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*Supplementary Material*

# Allelopathic Potential of Mangroves from the Red River Estuary against the Rice Weed *Echinochloa crus-galli* and Variation in Their Leaf Metabolome

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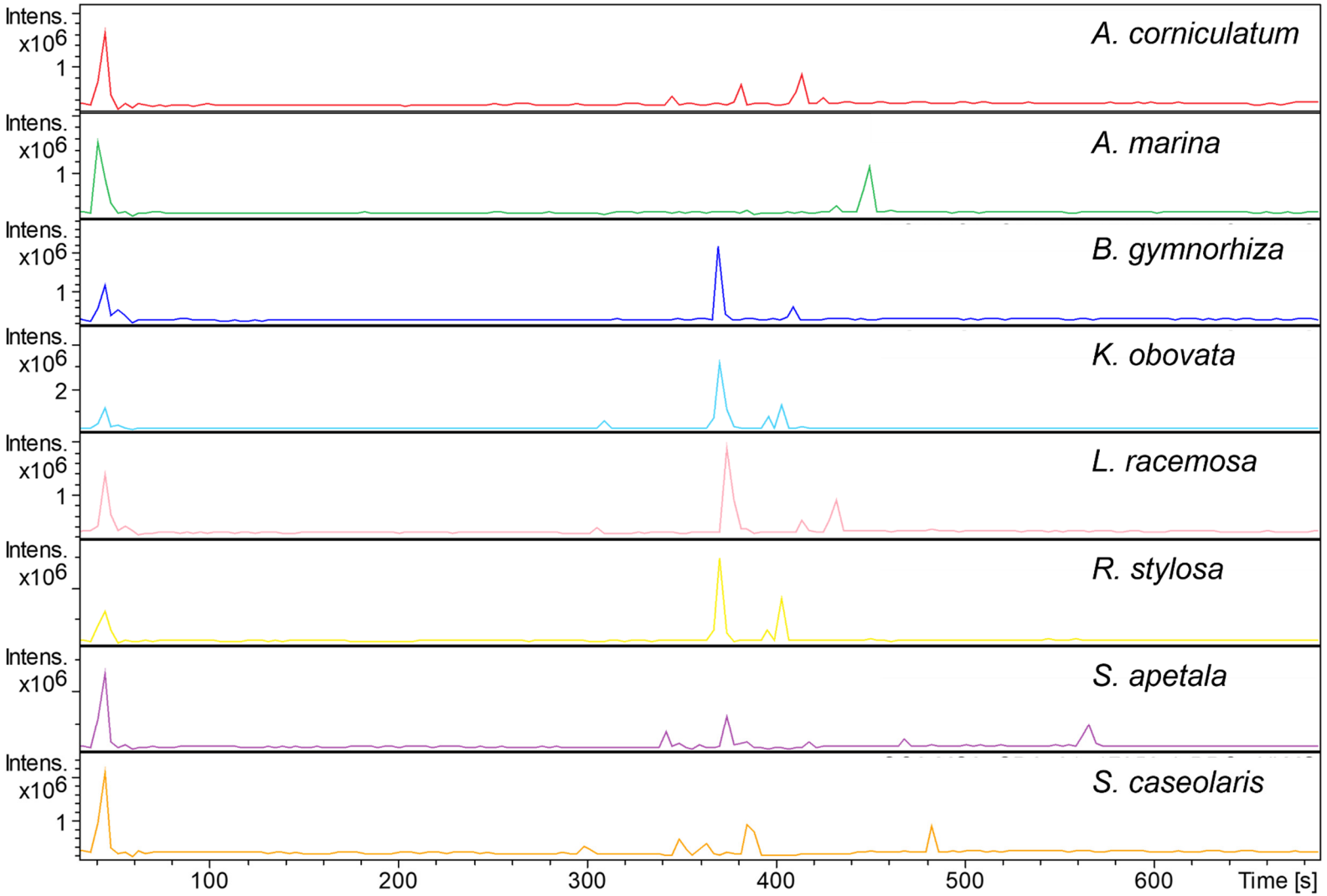


**Figure S1.** UHPLC-(-)ESI-qToF Base Peak Chromatograms (BPC) of leaf methanolic extracts from Aegiceras corniculatum, Avicennia marina, Bruguiera gymnorhiza, Kandelia obovata, Lumnitzera racemosa, Rhizophora stylosa, Sonneratia apetala and S. caseolaris.

