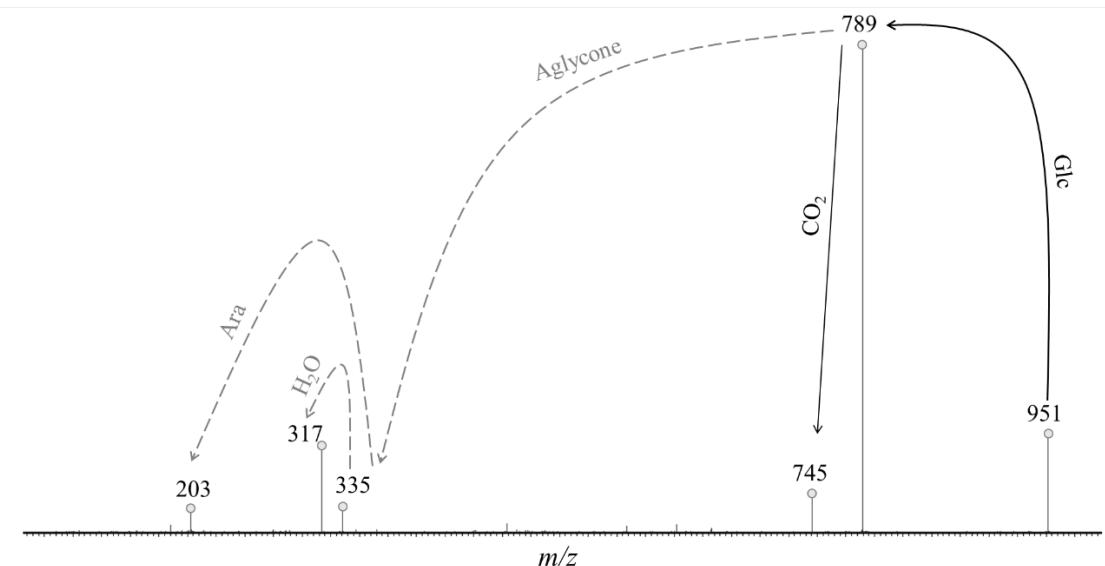


Part VIII. Appendix

1. Chenopodium quinoa

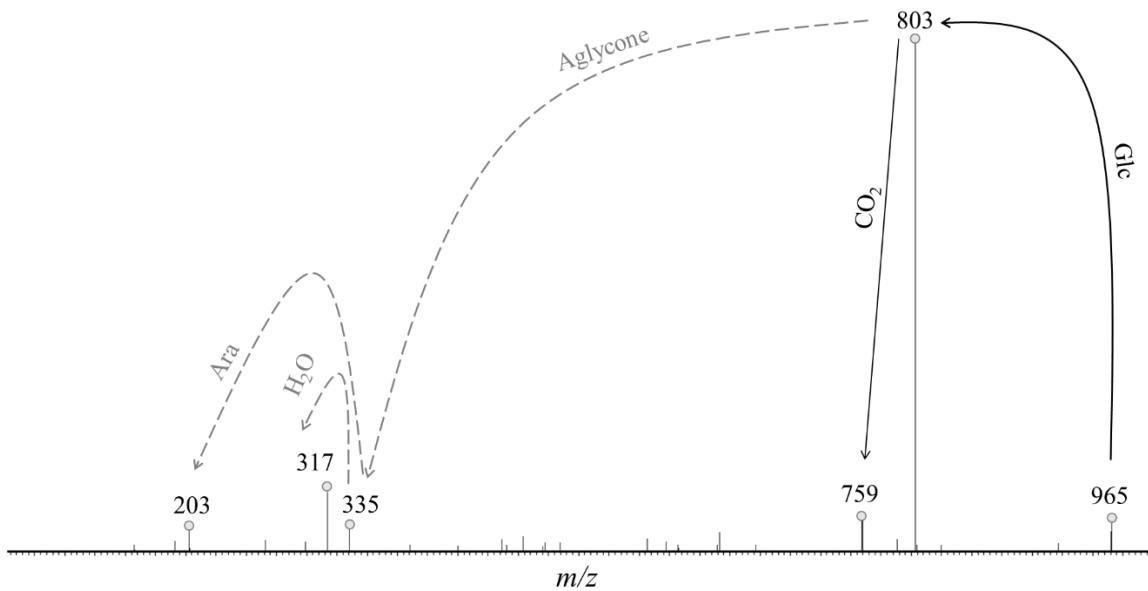
1.1. Mass spectrometry analyses

1.1.1. LC-MSMS analysis of original and modified saponins from *Aesculus hippocastanum*

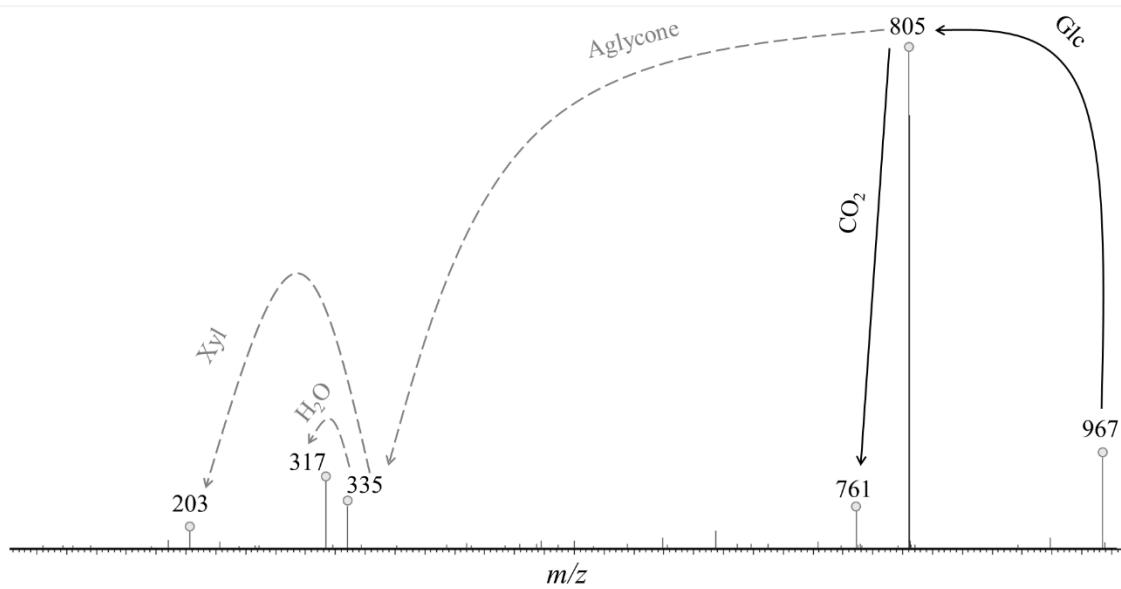


Appendix 1. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 951 precursor ions at 7.3 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin I.

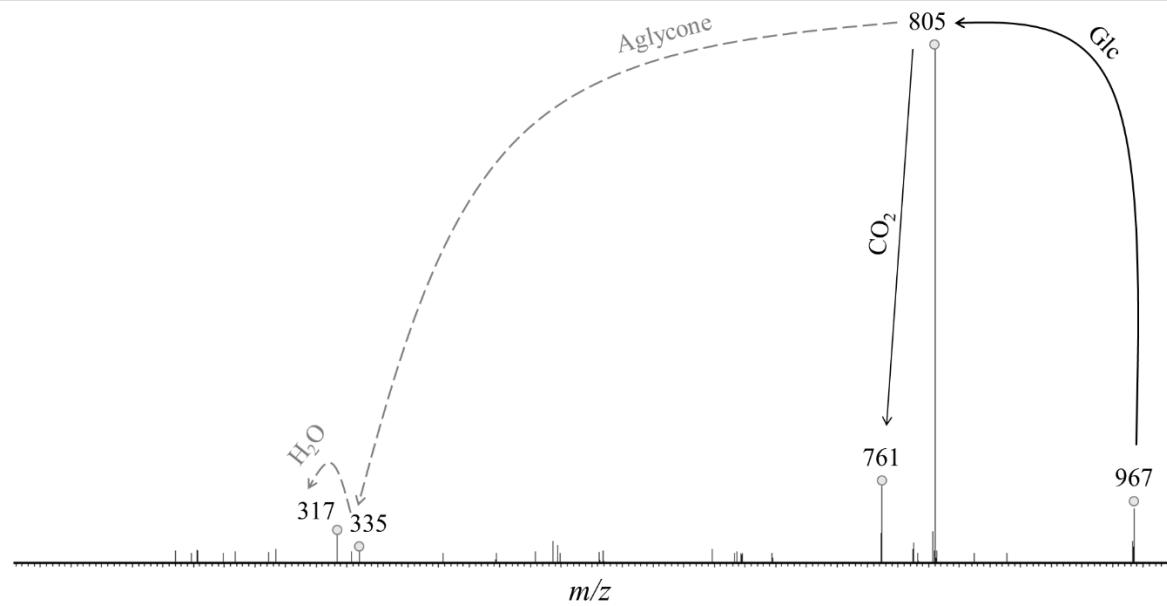
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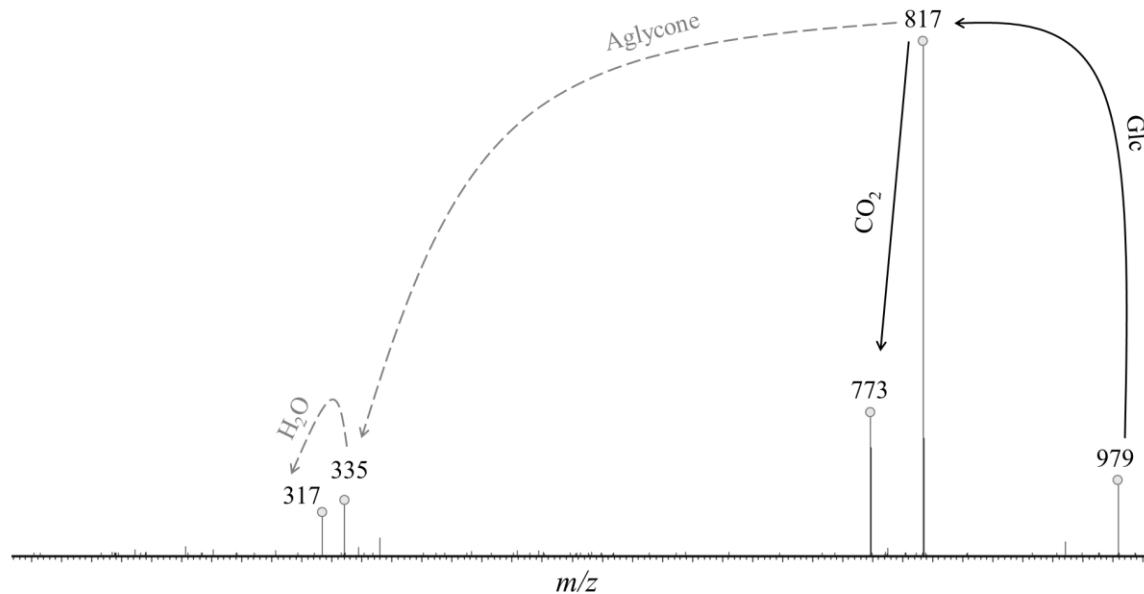
Appendix 2. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 965 precursor ions at 5.3 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin ?.



Appendix 3. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 967 precursor ions at 4 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin 19.

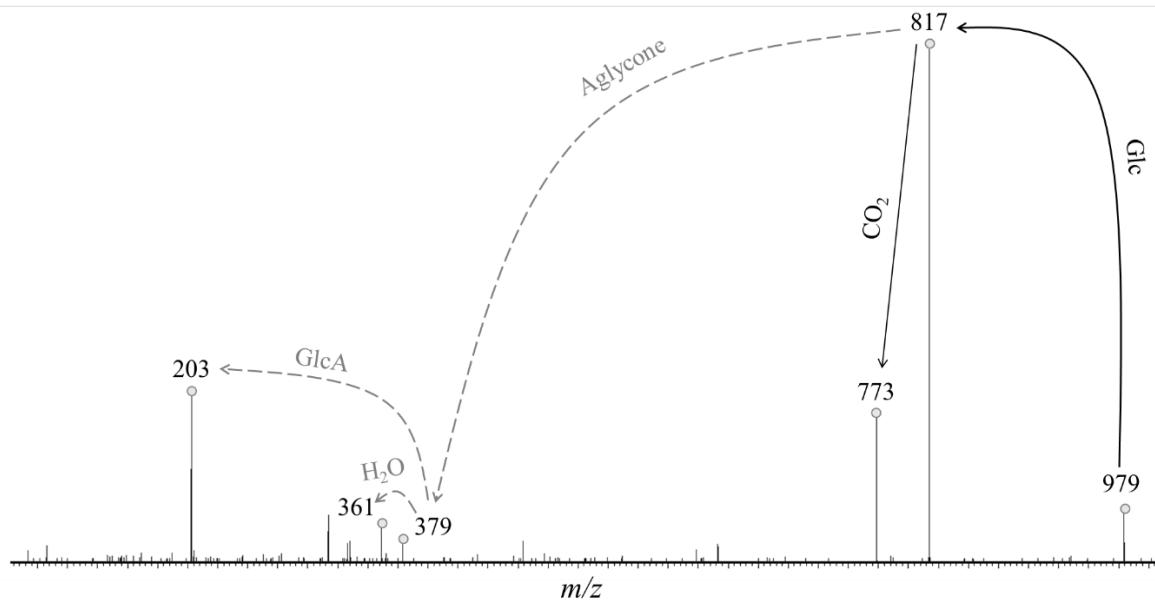


Appendix 4. LC-MSMS analysis of *Chenopodium quinoa* husk saponin extract: CID spectrum recorded for the m/z 967 precursor ions at 4.8 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin 19a.

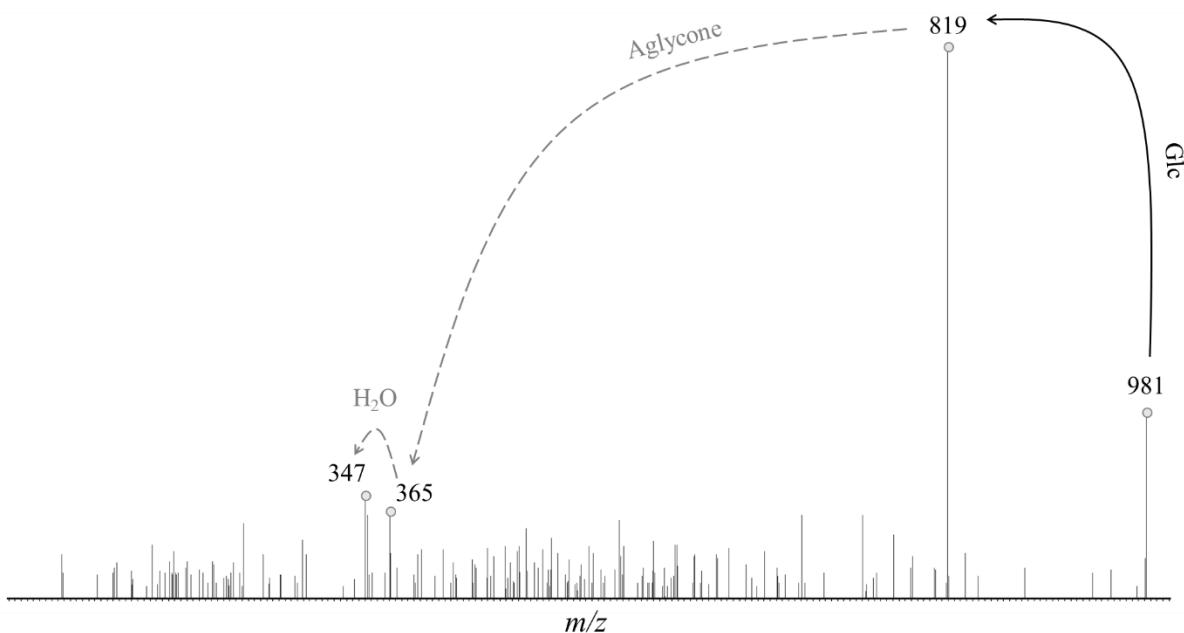


Appendix 5. LC-MSMS analysis of *Chenopodium quinoa* husk saponin extract: CID spectrum recorded for the m/z 979 precursor ions at 7.1 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin H.

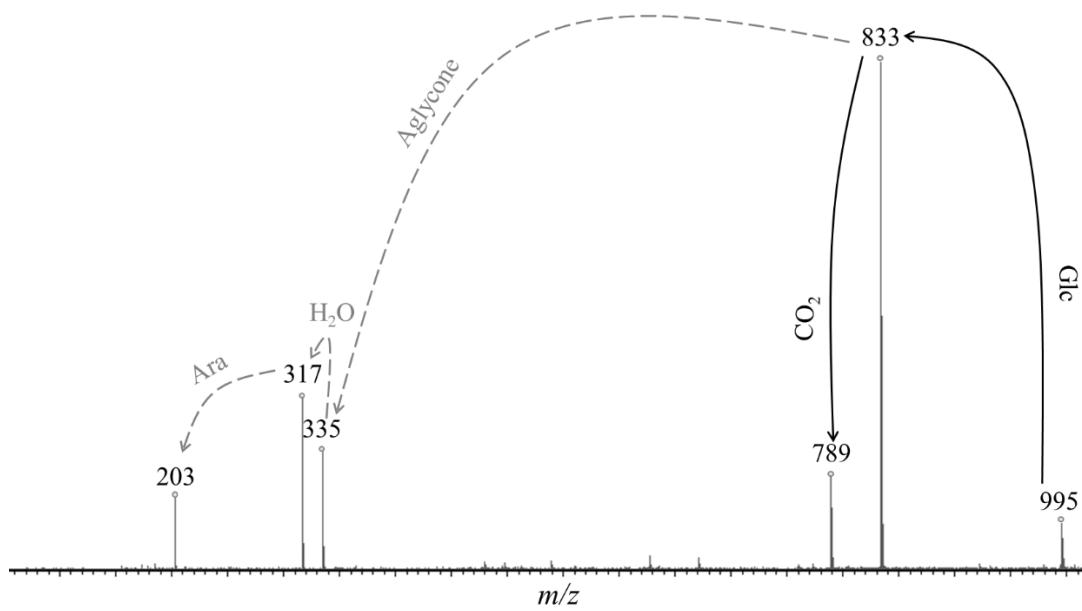
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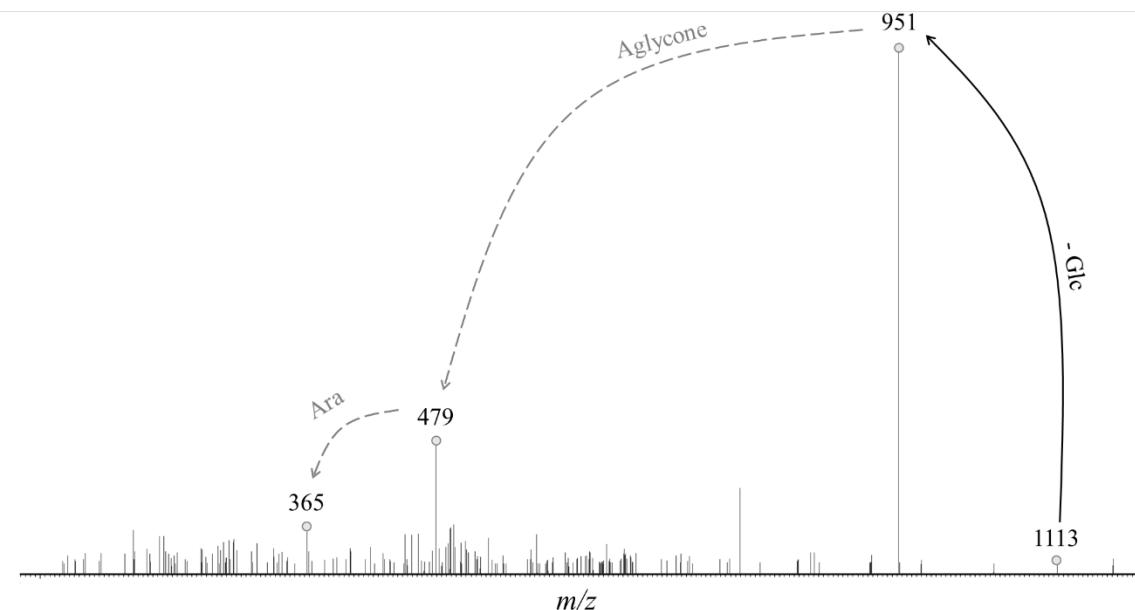
Appendix 6. LC-MSMS analysis of *Chenopodium quinoa* husk saponin extract: CID spectrum recorded for the m/z 979 precursor ions at 7.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin 70.



