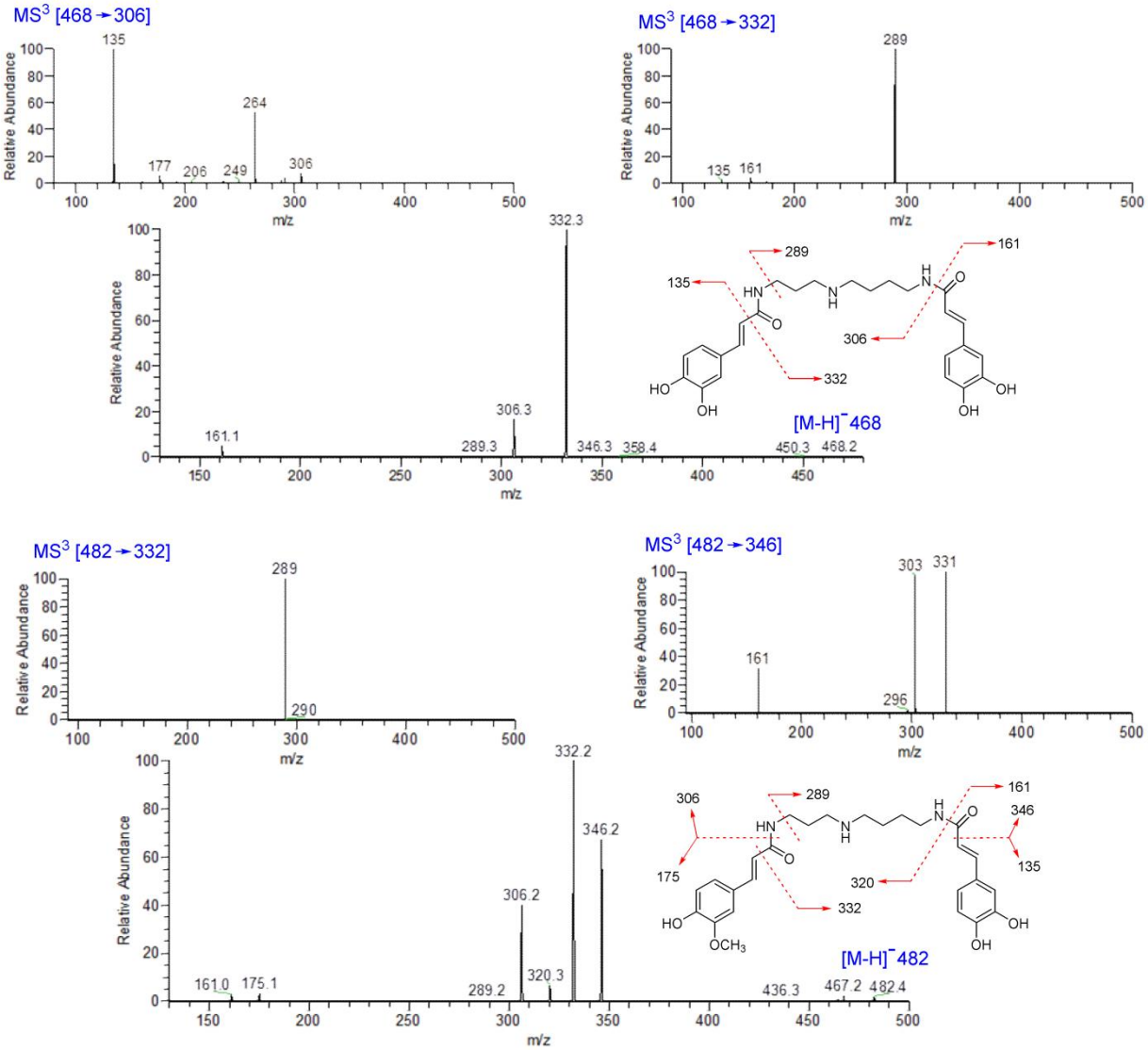

MS²[431]
(b)
MS³[431 - 269]
(b)
MS²[431]
(c)
MS³[431 - 269]
(c)
MS²[431]
(a)
MS²[431]
(d)
Relative Abundance
m/z
(a)
(b)
(c)
(d)
min
m/z 431
m/z 295
m/z 254
m/z 236
m/z 193
m/z 175
m/z 160
m/z 255
m/z 253
m/z 181
m/z 137
m/z 162
m/z 237
m/z 235
(m/z 413)
-H2O
-H2
-CO2
-CH3
-H

Figure 4



496
497 **Figure 5**