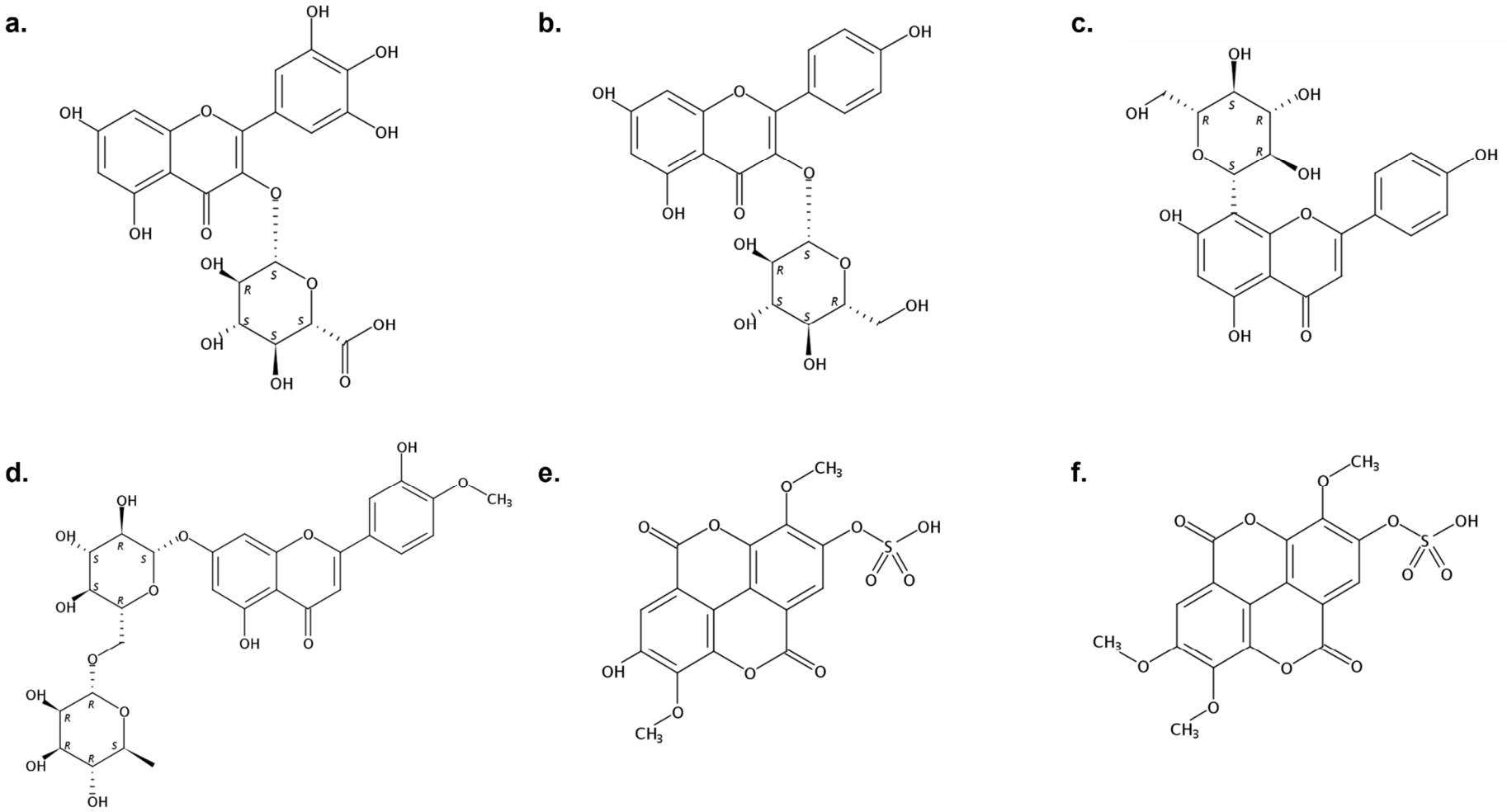


**Figure S2.** Chemical structures of some of the putatively annotated compounds: *Aegiceras corniculatum* compounds **Ac M1** (a, myricetin-3-glucuronide) and **Ac M3** (b, astragalin), and *Sonneratia apetala* compound **Sa M3** (c, vitexin), biomarker **M607T418 Sa M4 (d,** diosmin), compound **Sa M5** (e, dimethyl ellagic acid sulfate) and biomarker **M481T560 Sa M6** (f, 3,3′,4′-trimethyl ellagic acid 4-sulfate). All compounds and biomarkers were putatively annotated according to their HRMS/MS spectra.

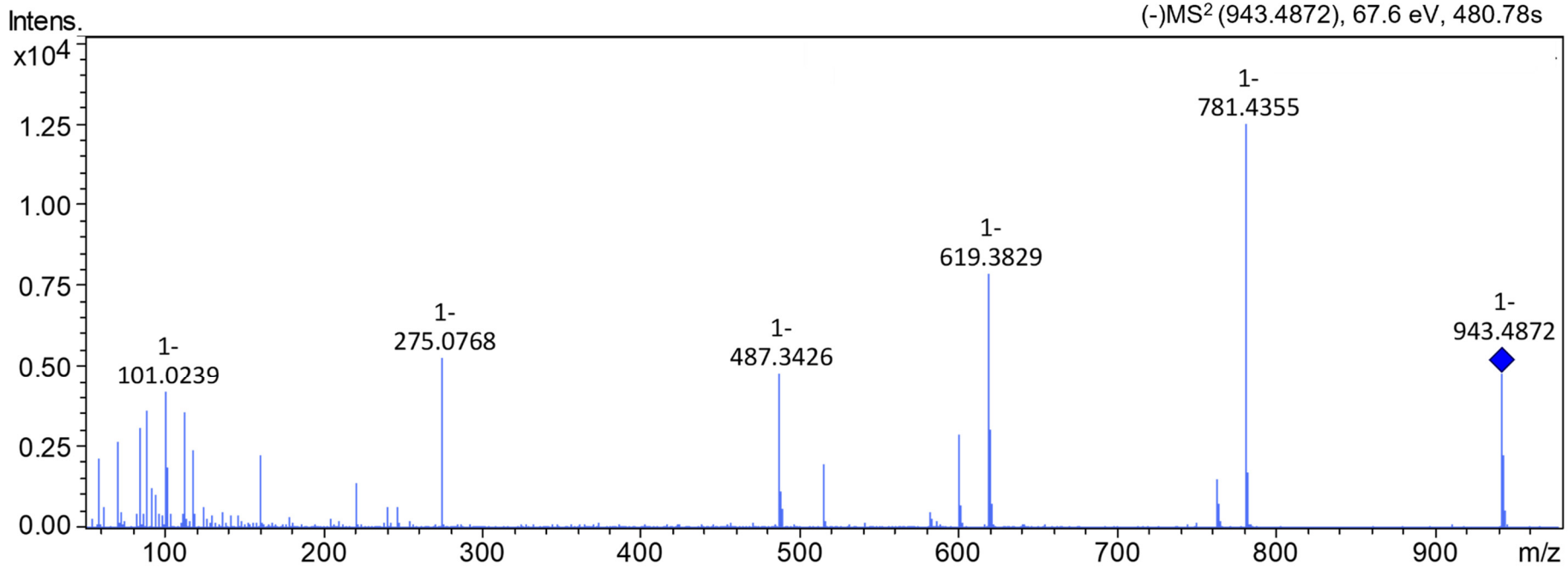


**Figure S3.** HRMS/MS spectrum of *Aegiceras corniculatum* biomarker M943T476 putatively annotated as (3β, 16α, 20α)-3,16,28-trihydroxyolean-12-en-29-oic acid 3-{O-β-D-glucopyranosyl (1→2)-O-[β-D-glucopyranosyl (1→4)]-α-L-arabinopyranoside}.

### *Detailed annotation*

Biomarker M943T476 gave a $[M-H]^-$ at *m*/*z* 943.4872 and allowed to deduce the molecular formula $C_{47}H_{76}O_{19}$. MS/MS spectrum (Figure S3) was almost superposable to that of M781T518 (Figure S5) with the same hexose and pentose unit losses and same deduced aglycone fragment (putative triterpenic acid, $C_{30}H_{47}O_5$), except for an additional hexose loss. Based on MS spectra comparison in literature, this compound could be assigned as (3β,16α,20α)-3,16,28-trihydroxyolean-12-en-29-oic acid 3-{O-β-D-glucopyranosyl-(1→2)-O-[ -D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside}, a saponin already described in leaves of *A. corniculatum* collected in Vietnam [41].