Appendix 7. LC-MSMS analysis of the *Chenopodium quinoa* husk saponin extract: CID spectrum recorded for the m/z 981 precursor ions at 6.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin Q.

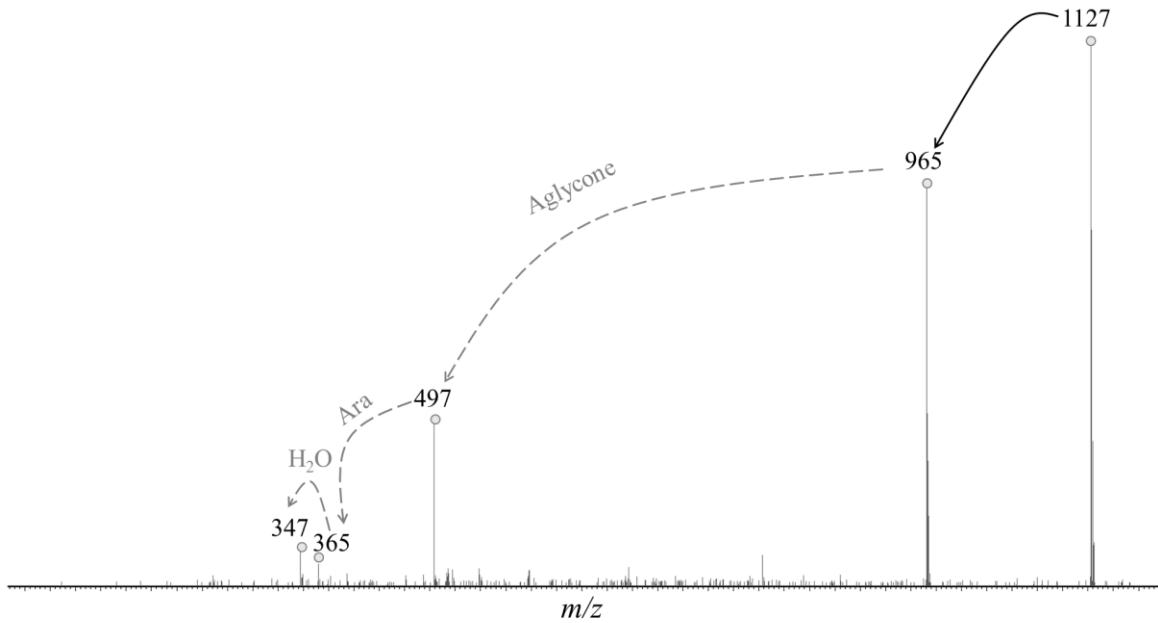


Appendix 8. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 995 precursor ions at 4.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin B.

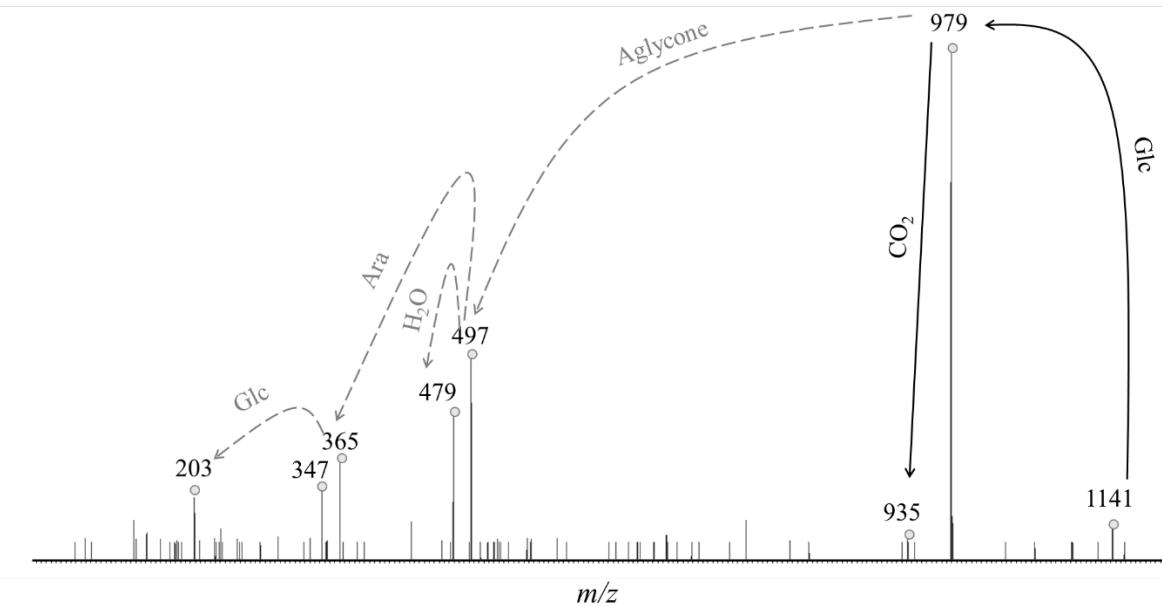


Appendix 9. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 1113 precursor ions at 5.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin 61.

Appendix

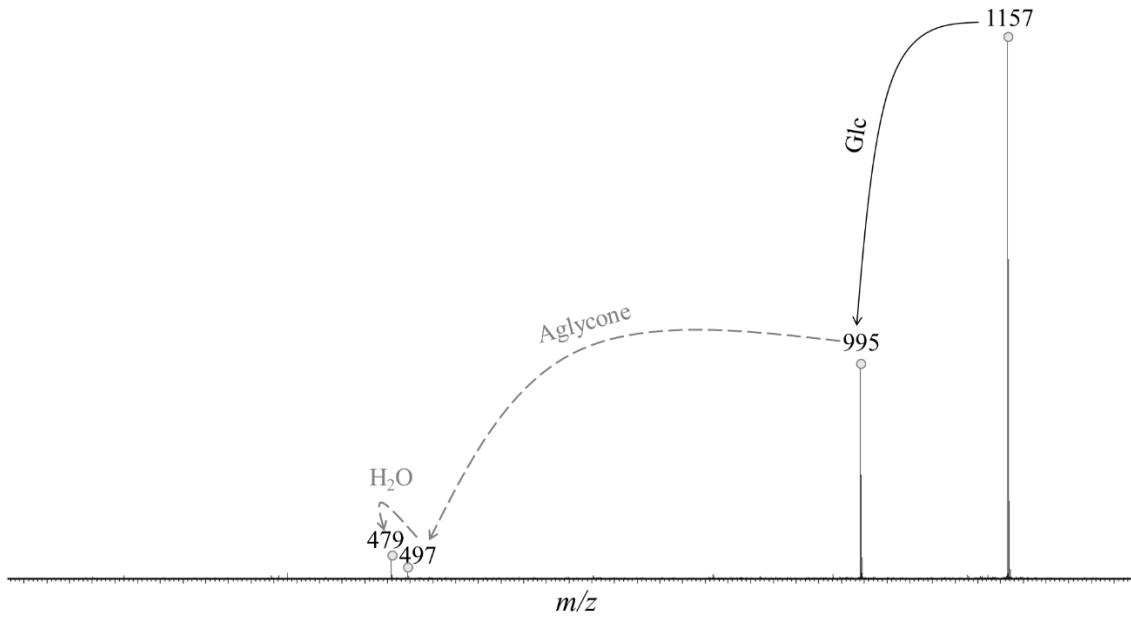


Appendix 10. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 1127 precursor ions at 4.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin ??.

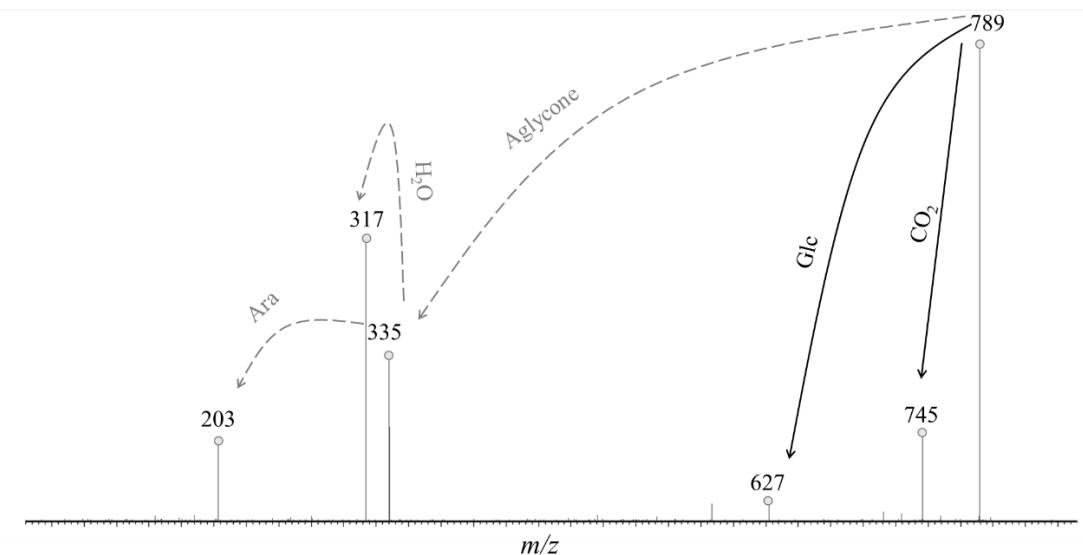


Appendix 11. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 1141 precursor ions at 5.7 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin G.

Appendix

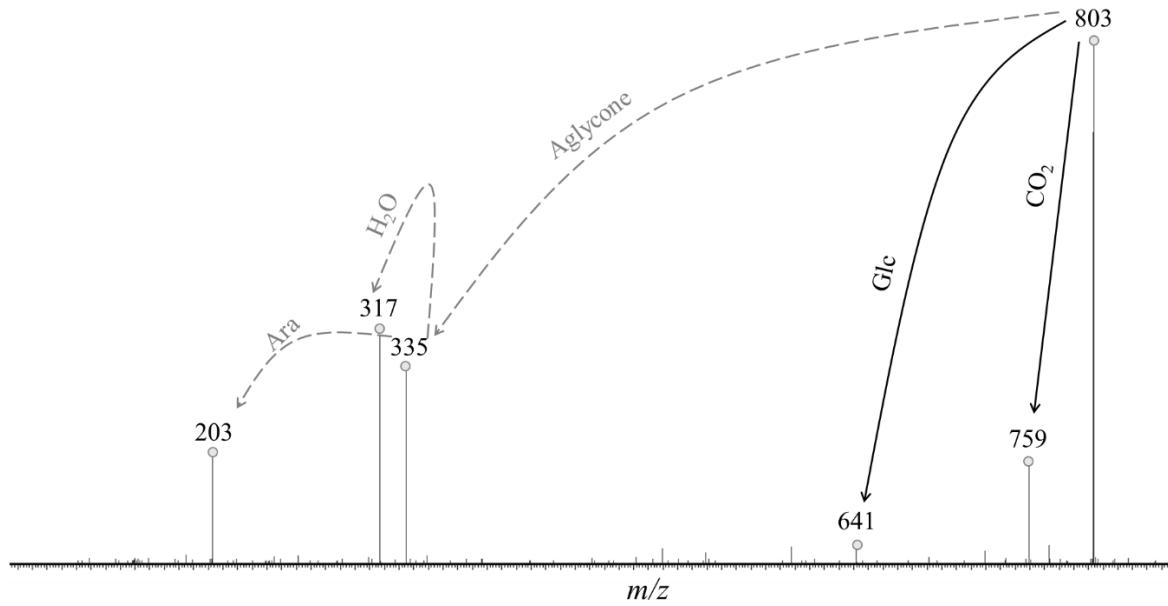


Appendix 12. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 1157 precursor ions at 4.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin O.

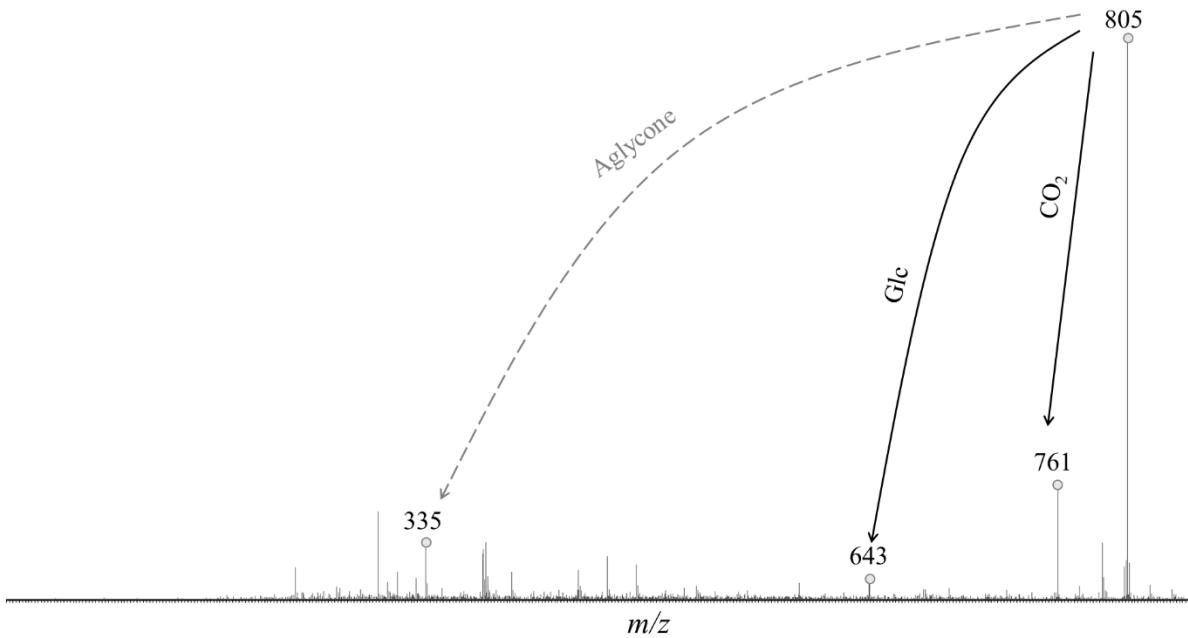


Appendix 13. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 789 precursor ions at 10.7 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin I^b.

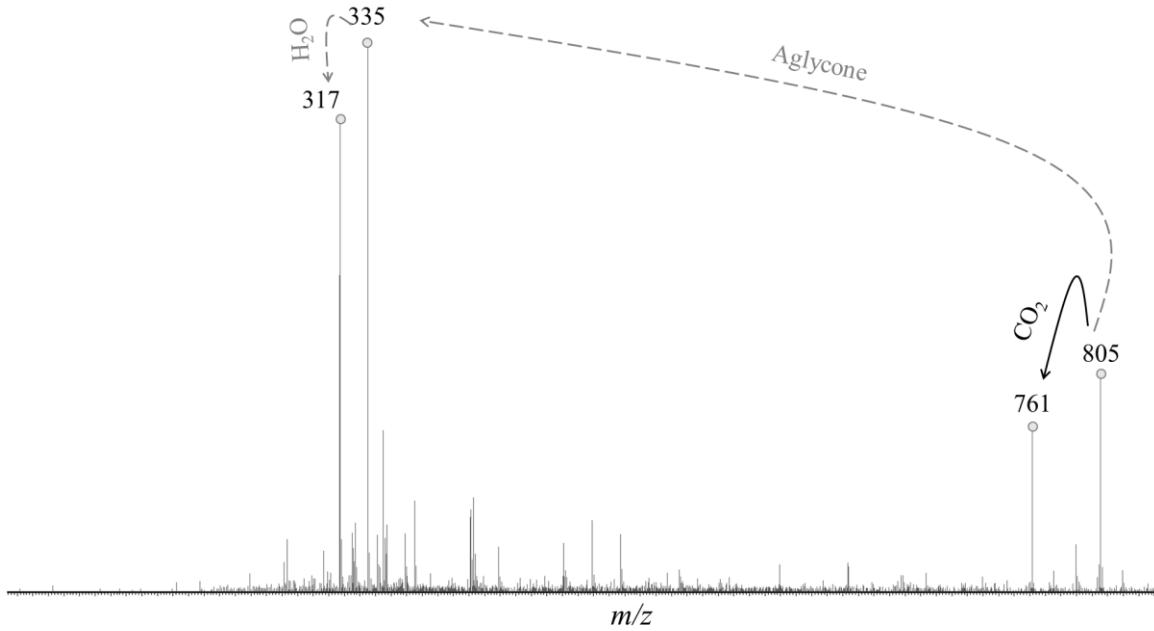
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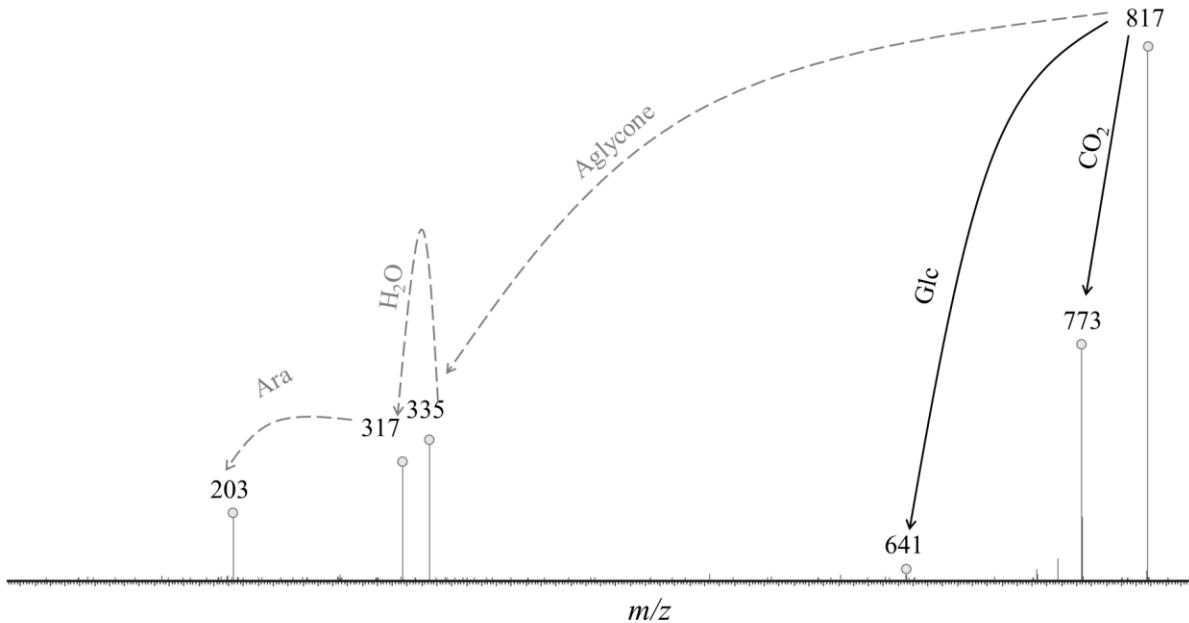
Appendix 14. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 803 precursor ions at 7.2 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Saponin ?^h.