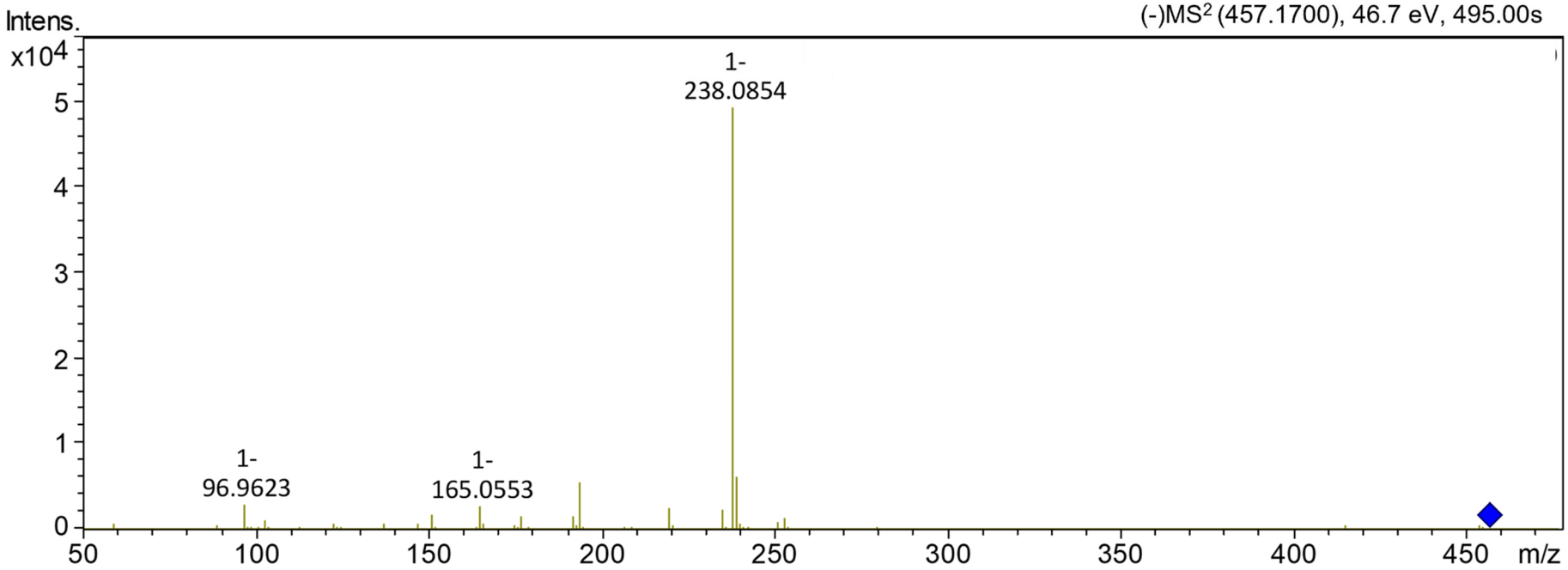


**Figure S4.** HRMS/MS spectrum of *Aegiceras corniculatum* unknown biomarker M457T495.

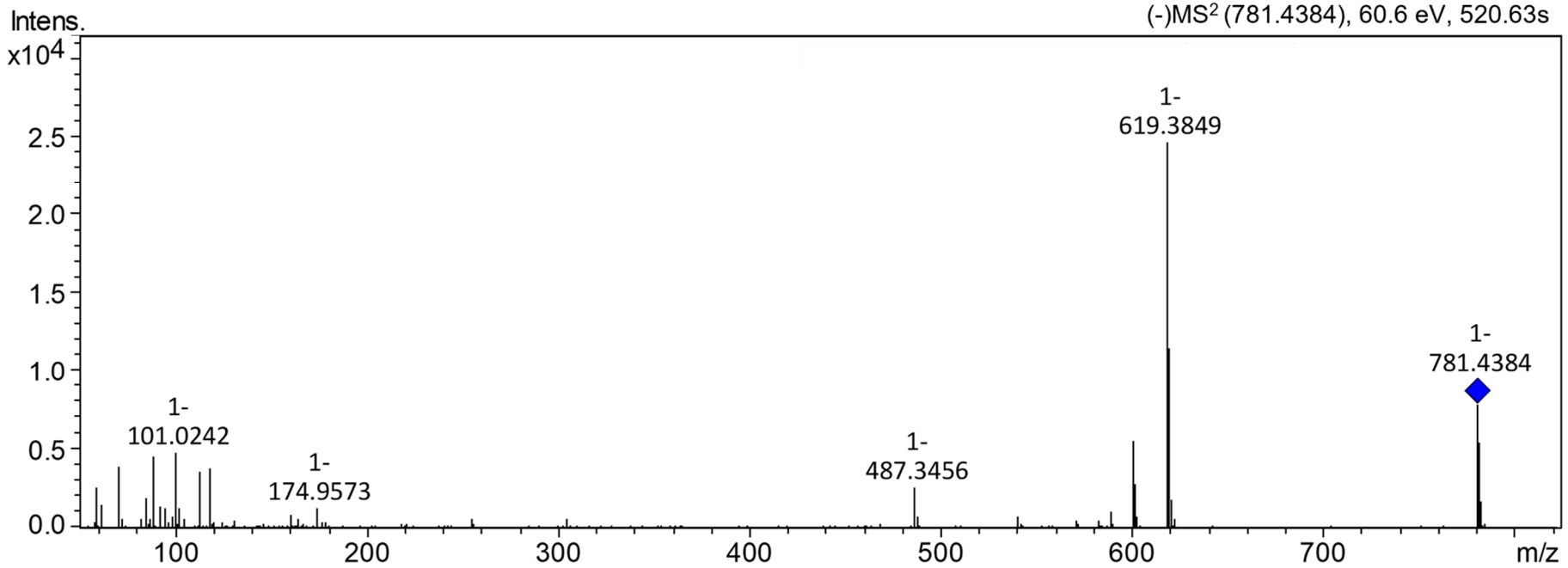


**Figure S5.** HRMS/MS spectrum of *Aegiceras corniculatum* biomarker M781T518 putatively annotated as a triterpenoid saponin.

### *Detailed annotation*

Biomarker M781T518 specific to *A. corniculatum* leaves produced a deprotonated ion $[M-H]^-$ at *m/z* 781.4384 along with a chloride $[M+Cl]^-$ and a sodium formate $[M+HCOONa-H]^-$ adducts. MS/MS spectrum (Figure S5) showed the successive losses of a hexose $[M-H-162]^-$ and a pentose moiety $[M-H-162-132]^-$. The corresponding aglycone at *m/z* 487.3456 ($C_{30}H_{47}O_5$) could correspond to a triterpenic acid according to database interrogation. This compound was therefore putatively annotated as a triterpenoid saponin derivative.

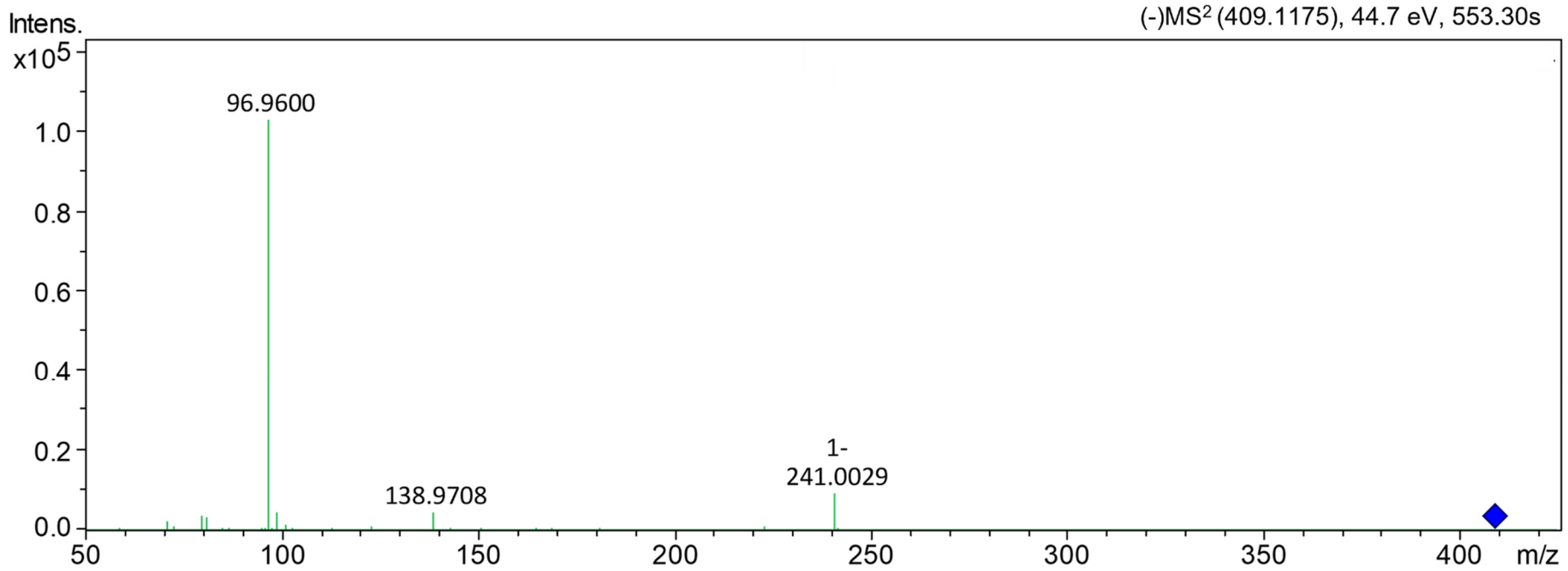


**Figure S6.** HRMS/MS spectrum of *Aegiceras corniculatum* biomarker **M410T546** putatively annotated as a **monoterpene sulfate.**