Appendix 15. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 805 precursor ions at 5.6 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Saponin 19^h.

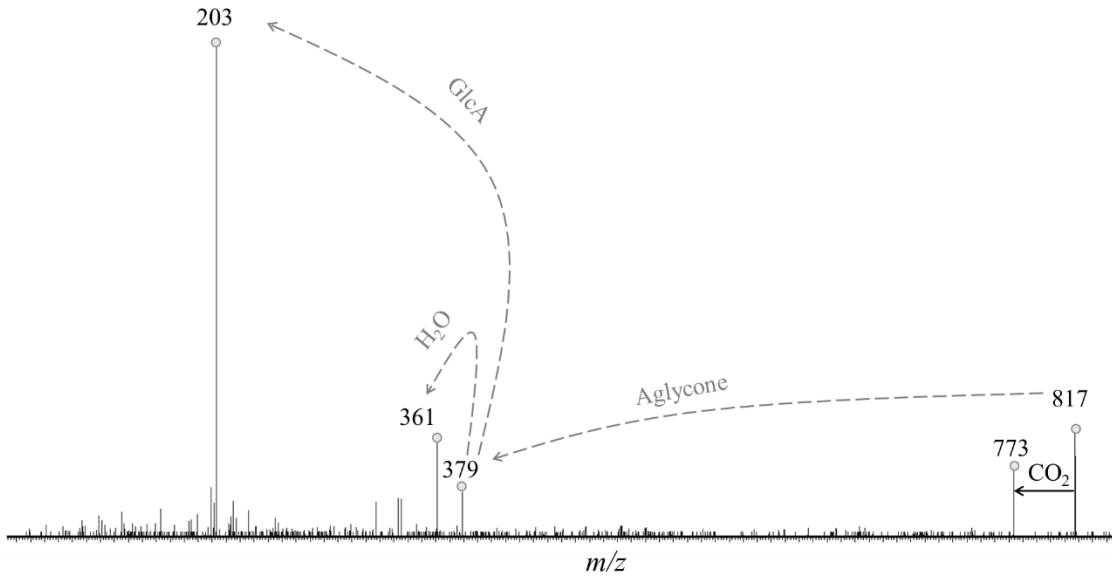


Appendix 16. LC-MSMS analysis of *Chenopodium quinoa* husk saponin extract: CID spectrum recorded for the m/z 805 precursor ions at 5.8 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin 19a^h.

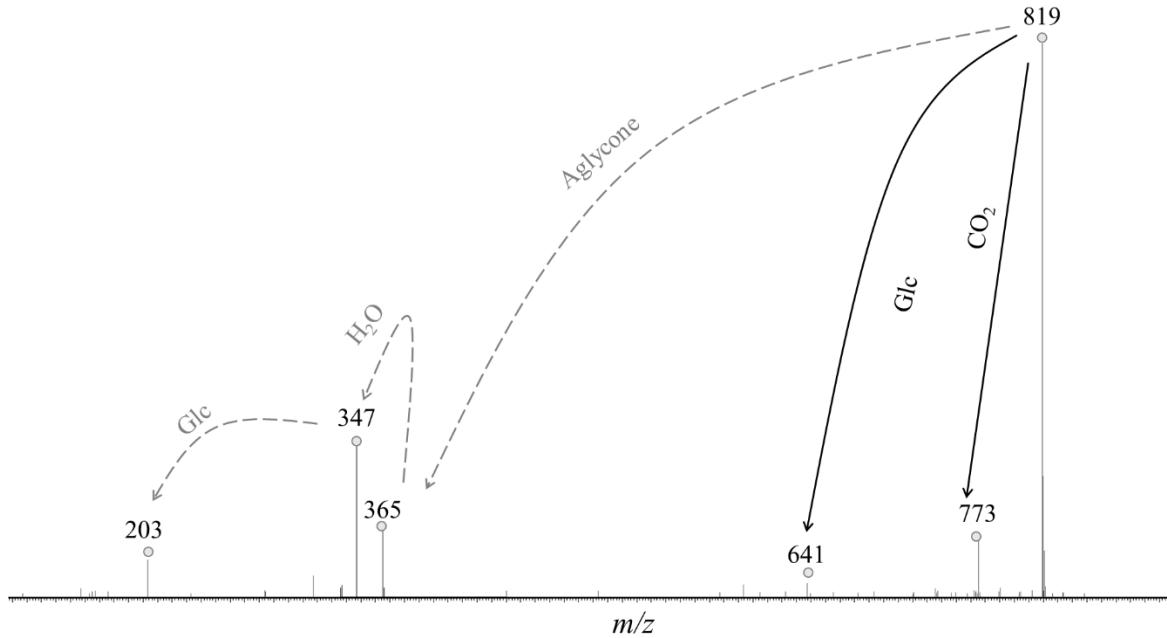


Appendix 17. LC-MSMS analysis *Chenopodium quinoa* husk saponin extract: CID spectrum recorded for the m/z 817 precursor ions at 9.4 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin H^h.

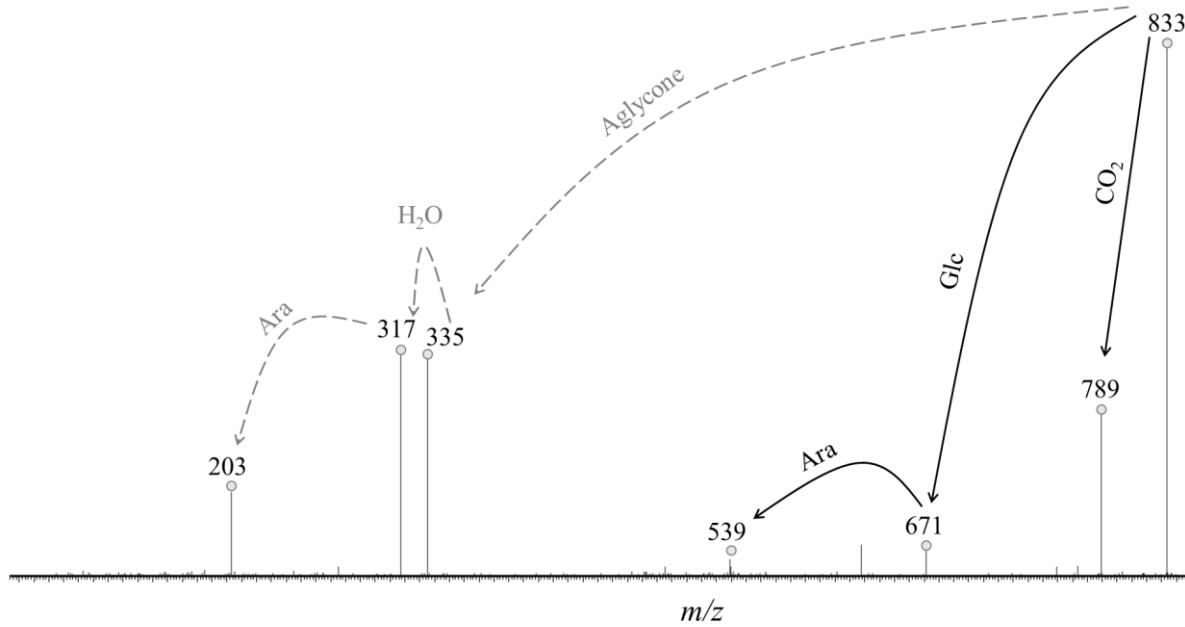
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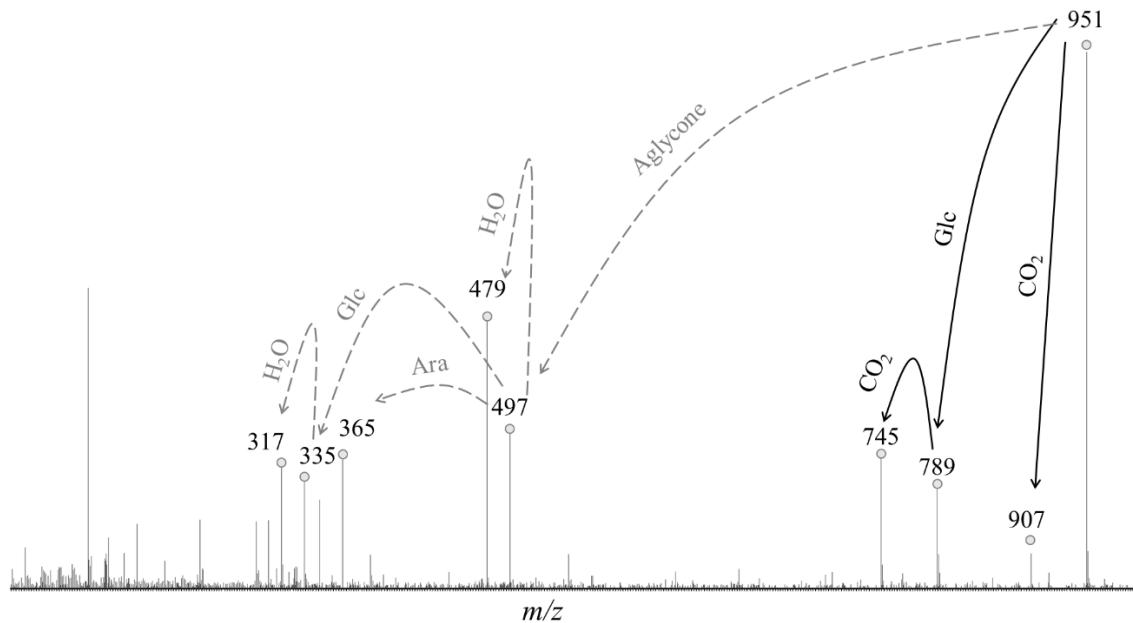
Appendix 18. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 817 precursor ions at 13.1 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Saponin 70^h.



Appendix 19. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 819 precursor ions at 9.4 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Saponin Q^h.

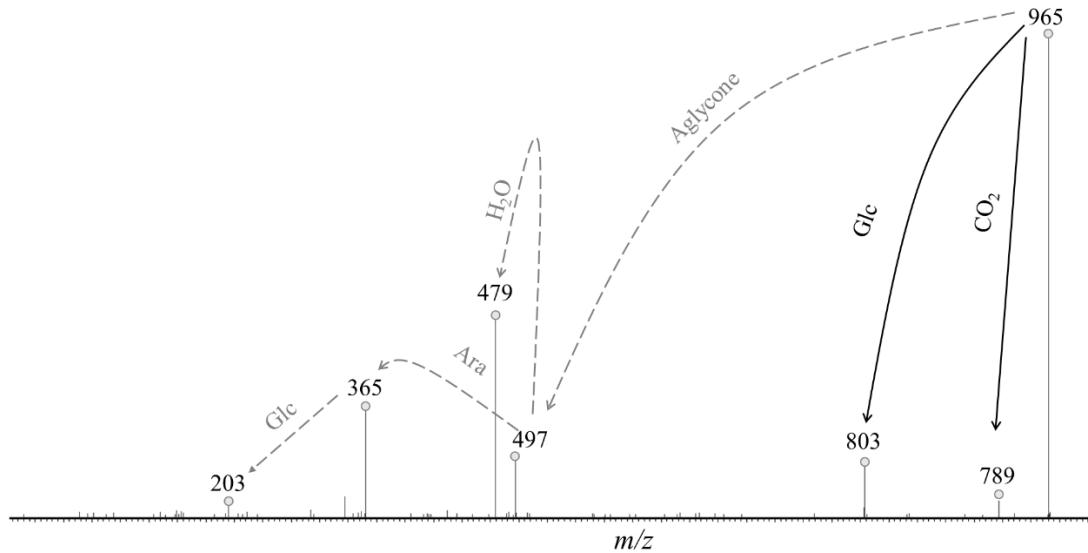


Appendix 20. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 833 precursor ions at 9.9 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Saponin B^h.

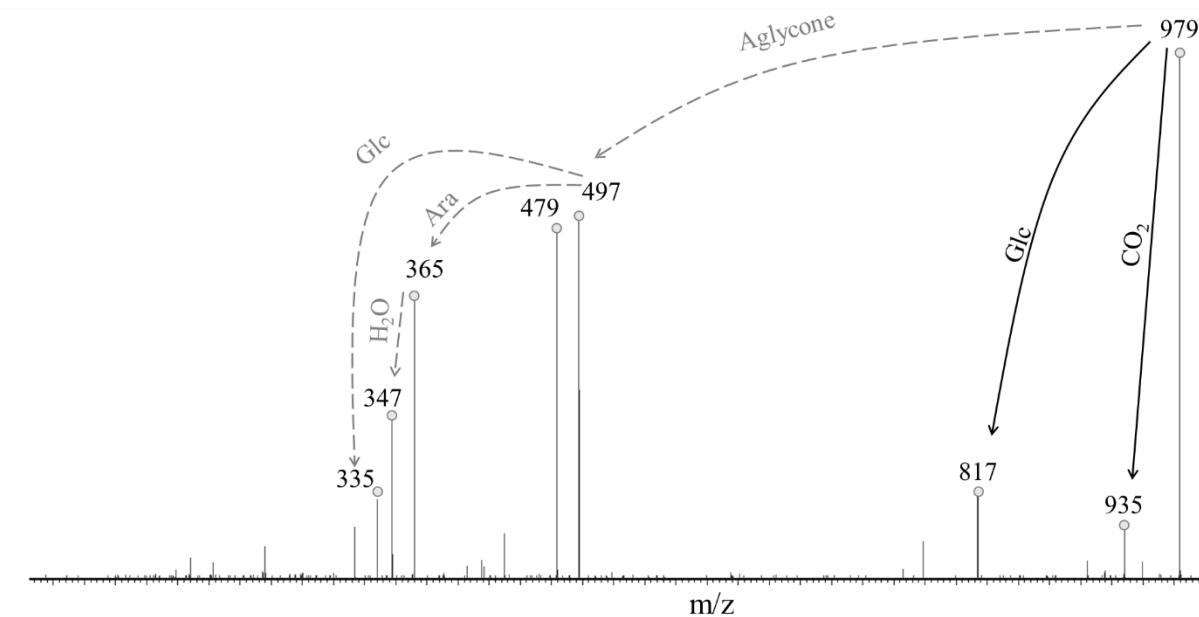


Appendix 21. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 951 precursor ions at 10.2 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Saponin 61^h.

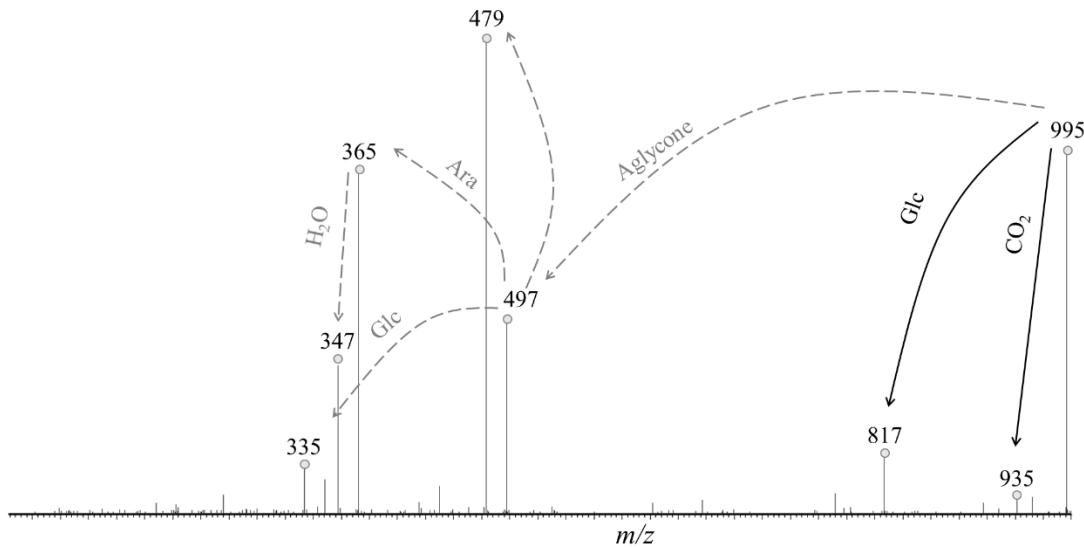
Appendix



Appendix 22. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 965 precursor ions at 7.1 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin ??^h.



Appendix 23. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 979 precursor ions at 9.4 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin G^h.



Appendix 24. LC-MSMS analysis of Chenopodium quinoa husk saponin extract: CID spectrum recorded for the m/z 995 precursor ions at 7.6 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Saponin O^h.