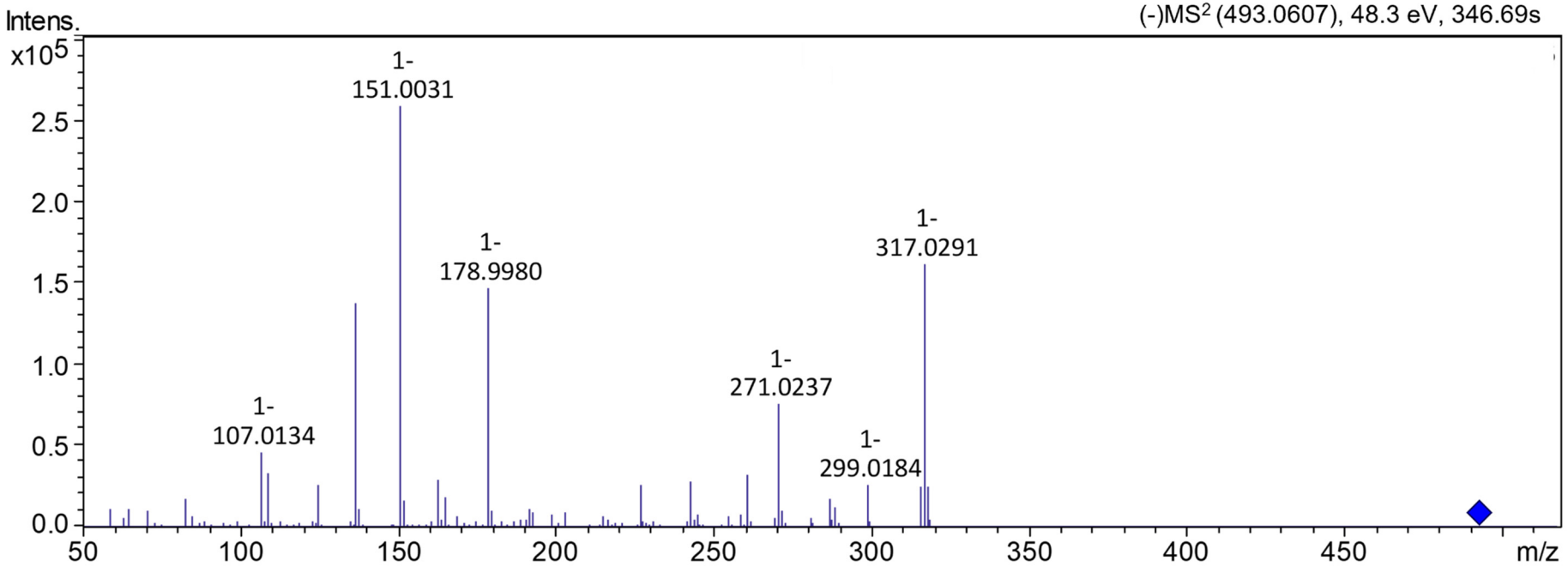


**Figure S7.** HRMS/MS spectrum of *Aegiceras corniculatum* major BPC compound **Ac M1** putatively annotated as **myricetin-3-glucuronide.**

### *Detailed annotation*

BPC major compound Ac M1 presented a pseudo molecular ion $[M-H]^-$ at *m/z* 493.0607 corresponding to a raw formula of $C_{21}H_{17}O_{14}$. MS/MS fragment ion at *m/z* 317.0291 highlighted the loss of a glucuronic acid unit $[M-H-176]^-$ indicating a possible glucoronid flavonoid. Fragments at *m/z* 178.9980 and 151.0031 suggested the aglycone moiety as myricetin [43]. With a common fragmentation (*m/z* 317.0291 and 271.0237) described by De Rosso et al, 2015 [52], this compound might be myricetin-3-glucoronid (Figure S2a), found in leaves of mangrove *Conocarpus erectus* [42].

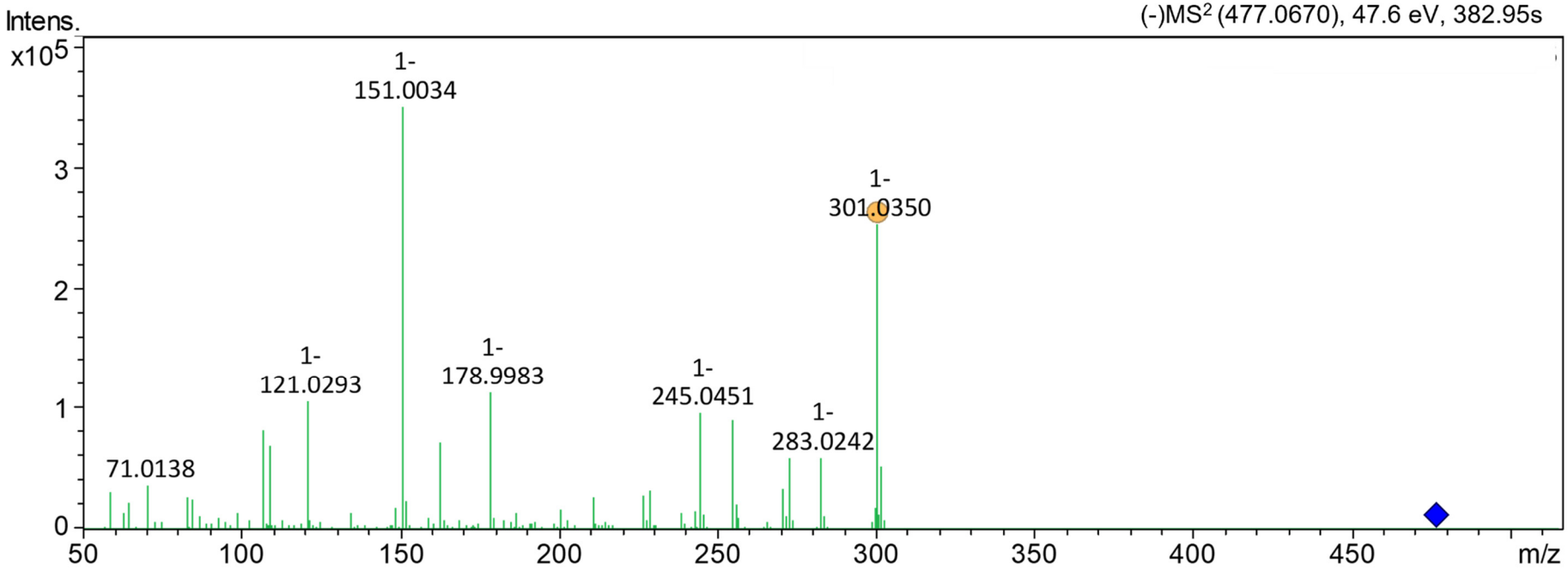


**Figure S8.** HRMS/MS spectrum of *Aegiceras corniculatum* major BPC compound **Ac M2** putatively annotated as **quercetin glucuronide** .

***Detailed annotation***

BPC major compound Ac M2 gave a $[M-H]^-$ at *m*/*z* 477.067 and showed a loss of glucuronic acid moiety as well with a fragment ion at *m*/*z* 301.0350 corresponding to the aglycone unit ($C_{15}H_9O_7$). According to literature, this metabolite could be a quercetin glucuronide already described in *Laguncularia racemosa* [43].