Appendix

1.2. Biological data of original and modified saponins from *Chenopodium quinoa*

1.2.1. Hemolytic activity assay

Concentration ($\mu\text{g.ml}^{-1}$)	Absorbance (450 nm) of natural extract saponin		Absorbance (450 nm) of Hydrolyzed saponin	
	Average	Standard deviation	Average	Standard deviation
0.5	0.0163	0.0021	0.0106	0.0008
1	0.0123	0.0029	0.0012	0.0042
2	0.0143	0.0048	0.0012	0.0015
3	0.0134	0.0041	0.0008	0.0065
4	0.0106	0.0030	0.0005	0.0018
5	0.0116	0.0036	0.0014	0.0012
10	0.0109	0.0027	0.0016	0.0046
20	0.0097	0.0136	0.0005	0.0039
30	0.0083	0.0042	0.0002	0.0007
40	0.0075	0.0012	0.0009	0.0069
50	0.0067	0.0003	0.0007	0.0005
100	0.0050	0.0050	0.0314	0.0004
200	0.0066	0.0071	0.3210	0.0130
300	0.0036	0.0008	0.7210	0.0812
500	0.0034	0.0018	/	/

Appendix 25. Hemolytic activity assay of the hydrolyzed and natural extract saponin. Average and standard deviation of the free heme absorbance (540 nm) with regards to the increasing saponin concentration.

1.2.2. Statistical data of antifungal classic assays

Concentration ($\mu\text{g.mL}^{-1}$)	5	10	20	50	100	200	500
Natural extract saponins	ns	ns	ns	ns	**	**	***
Hydrolyzed saponins	**	**	**	**	**	ns	***

Appendix 26. Statistical data of survival rate of C.albicans with quinoa saponins (Natural extract and hydrolyzed) where ns = non-significant. P_value in green = inhibitory effect and P_value in red = amplification effect. P-value : * <0.05 ; **<0.01;*<0,001.**

Concentration ($\mu\text{g.mL}^{-1}$)	5	10	20	50	100	200	500
Natural extract saponins	ns	ns	ns	ns	ns	ns	**
Hydrolyzed saponins	ns	ns	ns	ns	ns	ns	ns

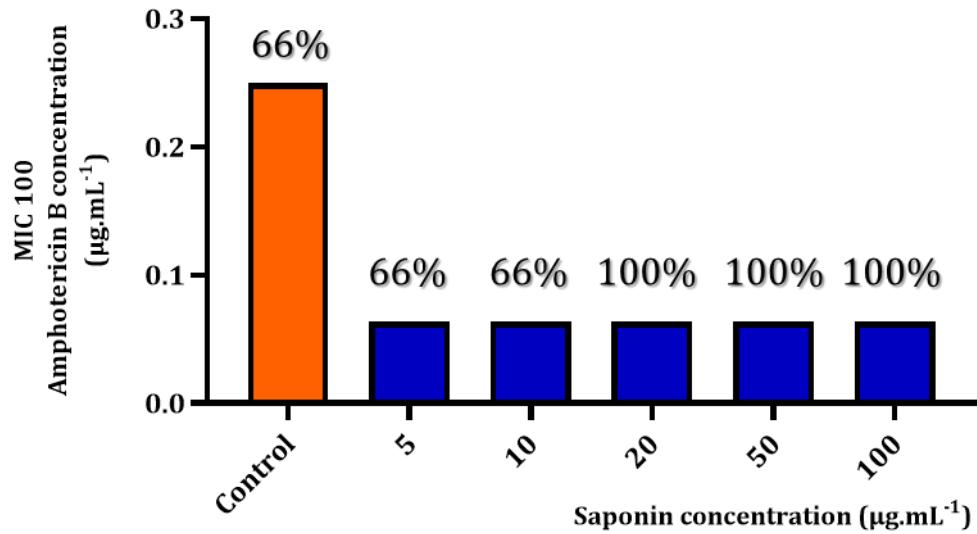
Appendix 27. Statistical data of survival rate of A.fumigatus with quinoa saponins (Natural extract and hydrolyzed) where ns = non-significant. P_value in green = inhibitory effect and P_value in red = amplification effect. P-value : * <0.05 ; **<0.01;*<0,001.**

Concentration ($\mu\text{g.mL}^{-1}$)	5	10	20	50	100	200	500
Natural extract saponins	ns	ns	ns	*	*	ns	ns
Hydrolyzed saponins	ns	ns	ns	ns	ns	ns	*

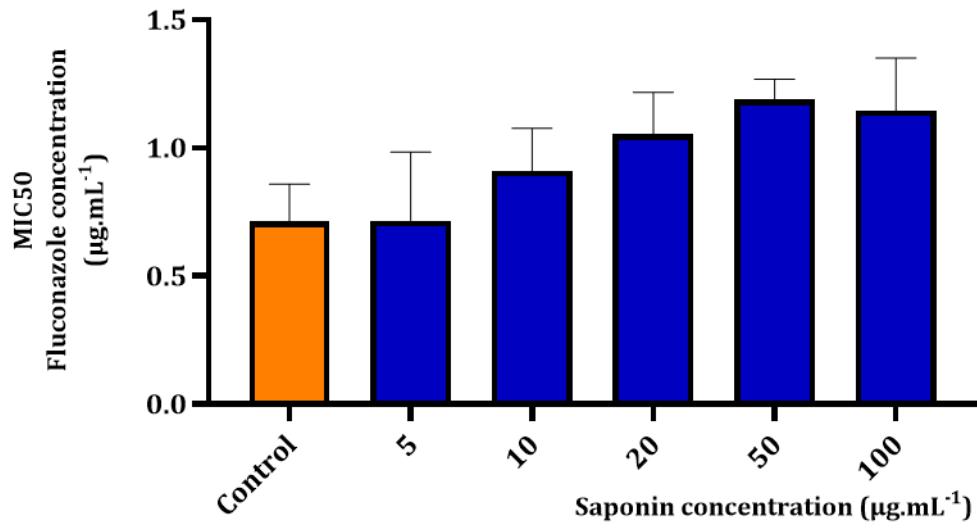
Appendix 28. Statistical data of survival rate of T.interdigitale with quinoa saponins (Natural extract and hydrolyzed) where ns = non-significant. P_value in green = inhibitory effect and P_value in red = amplification effect. P-value : * <0.05 ; **<0.01;*<0,001.**

Appendix

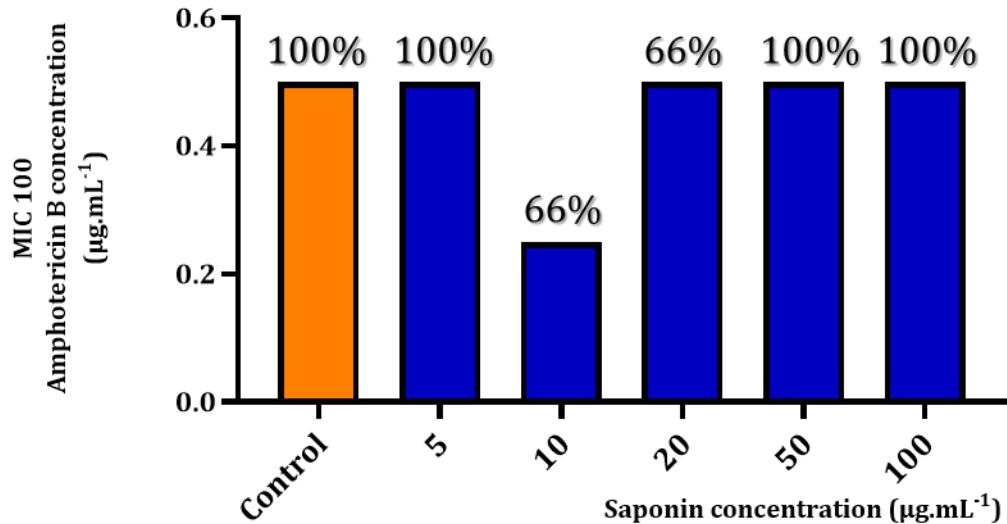
1.2.3. Additive assays



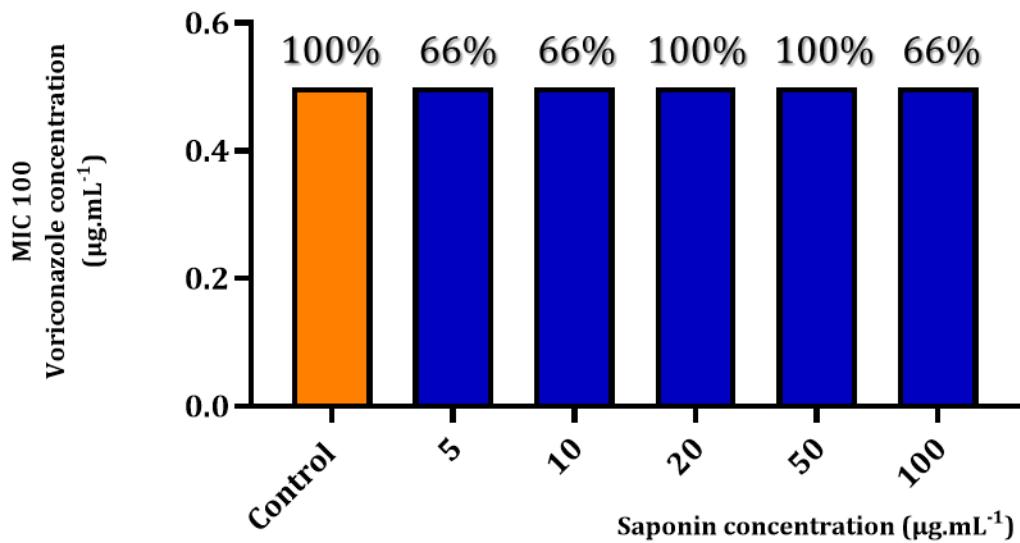
Appendix 29. Estimation of the antifungal activity of the natural extract saponins from Chenopodium quinoa combined with Amphotericin B on C. albicans. An additive effect is already observed at a saponin concentration of $5 \mu\text{g.mL}^{-1}$. The value above the column represents the reproducibility of the analysis (based on three assays)



Appendix 30. Estimation of the antifungal activity of the natural extract saponins from Chenopodium quinoa combined with Fluconazole on C. albicans. No additive effect is observed.

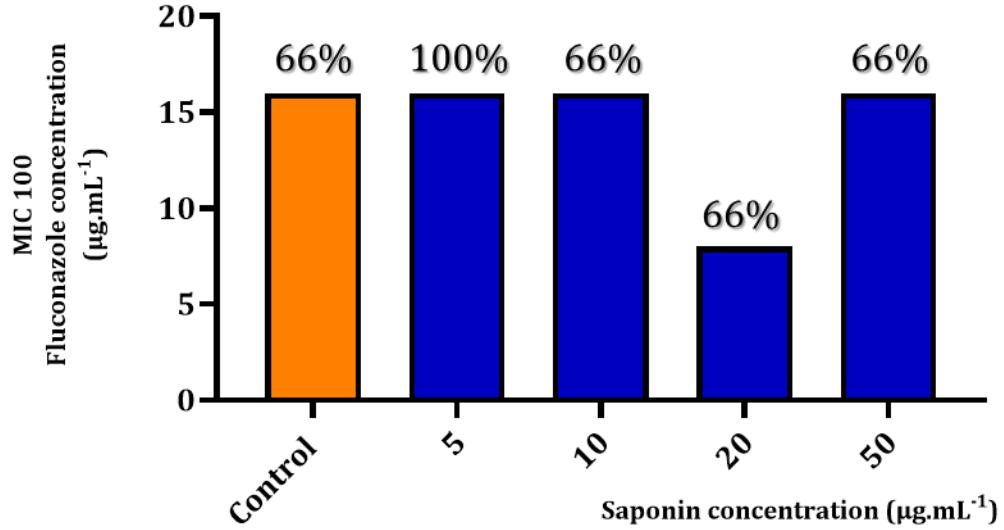


Appendix 31. Estimation of the antifungal activity of the natural extract saponins from Chenopodium quinoa combined with Amphotericin B on A. fumigatus. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)

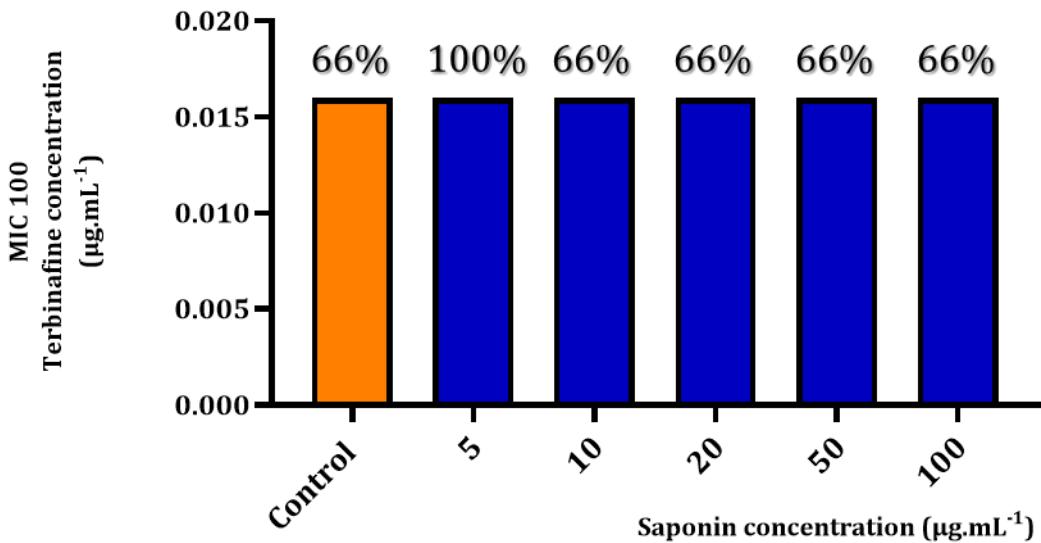


Appendix 32. Estimation of the antifungal activity of the natural extract saponins from Chenopodium quinoa combined with Voriconazole on A. fumigatus. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)

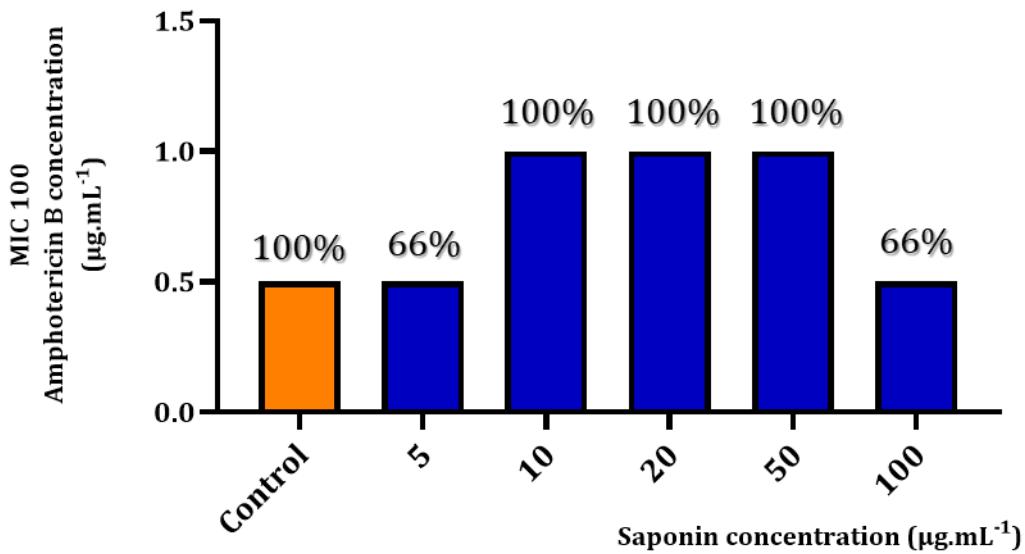
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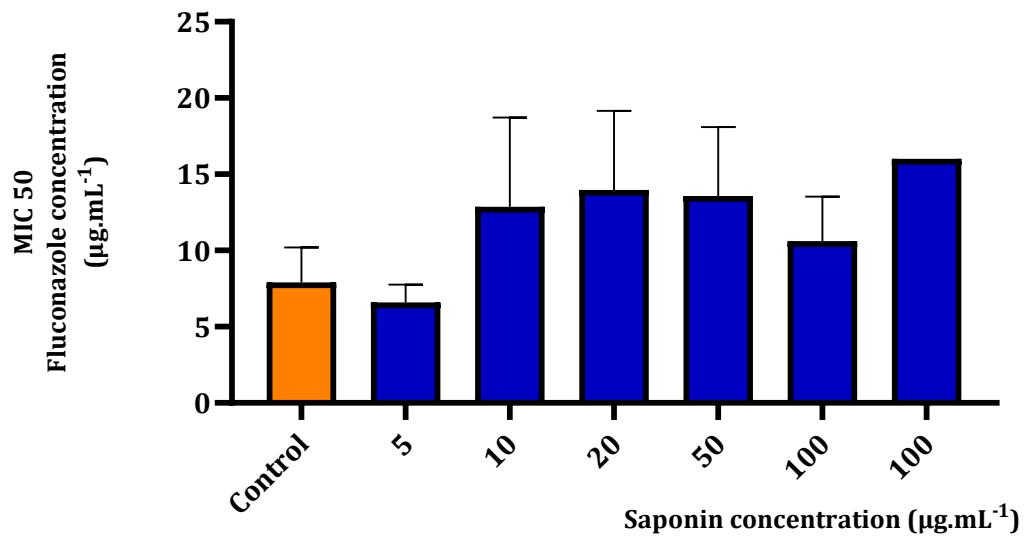
Appendix 33. Estimation of the antifungal activity of the natural extract saponins from Chenopodium quinoa combined with Fluconazole on *T. interdigitale*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)



Appendix 34. Estimation of the antifungal activity of the natural extract saponins from Chenopodium quinoa combined with Terbinafine on *T. interdigitale*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)

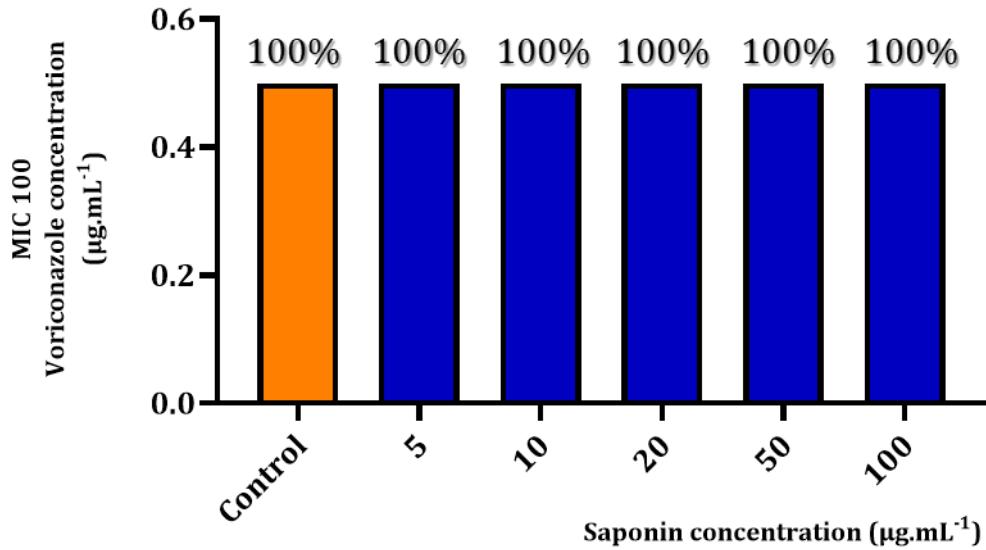


Appendix 35. Estimation of the antifungal activity of the hydrolyzed saponins from Chenopodium quinoa combined with Amphotericin B on C. albicans No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)

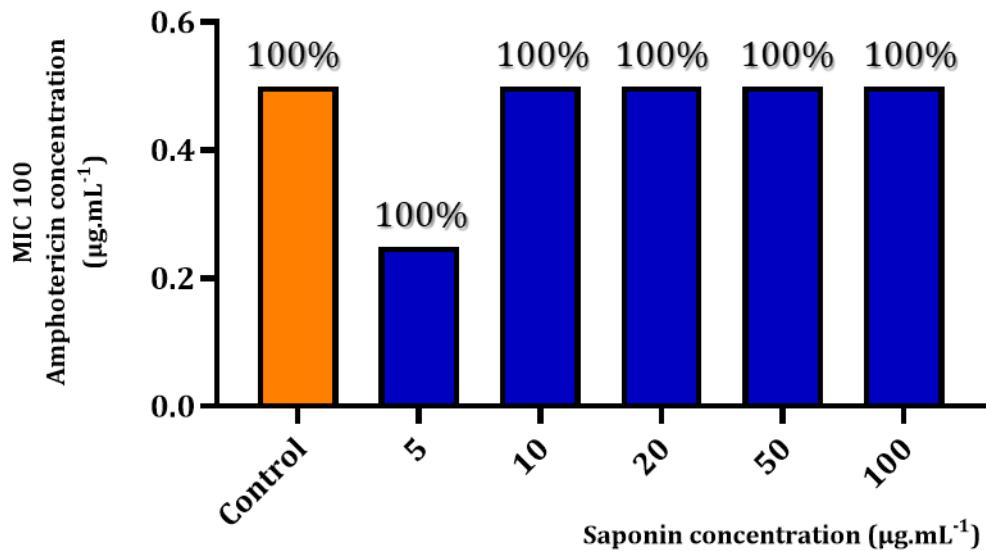


Appendix 36. Estimation of the antifungal activity of the hydrolyzed saponins from Chenopodium quinoa combined with Fluconazole on C. albicans. No additive effect is observed.