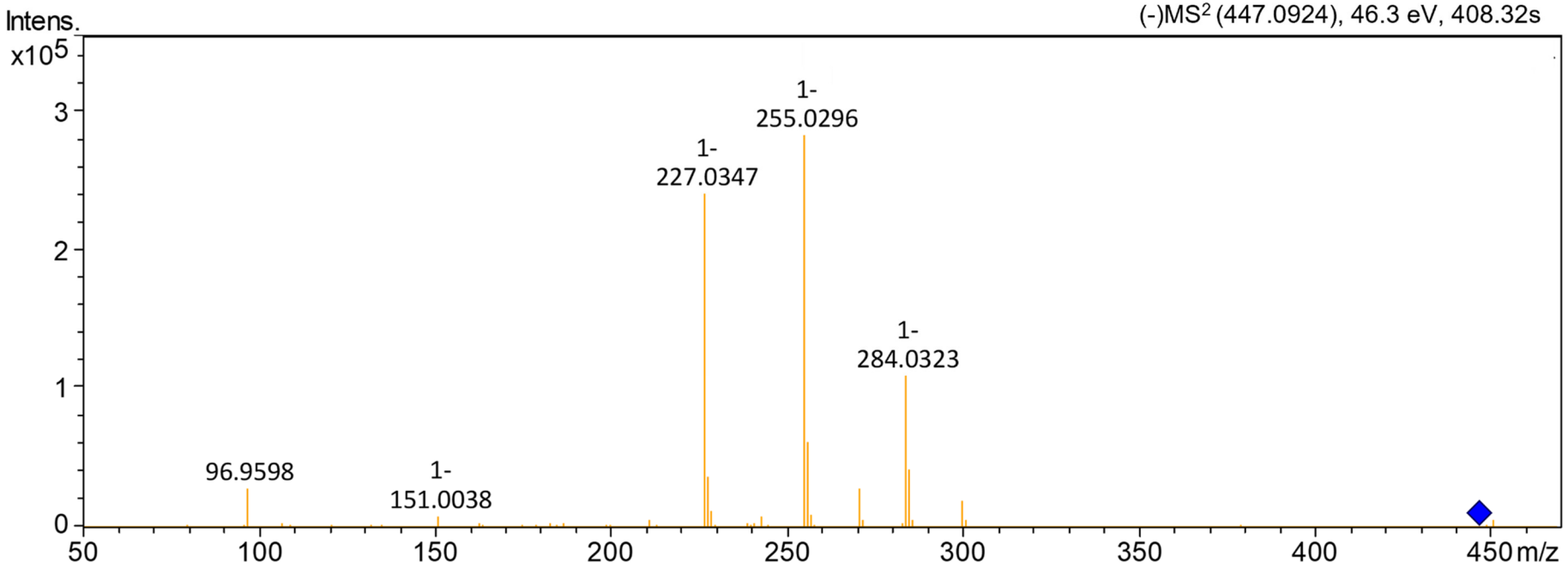


**Figure S9.** HRMS/MS spectrum of *Aegiceras corniculatum* major BPC compound Ac M3 putatively annotated as kaempferol-3-O-glucoside (astragalin)*. *Constructor statistical match factor (*m/z* defect and comparison of experimental vs theoretical isotopic patterns)

### *Detailed annotation*

BPC major compound Ac M3 showed a pseudo molecular ion at *m/z* 447.0924. MS/MS spectrum matched with constructor database (Bruker db) compound kaempferol-3-glucoside (Figure S2b) also known as astragalin (common fragment ions *m/z* 284.0323, 255.0296 and 227.0347). Vinh et al, 2019 reported this metabolite in *A. corniculatum* leaves, supporting our assumptions [41].