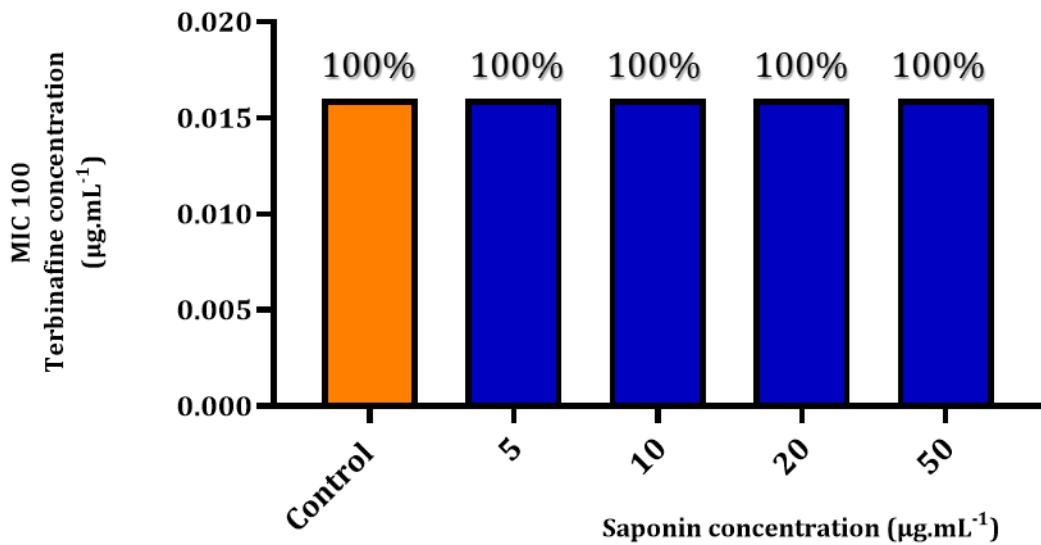
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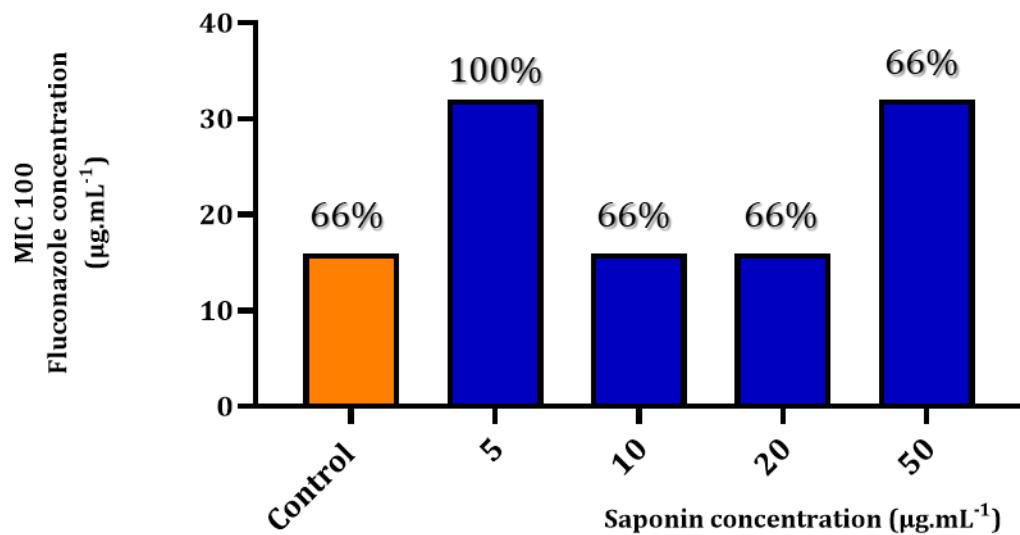
Appendix 37. Estimation of the antifungal activity of the hydrolyzed saponins from Chenopodium quinoa combined with Voriconazole on A. fumigatus. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)



Appendix 38. Estimation of the antifungal activity of the hydrolyzed saponins from Chenopodium quinoa combined with Amphotericin B on A. fumigatus. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)



Appendix 39. Estimation of the antifungal activity of the hydrolyzed saponins from Chenopodium quinoa combined with Terbinafine on T. interdigitale. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)



Appendix 40. Estimation of the antifungal activity of the hydrolyzed saponins from Chenopodium quinoa combined with Fluconazole on T. interdigitale. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)

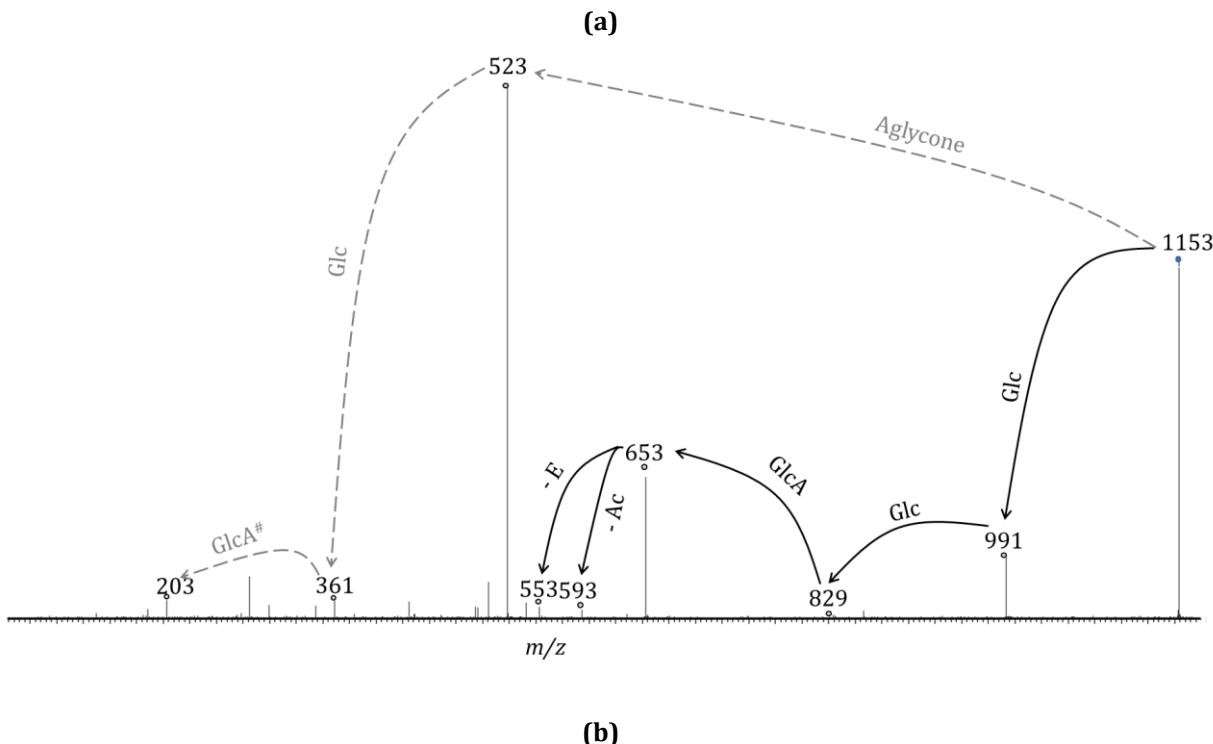
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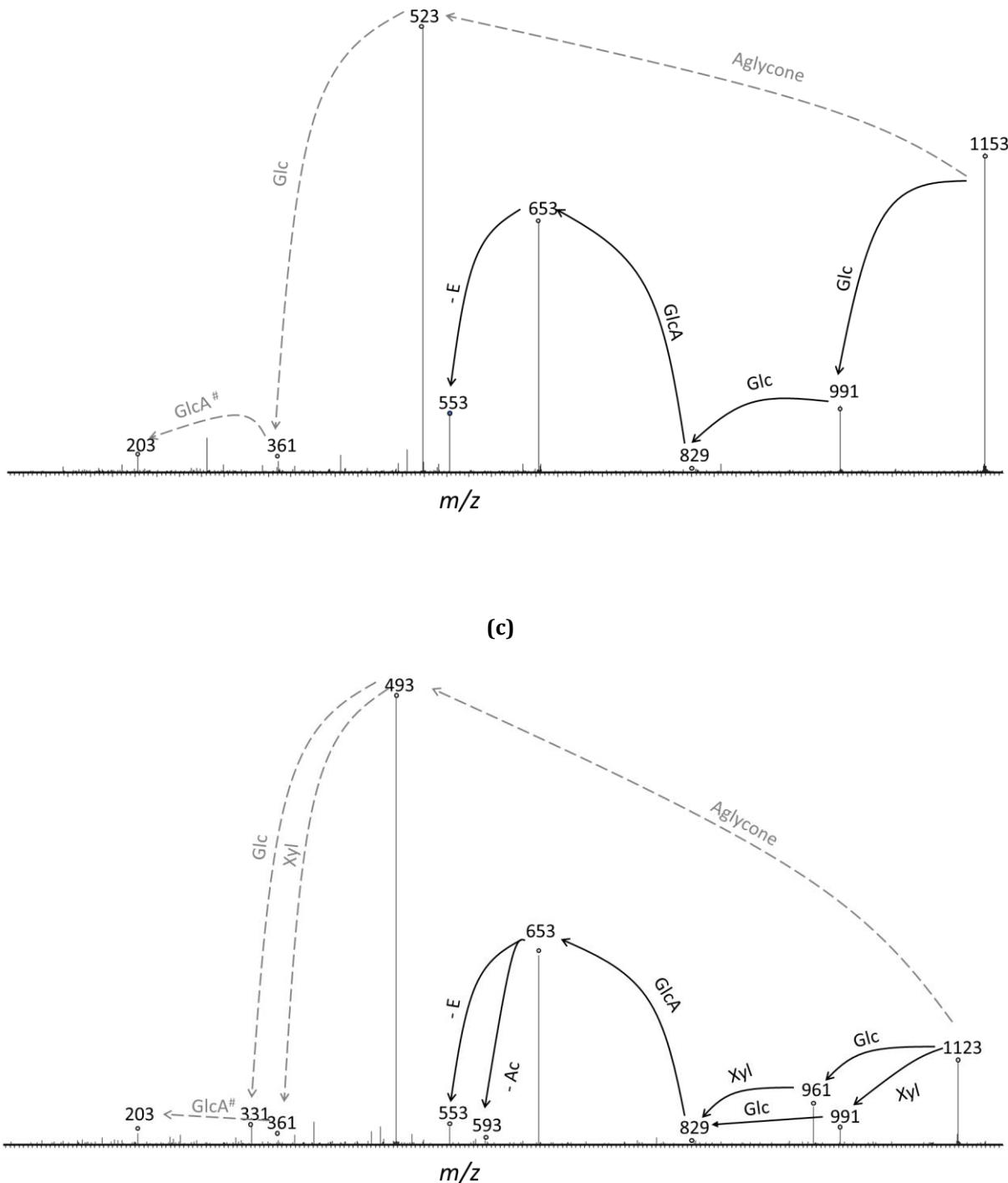
2. Aesculus hippocastanum

2.1. Mass spectrometry analyses

2.1.1. Characterization procedure

The oligosaccharide chain of the HC saponins is always a branched trisaccharide that is either Glc-GlcA-Glc or Glc-GlcA-Xyl with the GlcA residue being anchored at C-3 of the aglycone. These two oligosaccharides can be distinguished based on collision-induced dissociation (CID) experiments. Indeed, as presented in Appendix 41a-b (Escin 1a and Isoescin 1a), the four different Escin 1 ions, $[M+Na]^+$ at m/z 1153, predominantly expelled the neutral aglycone (630 u) leading to m/z 523 fragment ions that correspond to the trisaccharide ions, i.e. $[Glc-GlcA-Glc - H_2O + Na]^+$.



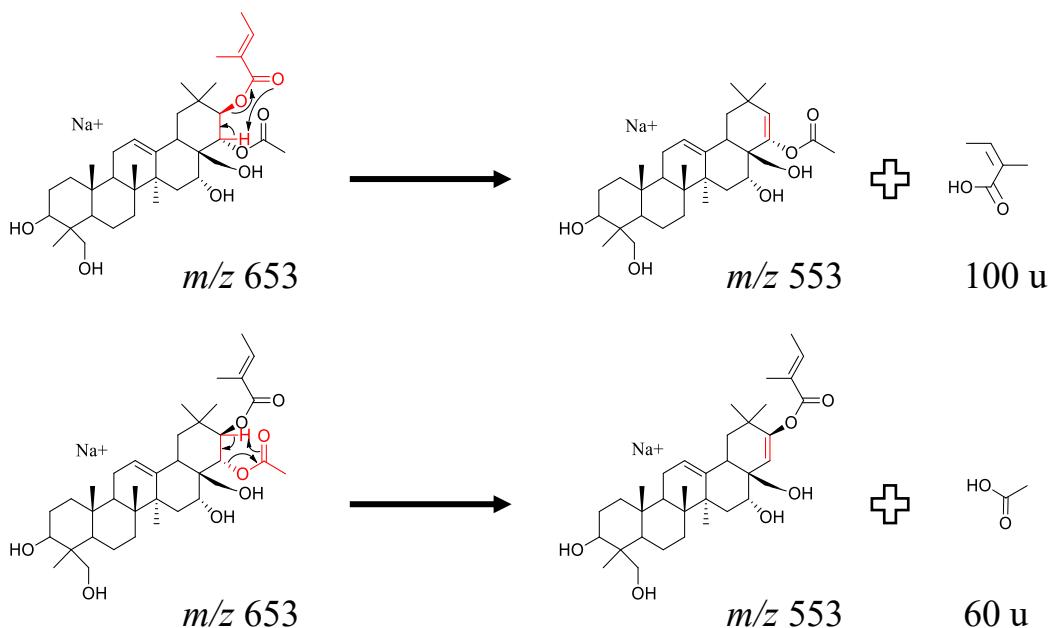


Appendix 41. LC-MSMS analyses of the horse chestnut saponin extract: CID spectra recorded for the m/z 1153 precursor ions at 6.9 min retention time (a), for the m/z 1153 precursor ions at 7.25 min retention time (b), and for the m/z 1123 precursor ions at 6.86 min retention time (c). The corresponding ions are assigned as $[M+Na]^+$ ions from Escin 1a, from Isoescin 1a, from Escin 2a, respectively.

Appendix

On the other hand, when exposed to LC-MSMS experiments, the presence of the Glc-GlcA-Xyl oligosaccharide chain in the Escin 2 isomers is clearly attested by the observation of the [Glc-GlcA-Xyl - H₂O + Na]⁺ fragment ions at *m/z* 493 in Figure 4c (Escin 2a). Table 8 summarizes the data and the oligosaccharide chain sequence is identified. The data indicates that (i) the two additional molecules detected at *m/z* 1123 belong to the Escin 2 family; (ii) the new molecules observed at *m/z* 1113 and at *m/z* 1083 are respectively members of the Escin 4 and 7 families, and (iii) that the new compositions (*m/z* 1111 and 1097) respectively contain a Glc-GlcA-Xyl and a Glc-GlcA-Glc oligosaccharide. The distinction between epimeric galactose and glucose is not achieved using CID experiments. Therefore, in Table 1, no definitive position is taken between glucose and galactose for the newly observed saponins, i.e. (Iso)escin 4 and Escin 9.

All the CID spectra are presented further and, except for the identification of the saccharide moiety, they are limited in structural information. Thus, in the CID spectra of the [M+Na]⁺ ions from Escin 1a and Isoescin 1a isomers presented in Appendix 41 a-b, most of the generated fragment ions are related to the glycone part of the saponins that is identical between the two isomers, making the CID spectra quasi superimposable. A closer inspection at the CID fragments, however, reveals some minor differences. The [M+Na]⁺ precursor ions associated to Escin 1a, Escin 1b, Isoescin 1a and Isoescin 1b (see Appendix 41 a-b) expel their oligosaccharide chains to produce the aglycone ions at *m/z* 653. From these ions, a 100 u loss (tiglic or angelic acid) is observed for the four isomers (see Table 8), whereas the loss of acetic acid (60 u) is only observed for the isomers eluting after 6.9 (Escin 1a) and 7.13 (Escin 1b) minutes. That is, this 60 u loss is specific for the Escin 1a and 1b isomers.



Appendix 42. Collision-induced dissociation of the m/z 653 aglycone ions from Escin 1a : McLafferty rearrangements leading to the a) 100 u loss and b) 60 u loss.

From Appendix 42, the 60 and 100 u losses can be associated to McLafferty rearrangements involving the breaking of the C-21-O and C-22-O bonds, respectively. Such a McLafferty rearrangement is not feasible for the regioisomeric Isoescin 1a and 1b ions, since no α hydrogen atom is present in agreement with the absence of the 60 u loss process. Interestingly, in the context of the present study, these dissociation reactions (60 u and 100 u losses) concern the substituents that contain the regioisomer/stereoisomer information. In Table SI 1, these telltale mass transitions are also added to afford pieces of information regarding the nature of the R₁, R₂ and R₃ substituents. Nevertheless, at this point of the discussion, based on the MALDI-MS, LC-MS and LC-MSMS data, the regioisomers and stereoisomers can hardly be distinguished within a given family (e.g., Escin I, Escin 1b, Isoescin 1a and Isoescin 1b).

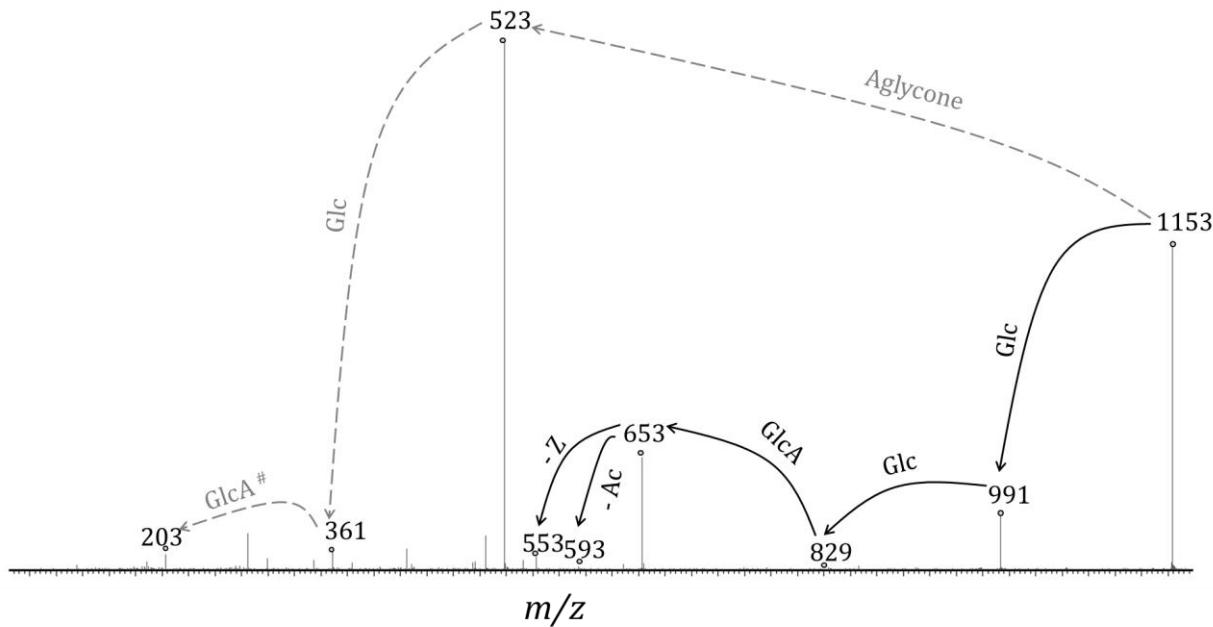
Identification of the HC saponins by combining the HRMS, LC, CID and IM data. The HC saponin congeners can now be identified using all the MS-based data gathered in Table 8. LC makes it possible to count the different isomers for each saponin composition confirmed by HRMS measurements. CID data allow (i) identification of the trisaccharide chains, either Glc-GlcA-Glc or Glc-GlcA-Xyl, (ii) identification of the nature of the R₁, R₂/R₃ side chains (100 u loss for Tig/Ang, 88 u loss for isopropylcarbonyl (A) or 60 u loss for acetyl) and (iii) assigning

Appendix

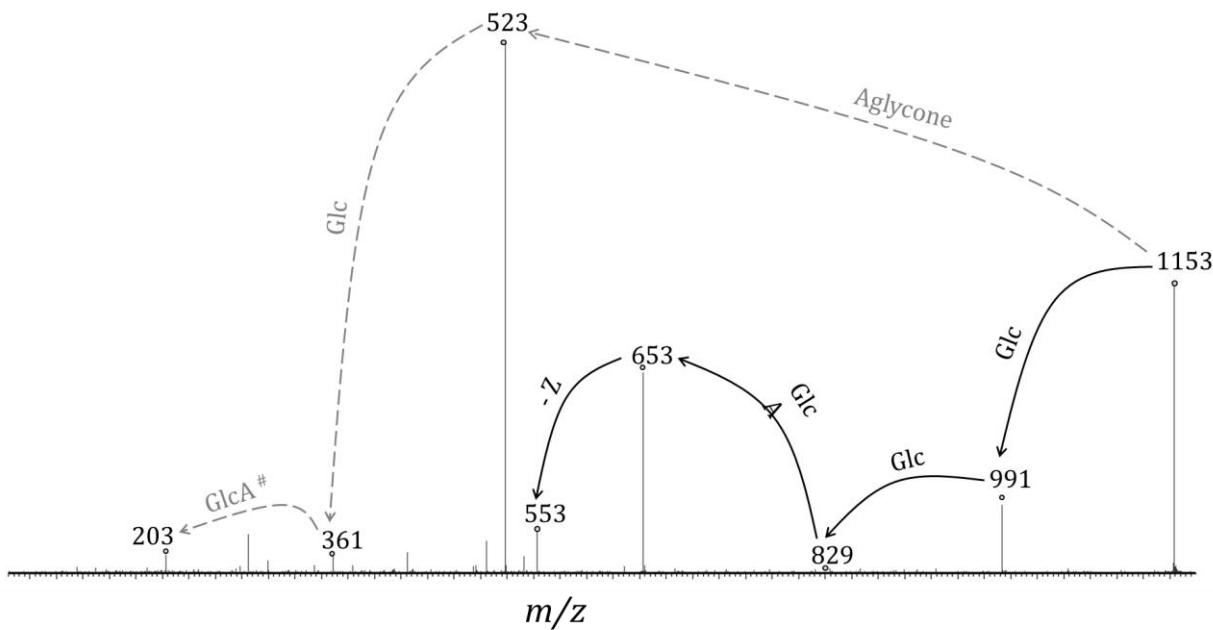
saponins with the acetyl group at C-22 (acetic acid loss) or at C-28 (no acetic acid loss), namely the Escin and Isoescin regioisomers. Special attention must be paid to the acetic acid loss that may also occur from position C-21 if an acetyl group is present. However, the ambiguity is removed when realizing that no other side chain loss is detected (100 u or 88 u losses for instance). Finally, the ion mobility data is of great help for discriminating the stereoisomer side chains, Tig vs Ang, with the Tig-containing stereoisomers being characterized by a longer t_A than the Ang-containing isomer ions.

As an example, the Escin 2 family contains four chromatographic peaks at retention times of 6.86, 7.1, 7.26 and 7.44 min (Table 8). Beside Escin 2a and Escin 2b that were already described in the literature, the CID data confirms that the additional isomers are Isoescin 2a and Isoescin 2b (no 60 u loss and m/z 493 for the glycone fragment ions). Further, the IMS data in Table 2 allows defining the configuration of the side chain (Tig vs Ang). The same procedure is applied to the Escin 4 and Escin 7 new isomers, that are confirmed to be Isoescin 4 and Isoescin 7. Note that for these two molecules, the absence of the Tig/Ang side chain (no 100 u loss) already ruled out the presence of stereoisomers (Escin 4b and Escin 7b for instance are impossible). Finally, for Escin 8, the acetyl function must be in C-22 (60 u loss), whereas for the new Escin 9, acetyl groups are present on C-21 and C-22.

2.1.2. LC-MSMS analysis of the horse chestnut saponin extract

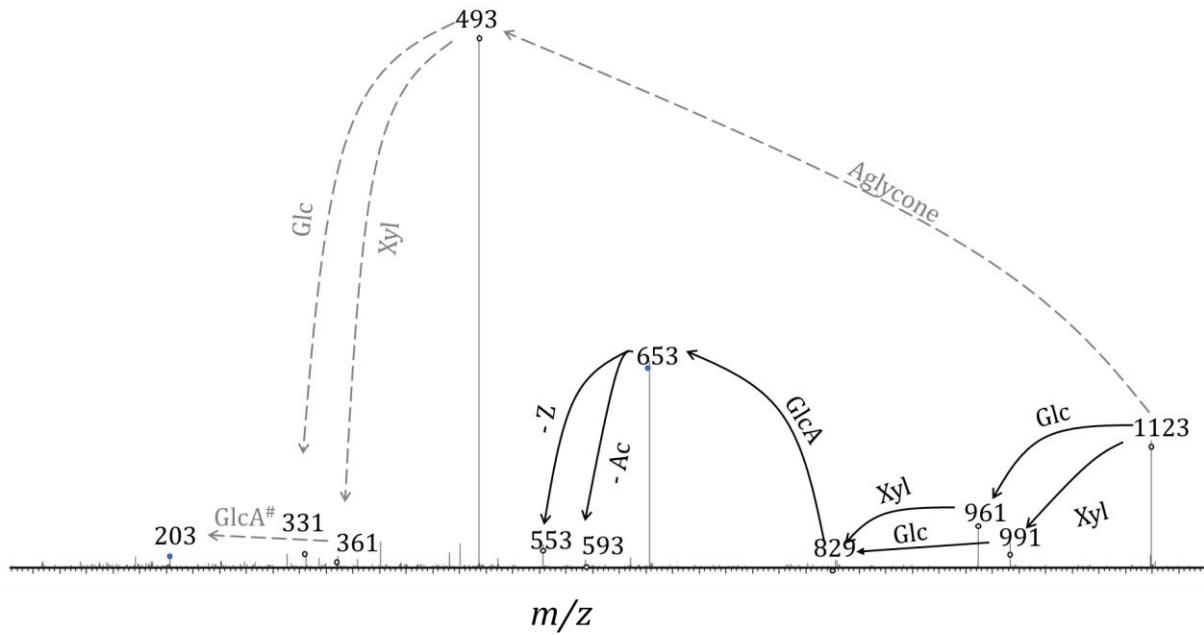


Appendix 43. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1153 precursor ions at 7.13 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Escin 1b.

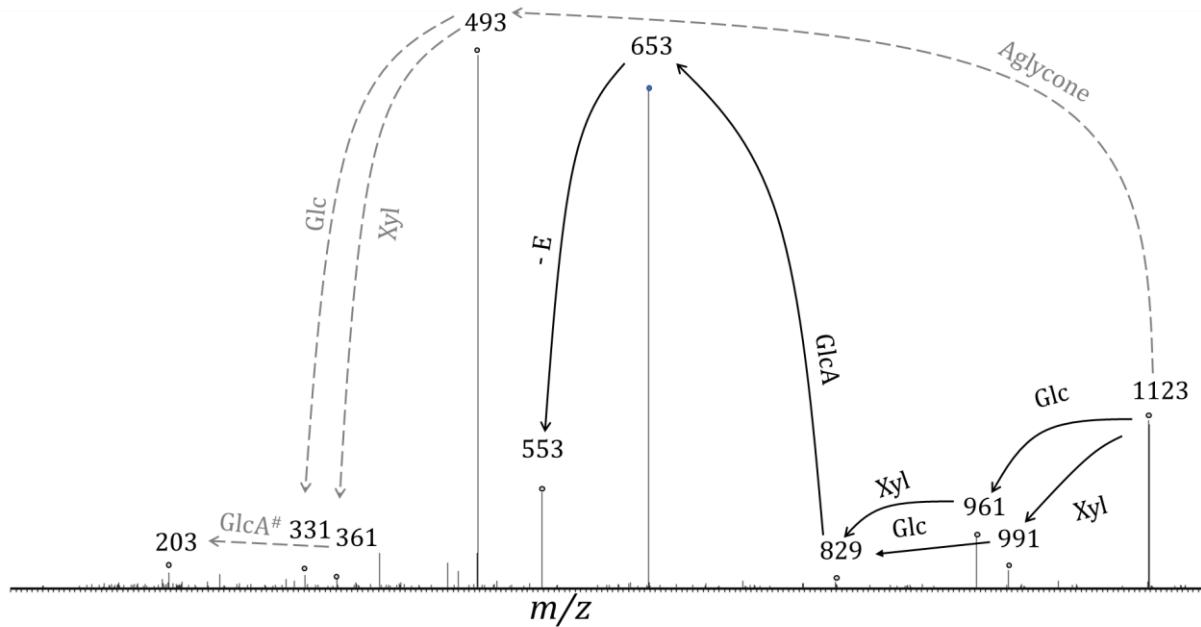


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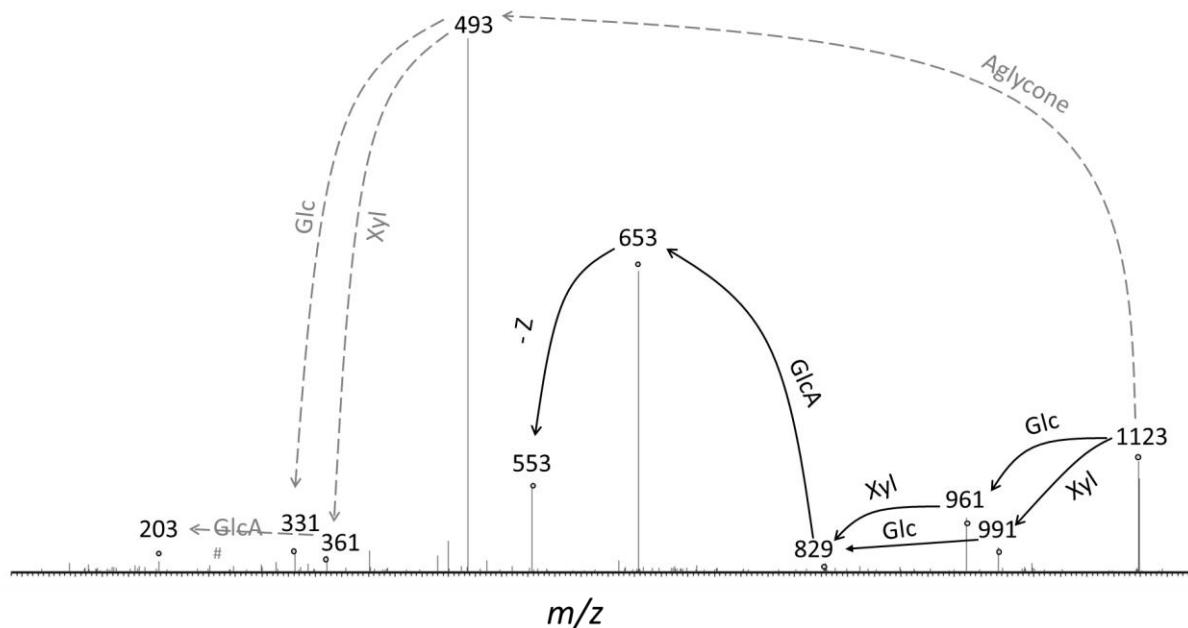
Appendix 44. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1153 precursor ions at 7.44 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Isoescin 1b.



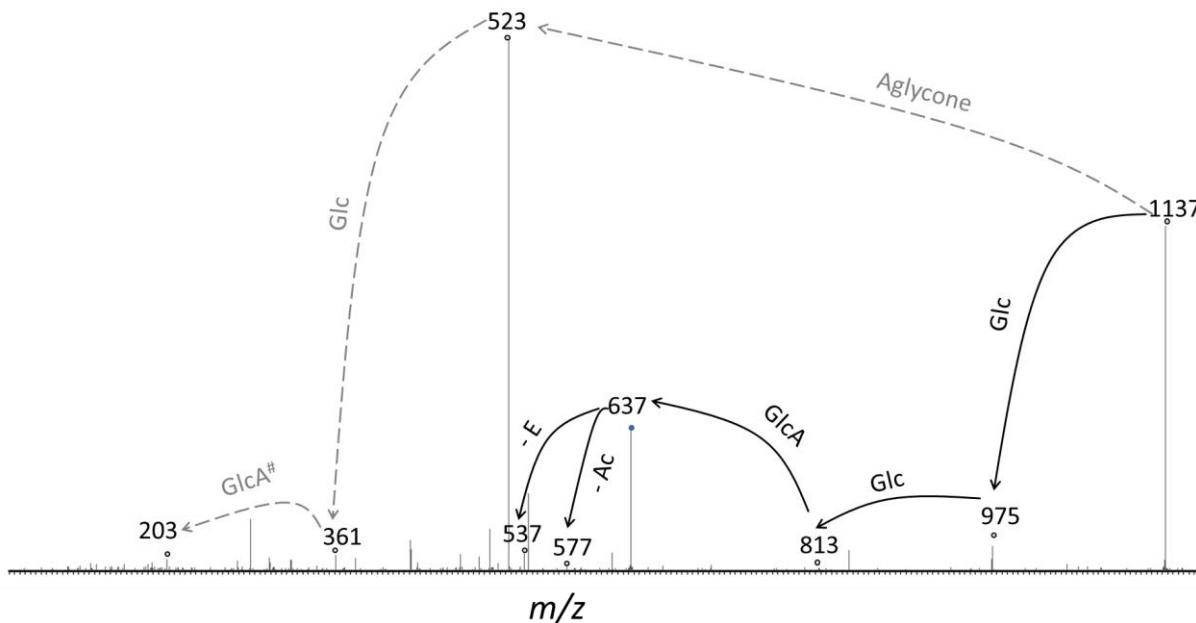
Appendix 45. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1123 precursor ions at 7.1 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 2b.



Appendix 46. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1123 precursor ions at 7.26 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Isoescin 2a.