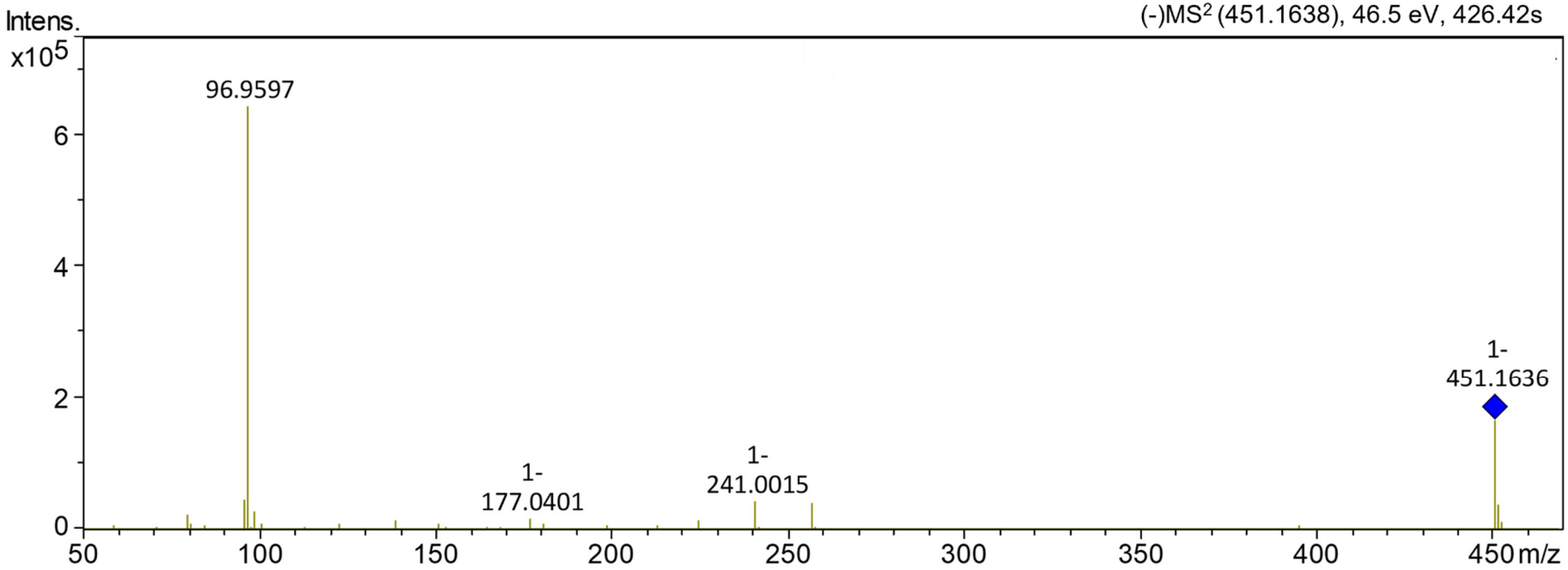


**Figure S10.** HRMS/MS spectrum of *Aegiceras corniculatum* **unknown** major BPC compound **Ac M4.**

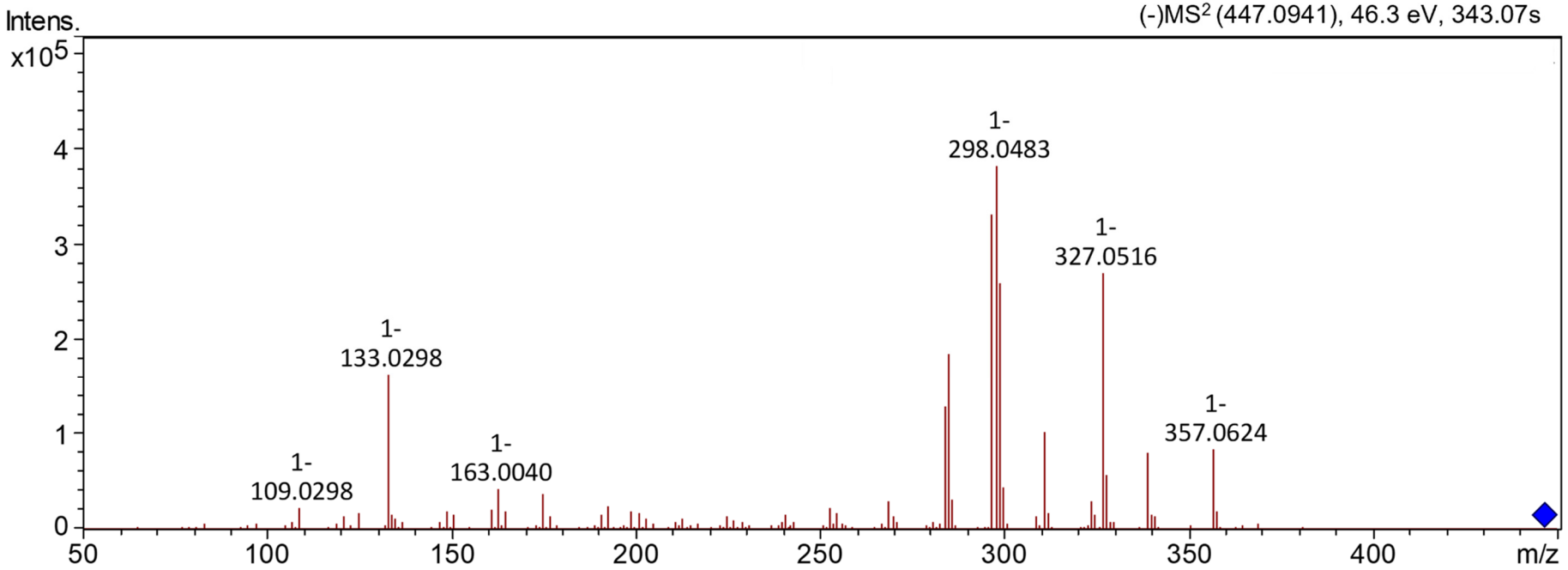


**Figure S11.** HRMS/MS spectrum of *Sonneratia apetala* major BPC compound **Sa M1** (biomarker **M895T343**) putatively annotated as **luteolin-7-O-β-glucoside.**

### *Detailed annotation*

Biomarker M895T343 (also compound Sa M1) showed a deprotonated molecular ion $[M-H]^-$ at *m/z* 447.0941 consistent with a molecular formula of $C_{21}H_{20}O_{11}$. Precursor ion fragmentation led to the loss of a hexose moiety producing a deprotonated aglycone ion at *m/z* 285.0405 with a raw formula of $C_{15}H_9O_6$, which could correspond to luteolin or kaempferol. Characteristic fragment ions (*m/z* 199.0407, 175.0405, 151.0039 and 133.0298) observed in MS/MS spectrum [46] and luteolin already described in *S. apetala* fruits [47] allowed to suggest a luteolin glucoside. Although specifically more abundant in *S. apetala*, biomarker M895T343 was also detected in *S. caseolaris* and *B. gymnorhiza* extracts (Main text Figure 1b). For these additional reasons, this compound could be a luteolin 7-O-β-glucoside already described in *S. caseolaris* leaves and fruits [48,49].

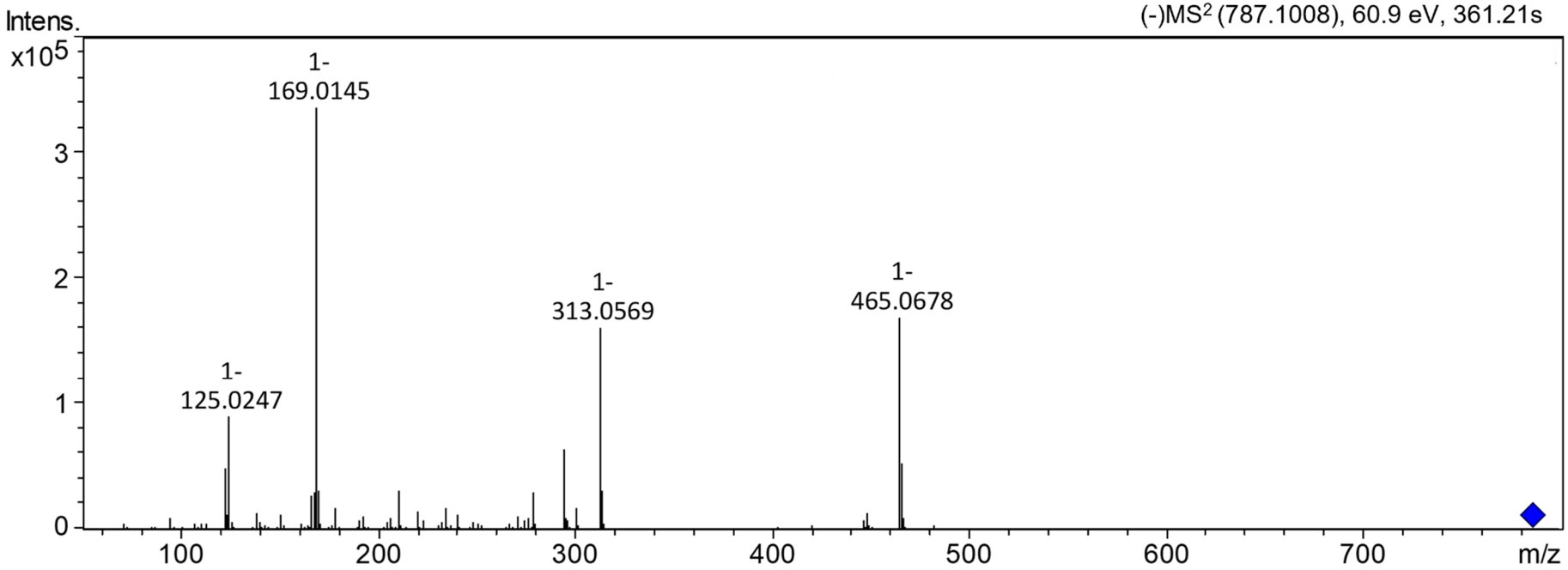


**Figure S12.** HRMS/MS spectrum of *Sonneratia apetala* major BPC compound **Sa M2** putatively annotated as **tetragalloyl glucose.**

### *Detailed annotation*

Compound Sa M2 gave a deprotonated molecular ion $[M-H]^-$ at *m/z* 787.1008 which gave a raw formula of $C_{34}H_{27}O_{22}$. Online database interrogation led to matches with gallic acid derivatives. Characteristic fragment ions of tetragalloyl glucose [54] were detected at *m/z* 635.0915, 483.0789, 465.0678, 313.0569, 295.0463 and 169.0145 and showed successive galloyl moiety losses [M-H-152] [53]. This compound might be a 1,3,4,6-tetra-O-galloyl-β-D-glucose previously reported in the mangrove species *Excoecaria agallocha* [51].