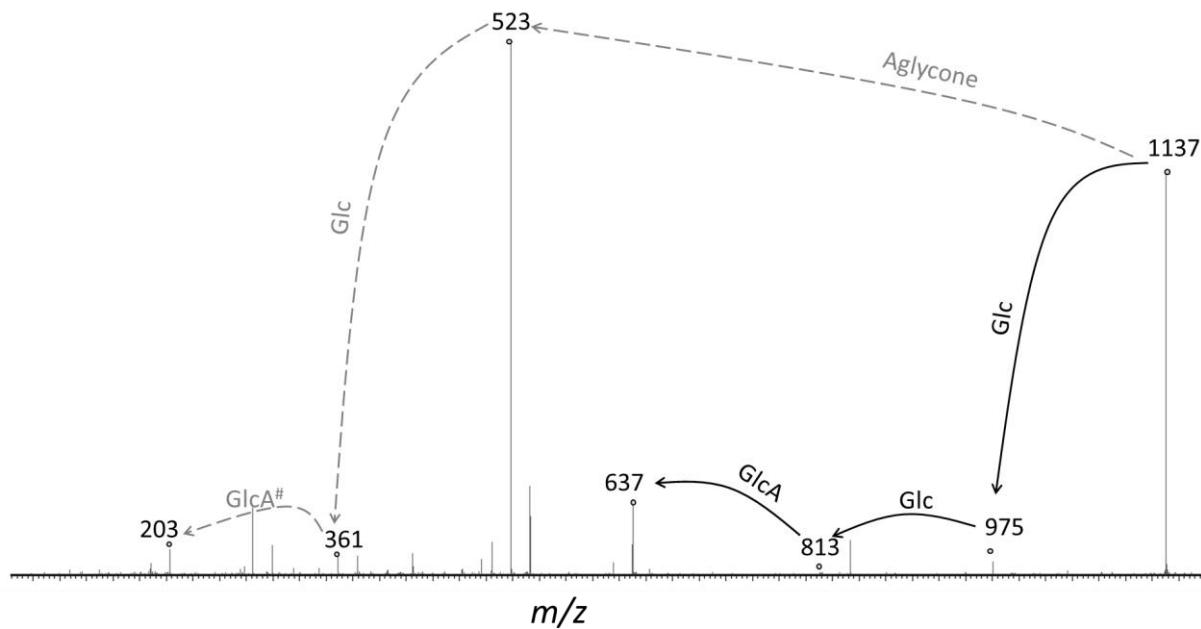


Appendix 47. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1123 precursor ions at 7.44 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Isoescin 2b.

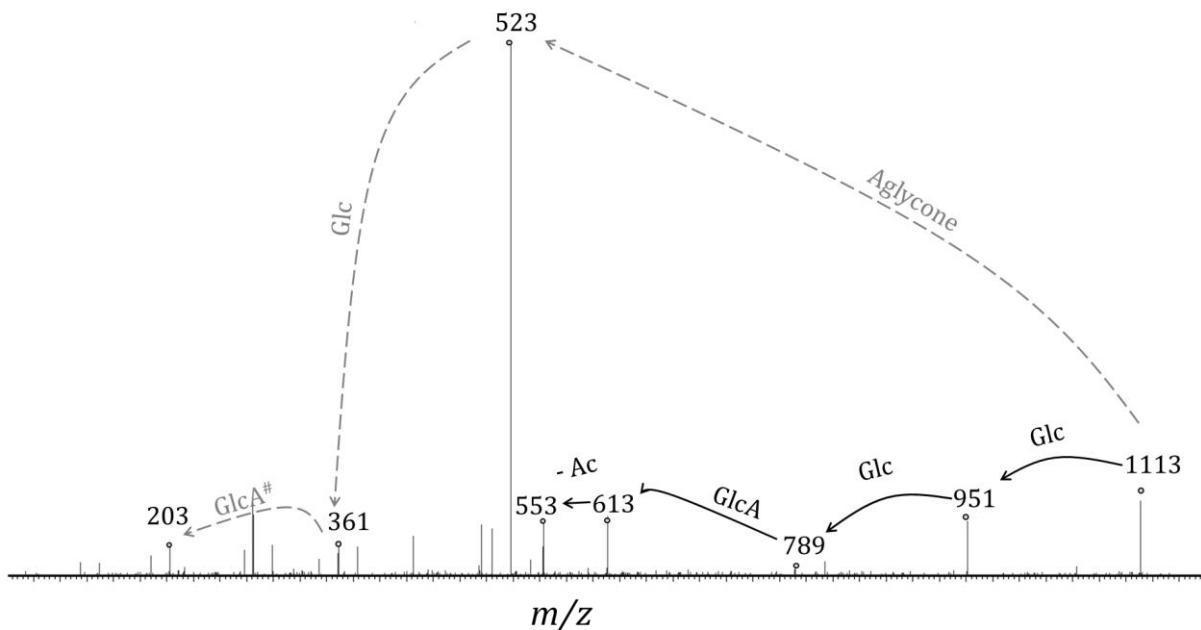


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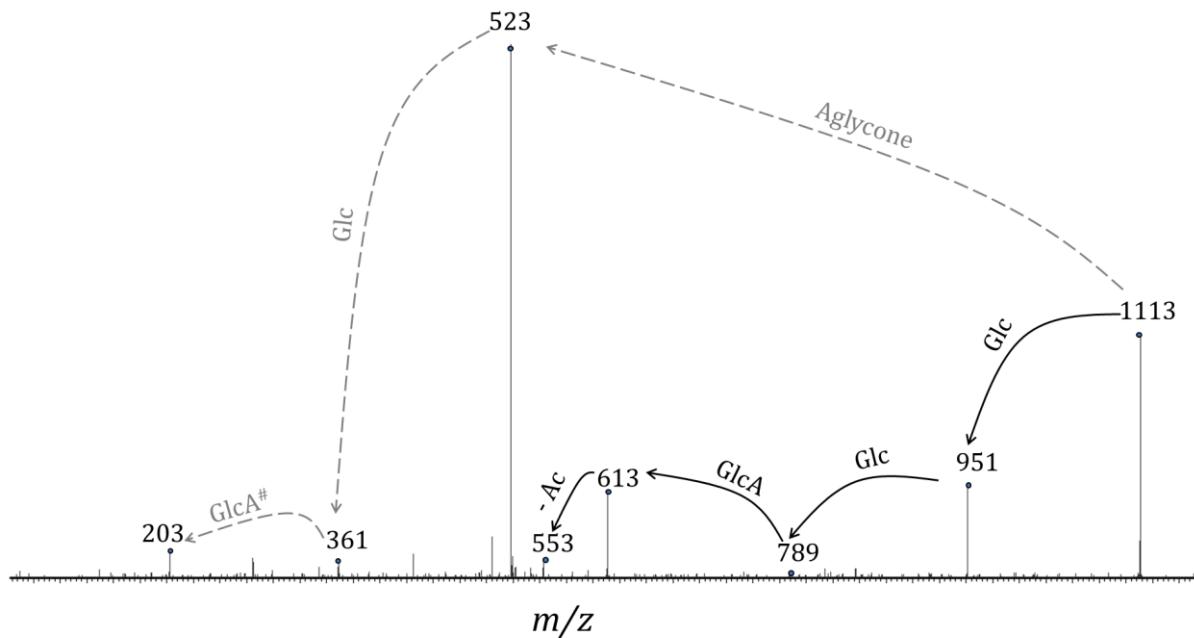
Appendix 48. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1137 precursor ions at 7.25 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 3a.



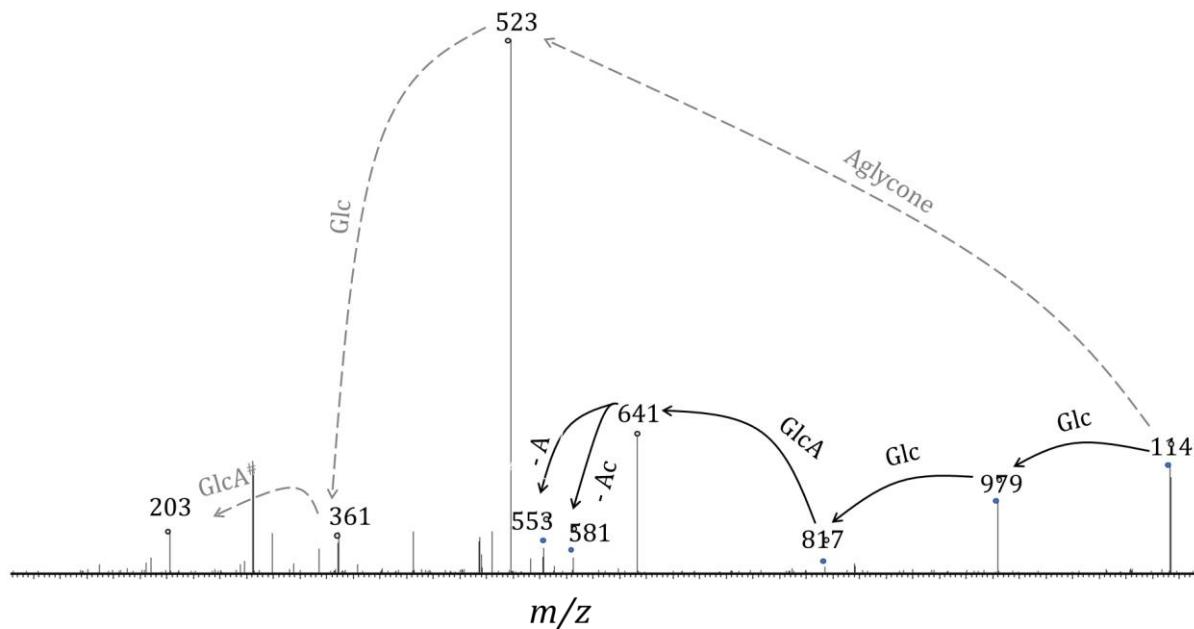
Appendix 49. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1137 precursor ions at 7.49 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 3b.



Appendix 50. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1113 precursor ions at 4.77 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 4.

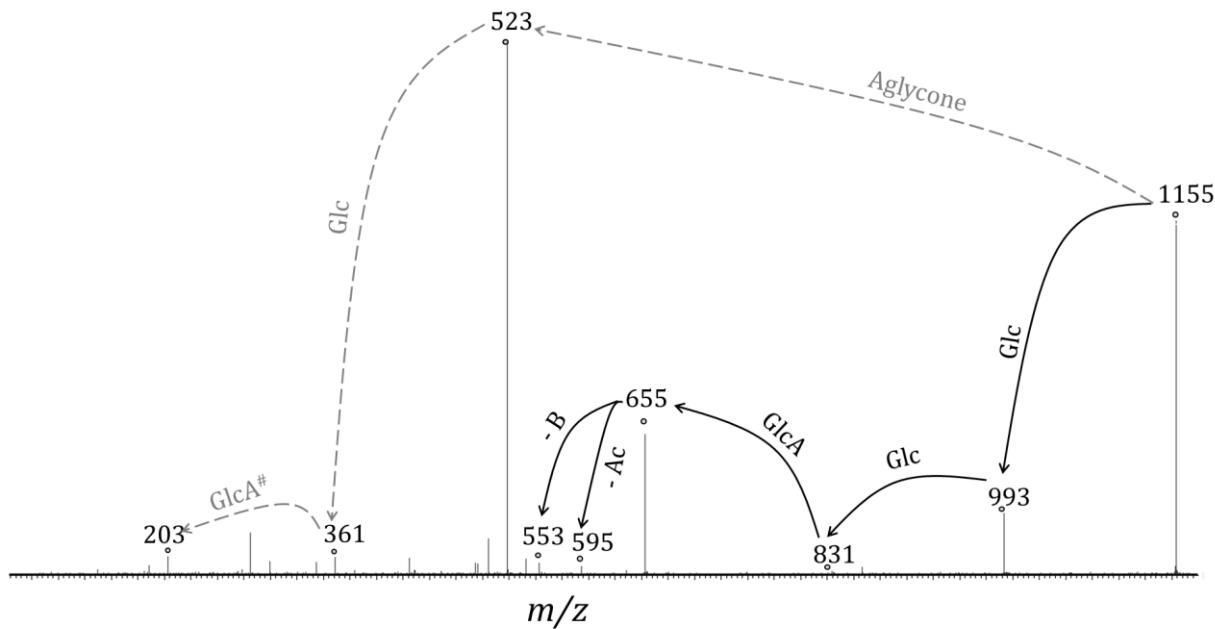


Appendix 51. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1113 precursor ions at 5.26 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Isoescin 4.

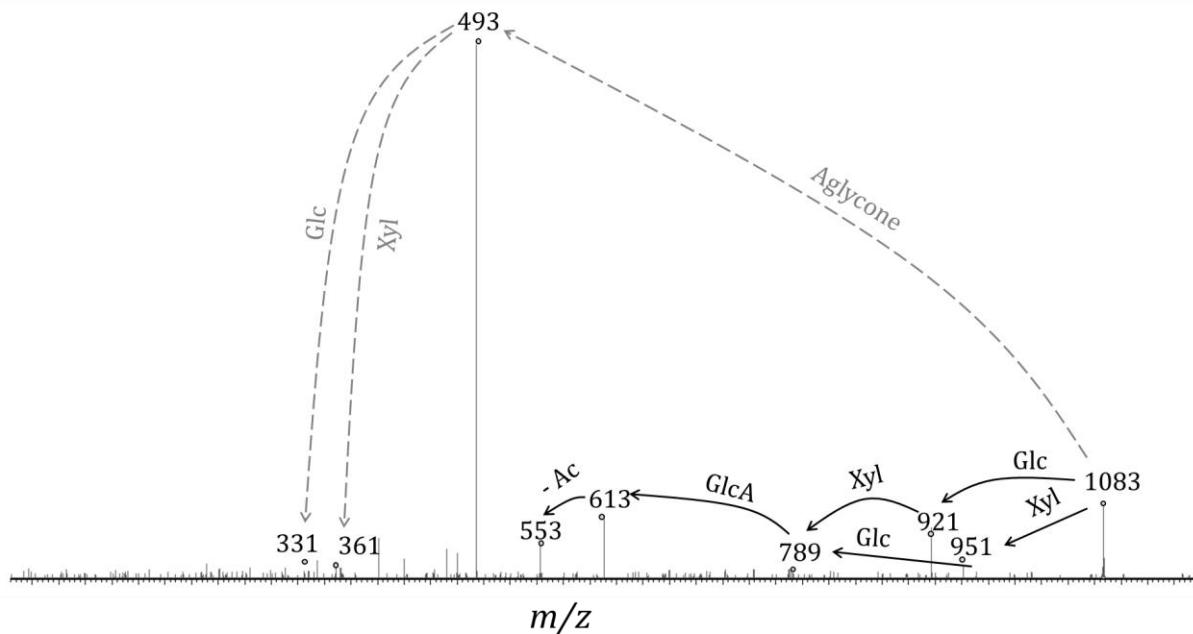


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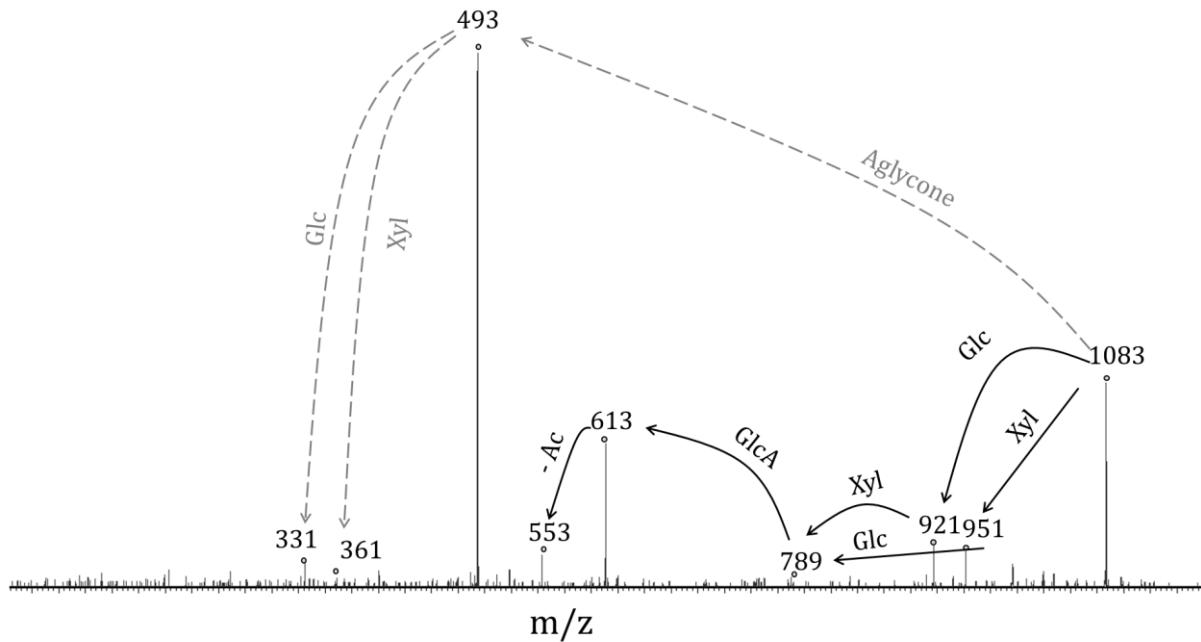
Appendix 52. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1141 precursor ions at 6.54 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 5.



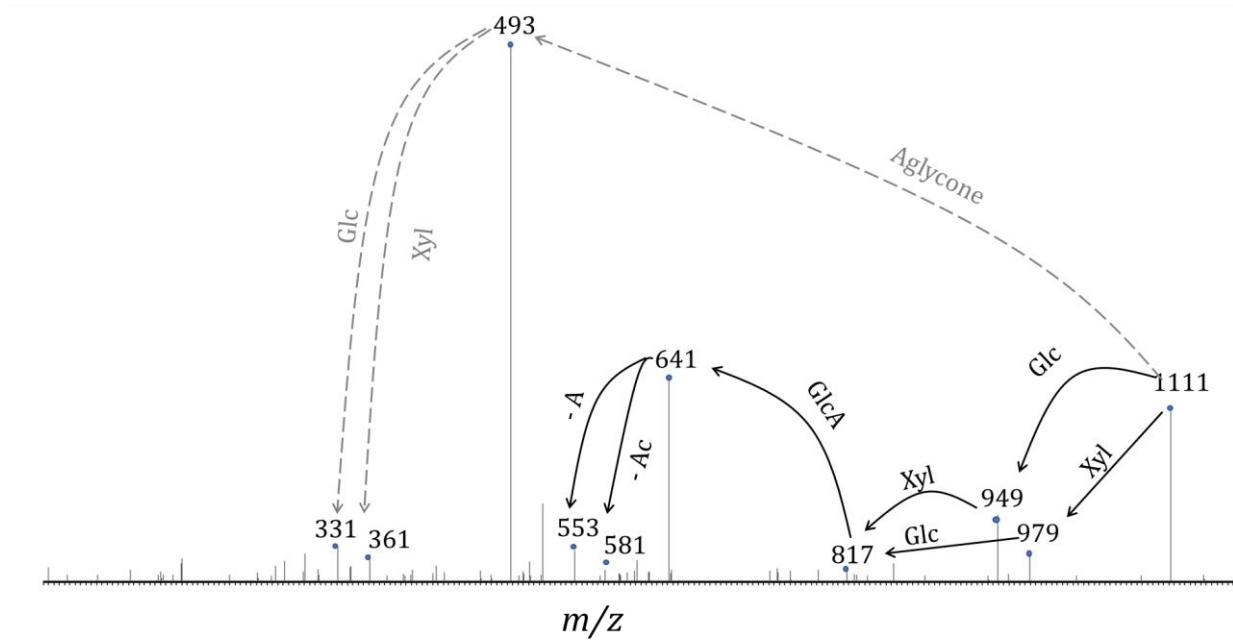
Appendix 53. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1155 precursor ions at 7.44 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 6.