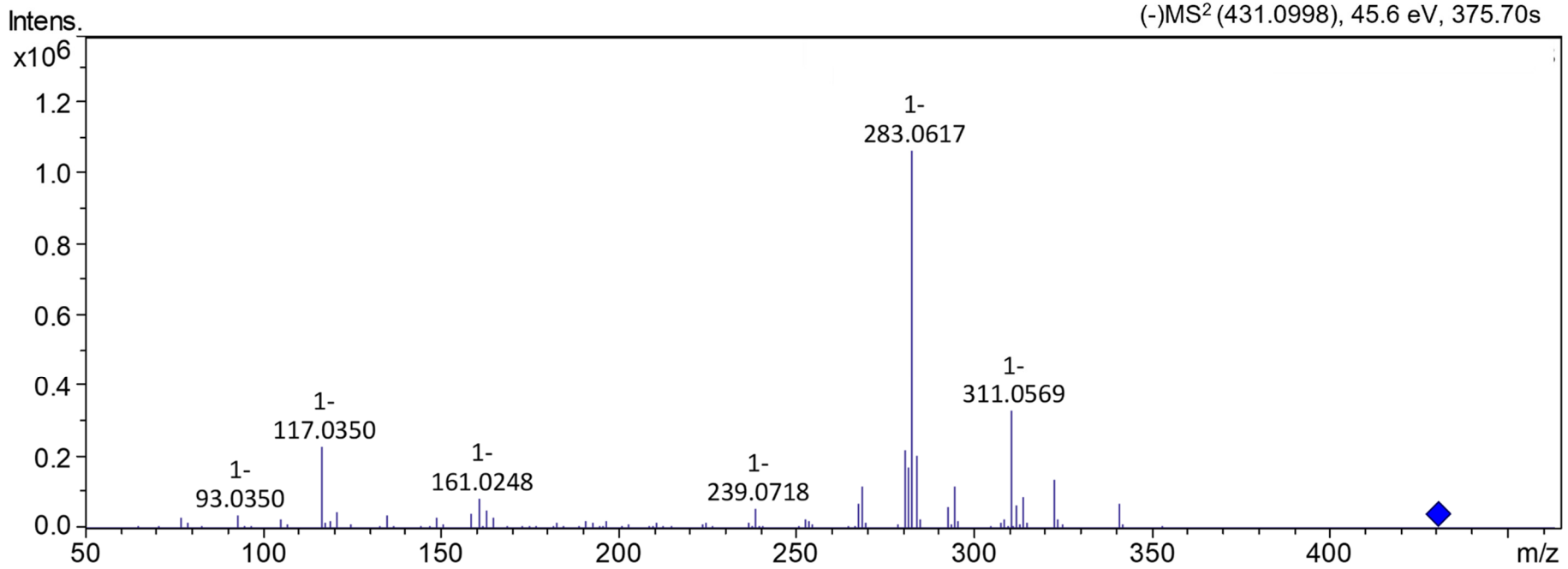


**Figure S13.** HRMS/MS spectrum of *Sonneratia apetala* major BPC compound **Sa M3** putatively annotated as **vitexin*.** * Constructor statistical match factor (*m/z* defect and comparison of experimental vs theoretical isotopic patterns)

### *Detailed annotation*

Compound Sa M3 produced a deprotonated molecular ion at *m/z* 431.0998 allowing to deduce a molecular formula of $C_{21}H_{20}O_{10}$. MS/MS spectrum matched with those of vitexin (Figure S2c), a flavone glycoside, in the constructor database. Fragmentation showed two major product ions at *m/z* 311.0569 $[M-H-120]^-$ and 283.0617 common to the reference standard from database. This compound was already described in *S. apetala* leaves and branches collected in China [50].

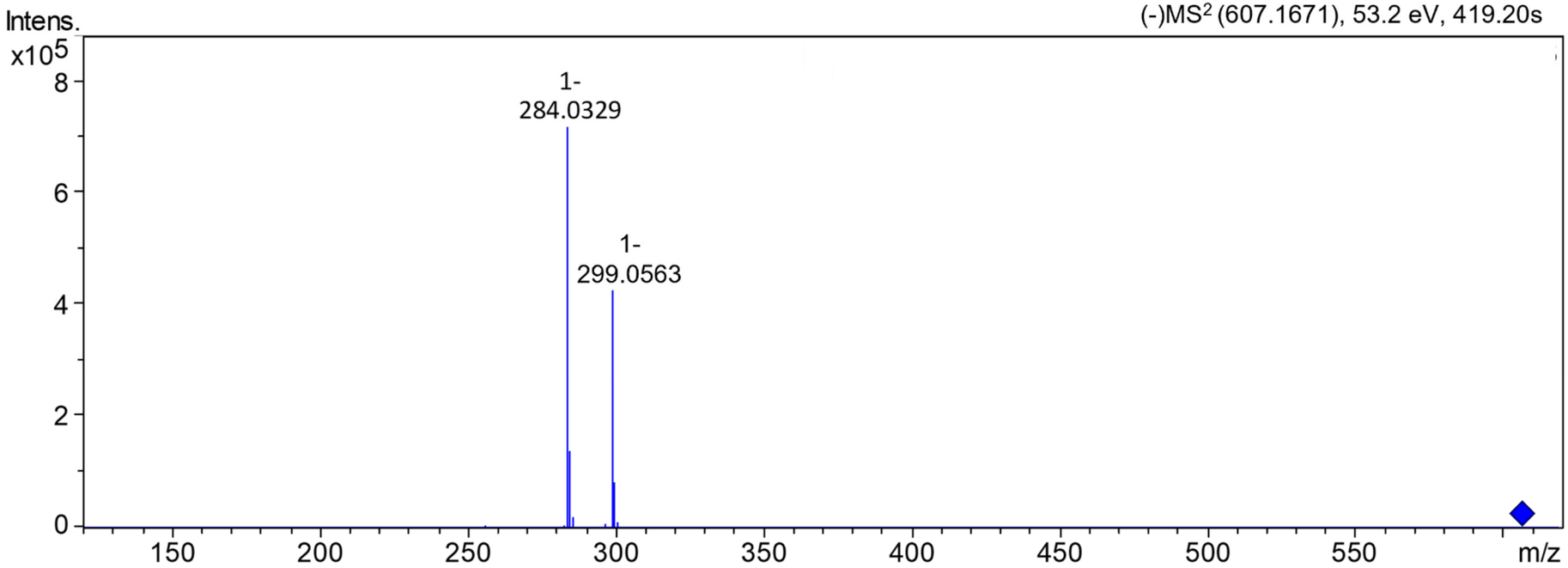


**Figure S14.** HRMS/MS spectrum of *Sonneratia apetala* major BPC compound **Sa M4** (biomarker **M607T418**) putatively annotated as **diosmin***.* Confirmed with commercial standards (in-house MS/MS spectra database)

### *Detailed annotation*

Compound M607T418 (also compound Sa M4) gave a deprotonated molecular ion $[M-H]^-$ at *m/z* 607.1671 corresponding to the raw formula $C_{28}H_{32}O_{15}$. Interrogation of in-house database led to a match of MS/MS spectrum with diosmin (Figure S2d), a disaccharide derivative consisting of diosmetin substituted by a rutinose at position 7 via a glycosidic linkage. This biomarker broke down into two major product ions at *m/z* 299.0563 and 284.0329, confirming the characteristic loss of rutinose followed by a loss of a methyl. Finally, this assumption was further reinforced by the presence of diosmetin reported in two species from *Sonneratia* genus: *S. paracaseolaris* aerial parts [44] and *S. alba* leaves [45].

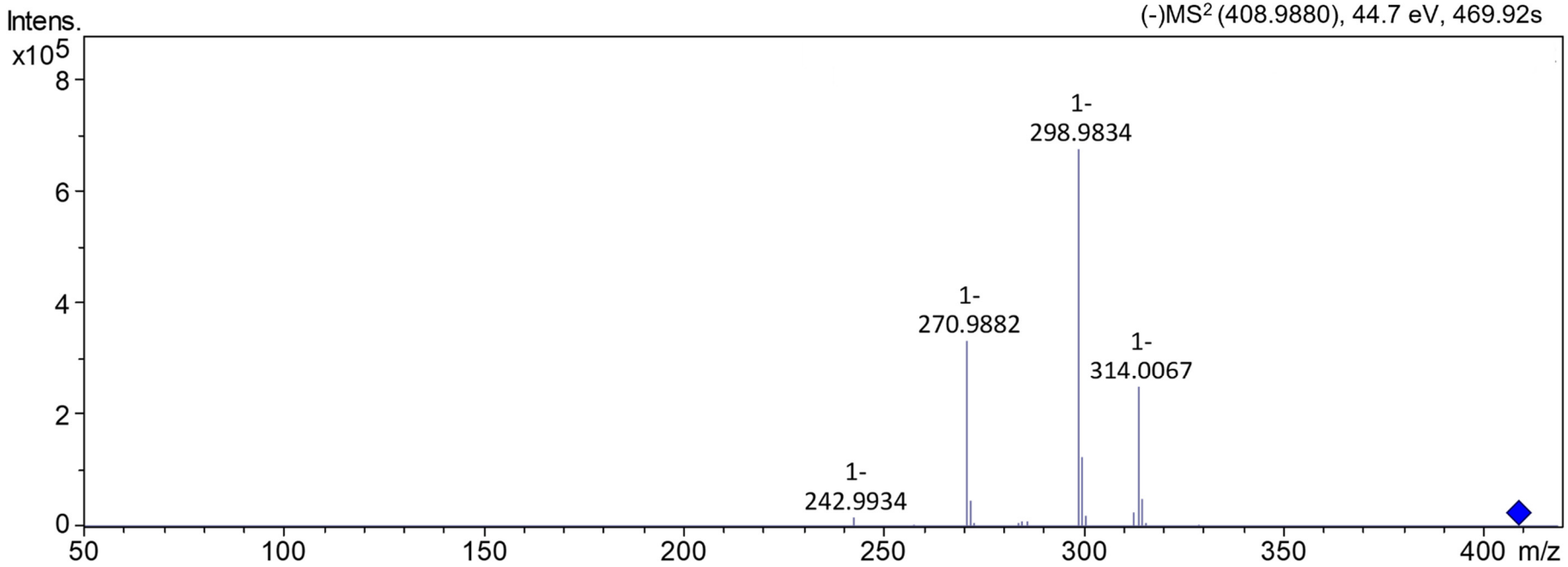


**Figure S15.** HRMS/MS spectrum of *Sonneratia apetala* major BPC compound **Sa M5** putatively annotated as **dimethyl ellagic acid sulfate.**

### *Detailed annotation*

According to Manurung et al. 2021 [40], compound Sa M5 could be assigned to a dimethyl-ellagic acid sulfate (Figure S2e) since it followed the same fragmentation pattern as Sa M6 (Figure S16) (loss of a sulfate and methyl moieties) with a produced deprotonated molecular ion $[M-H]^-$ at *m*/*z* 408.9880 ($C_{16}H_{10}O_{11}S$).

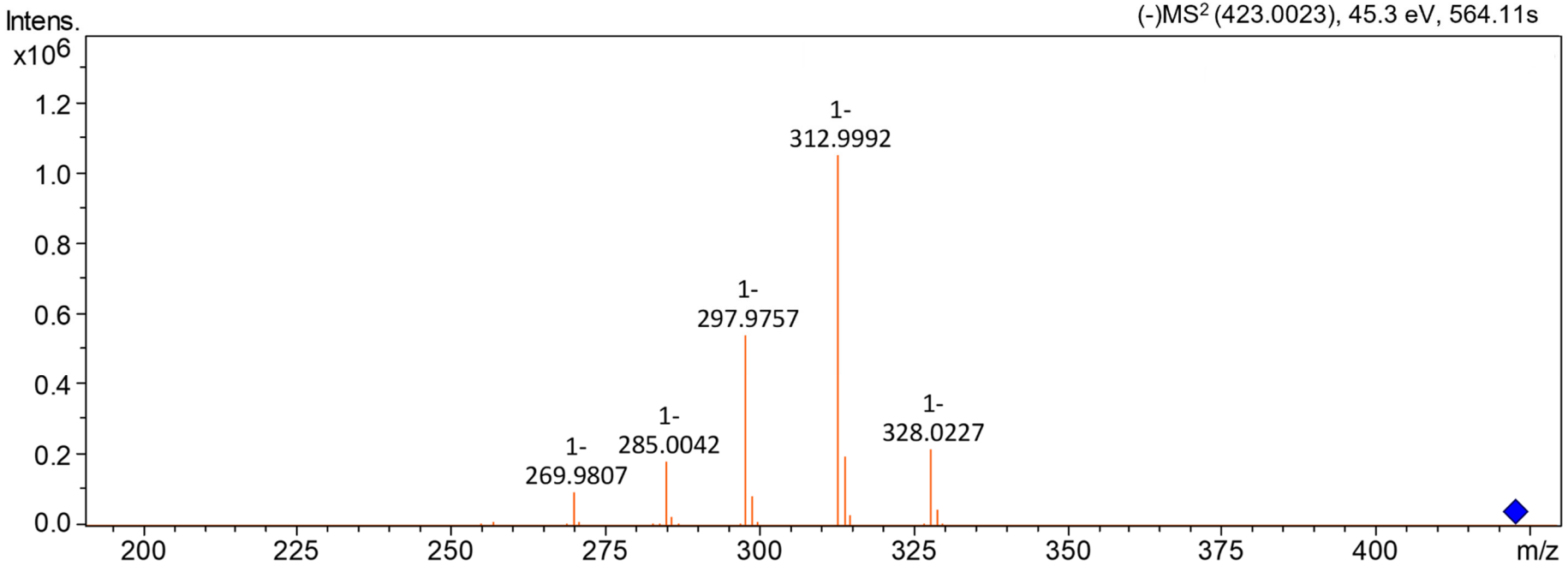


**Figure S16.** HRMS/MS spectrum of *Sonneratia apetala* major BPC compound **Sa M6** (biomarker **M481T560**) putatively annotated as **3,3′,4′-trimethylellagic acid 4-sulfate.**

## *Detailed annotation*

Biomarker M481T560 (also BPC major compound Sa M6) of *S. apetala* leaves presented a deprotonated molecular ion $[M-H]^-$ at *m*/*z* 423.0023 with an isotopic pattern indicating the possible presence of a sulfur atom. The presence of a $[M-H-SO_3]^-$ fragment ion at *m*/*z* 343.0471 confirmed this assumption and suggested a sulfated metabolite with a raw formula of $C_{17}H_{12}SO_{11}$. Fragmentation pattern showed the successive loss of 3 methyl groups (*m*/*z* 328.0227, 312.9992, 297.9757). After comparison with literature, this compound was putatively annotated as 3,3',4′-tri-O-methyl-ellagic acid 4-sulfate (Figure S2f) which have recently been reported for the first time in mangrove species in the roots of *Lumnitzera littorea* and *L. racemosa* from Indonesia [40].

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