Appendix 54. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1083 precursor ions at 4.72 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 7.

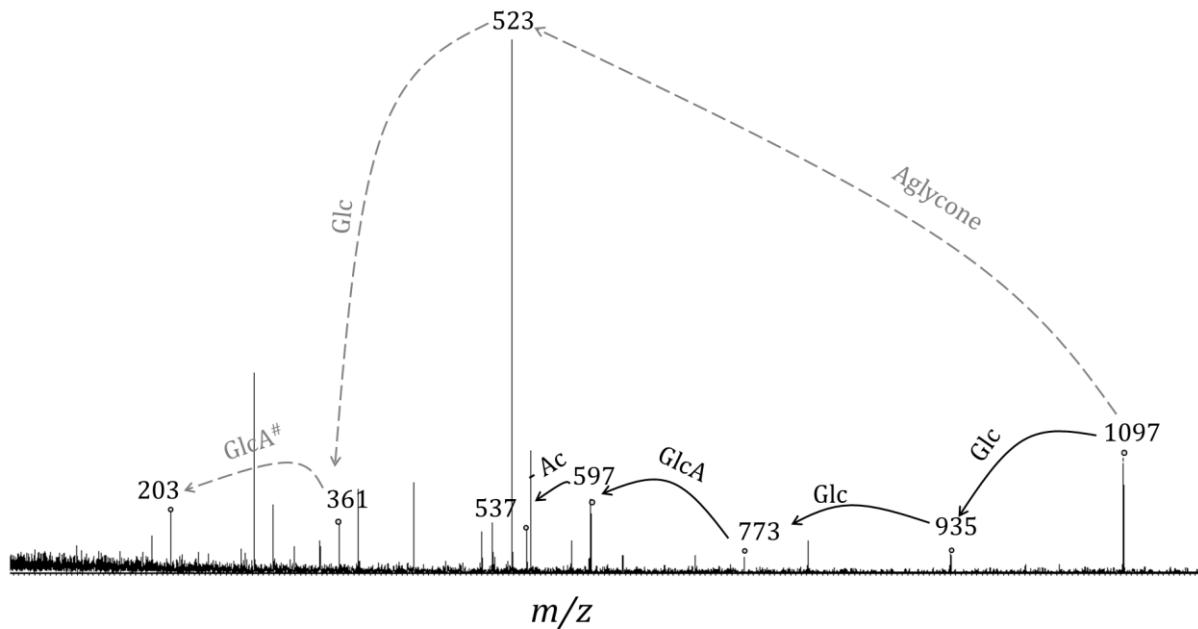


Appendix 55. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1083 precursor ions at 5.24 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Isoescin 7.



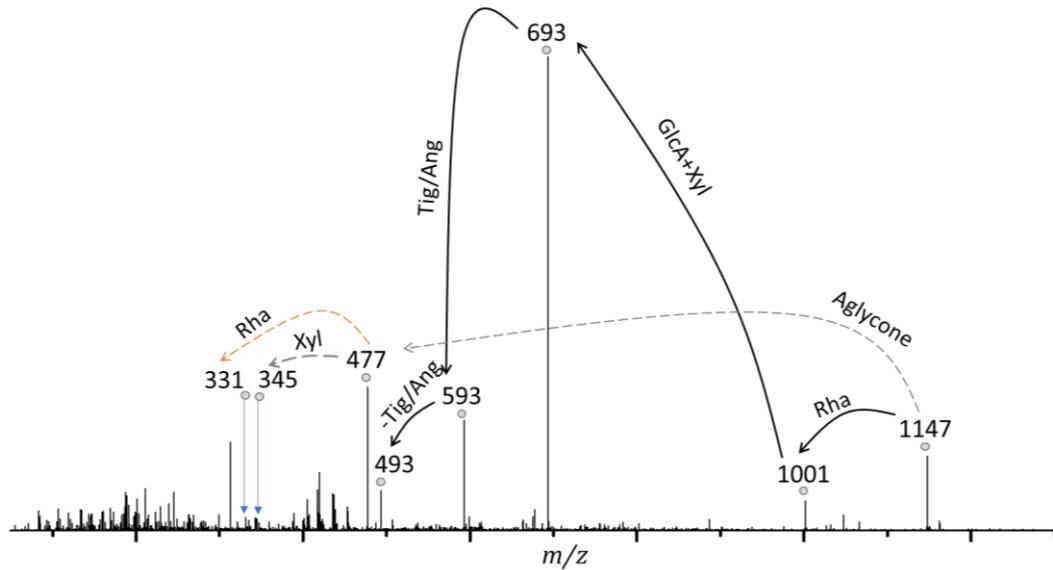
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Appendix 56. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1111 precursor ions at 6.53 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 8.

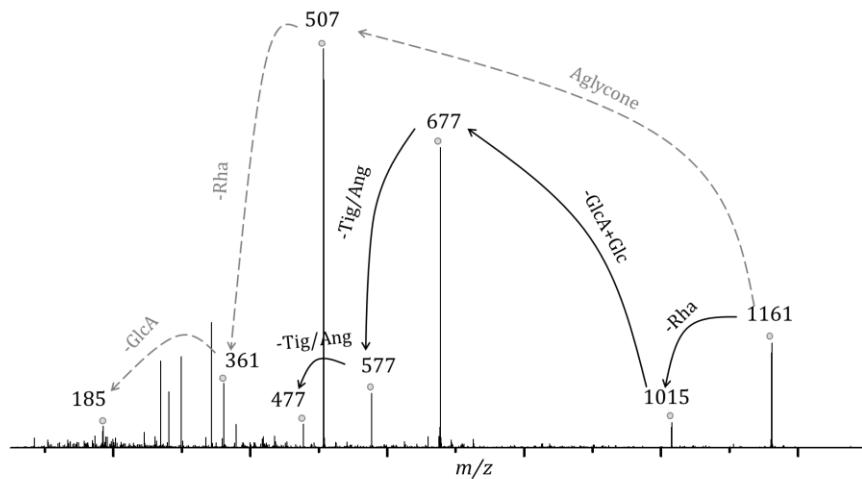


Appendix 57. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1097 precursor ions at 5.22 min retention time. The corresponding ions are assigned as [M+Na]⁺ ions from Escin 9.

2.1.3. MALDI-MSMS analysis from the burr saponin extract from *Aesculus hippocastanum*

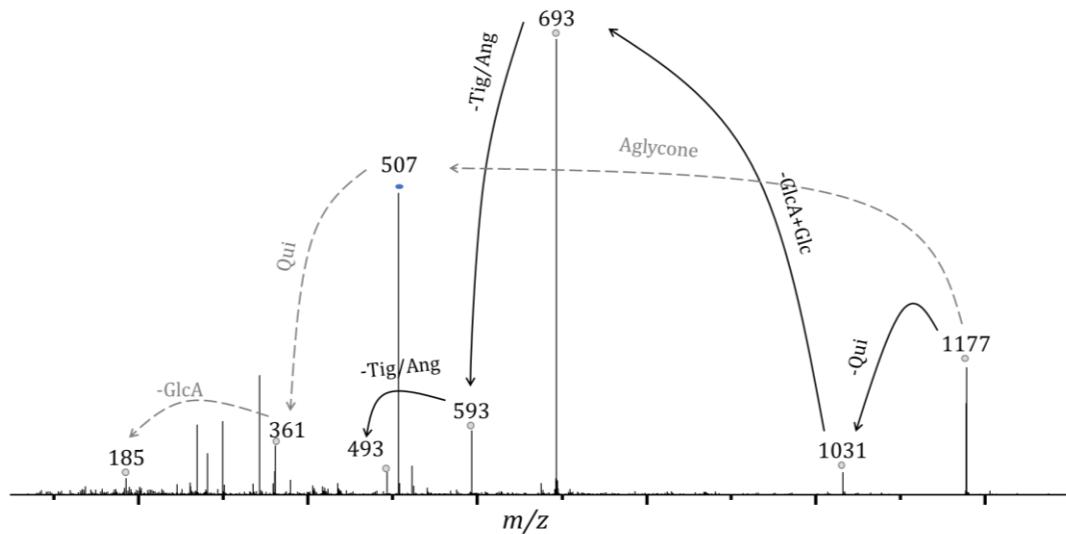


Appendix 58. MALDI-MSMS analyses of mass-selected m/z 1161.5 precursor ions - $[M+Na^+]$ BU-2 ions - from the burr saponin extract

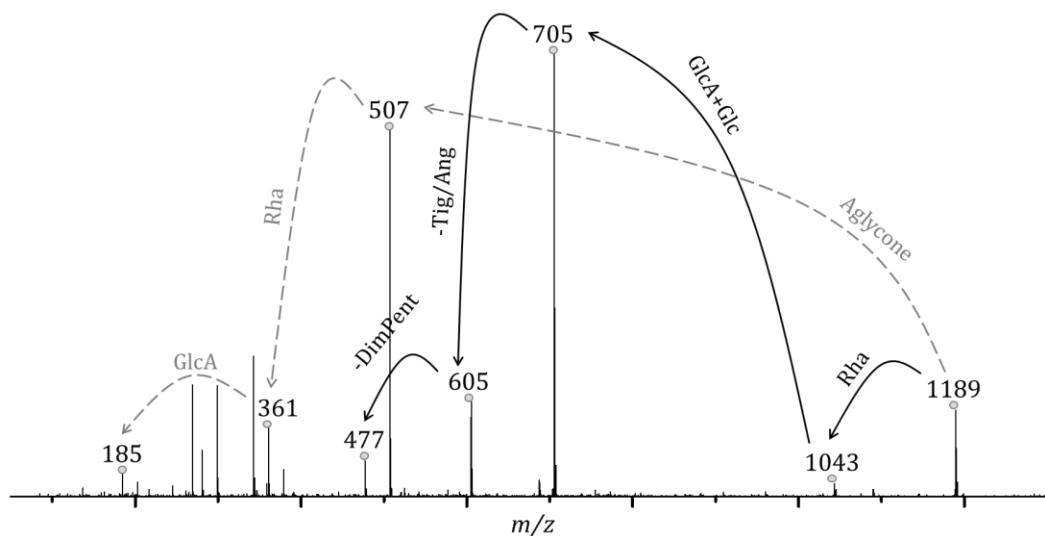


Appendix 59. MALDI-MSMS analyses of mass-selected m/z 1161.5 precursor ions - $[M+Na^+]$ BU-2 ions - from the burr saponin extract

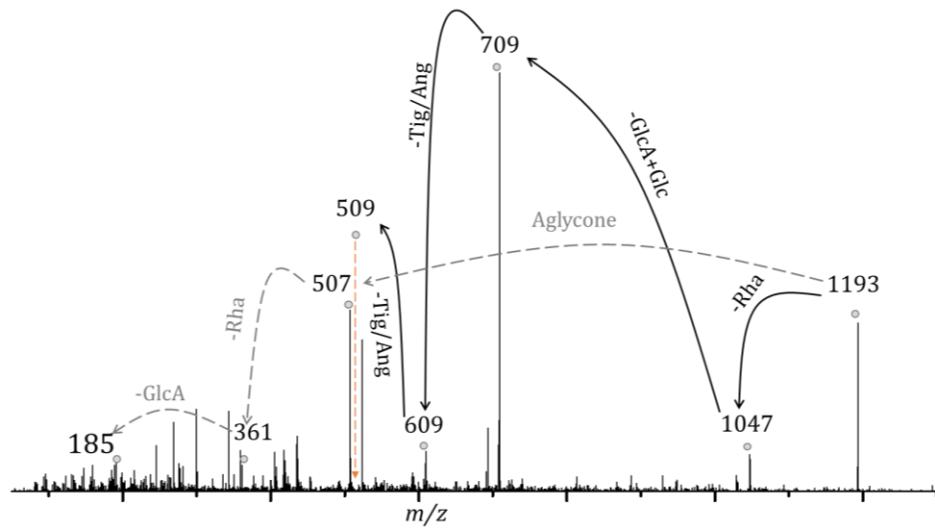
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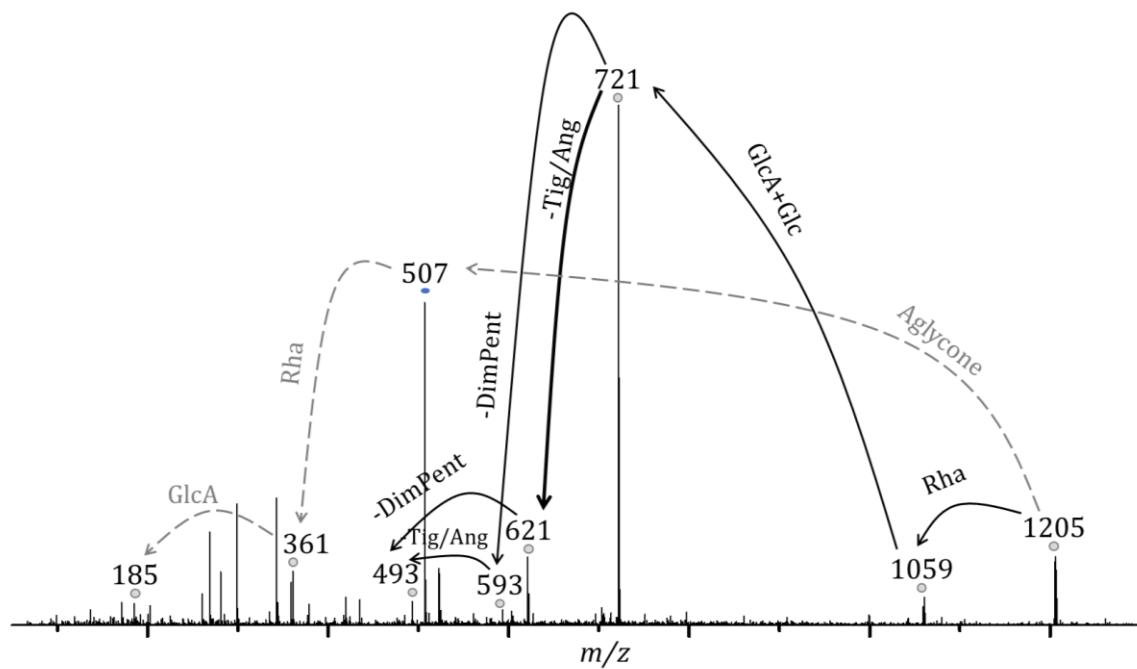
Appendix 60. MALDI-MSMS analyses of mass-selected m/z 1177.5 precursor ions - $[M+Na^+]$ BU-3 ions - from the burr saponin extract



Appendix 61. MALDI-MSMS analyses of mass-selected m/z 1189.5 precursor ions - $[M+Na^+]$ BU-4 ions - from the burr saponin extract



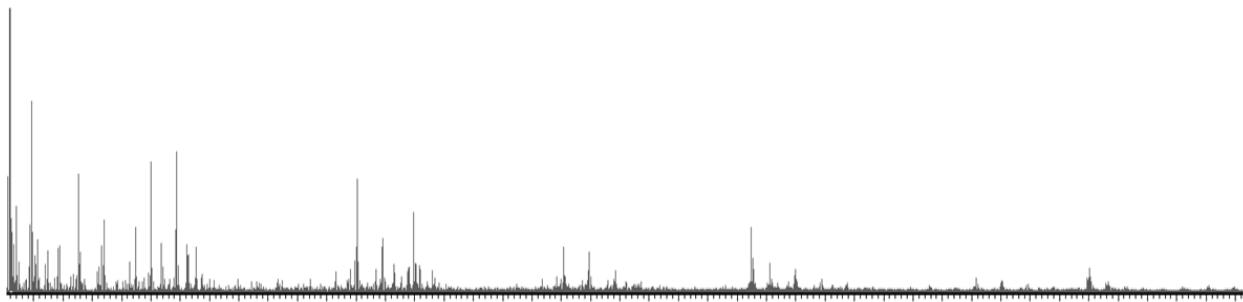
Appendix 62. MALDI-MSMS analyses of mass-selected m/z 1193.5 precursor ions - $[M+Na^+]$ BU-5 ions - from the burr saponin extract



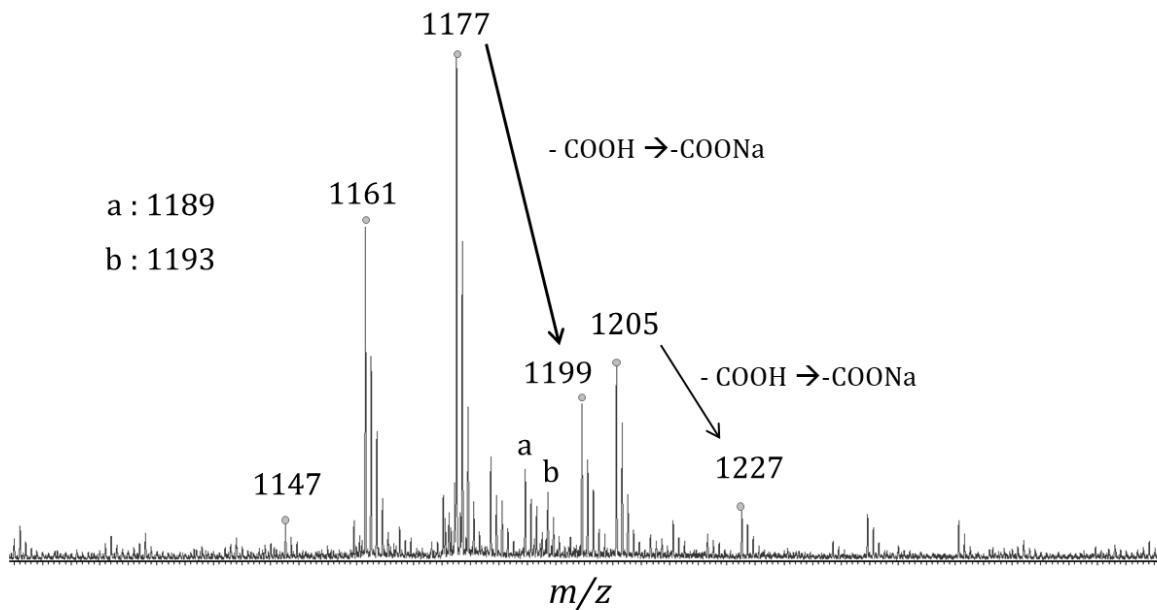
Appendix 63. MALDI-MSMS analyses of mass-selected m/z 1205.5 precursor ions - $[M+Na^+]$ BU-6 ions - from the burr saponin extract

Appendix

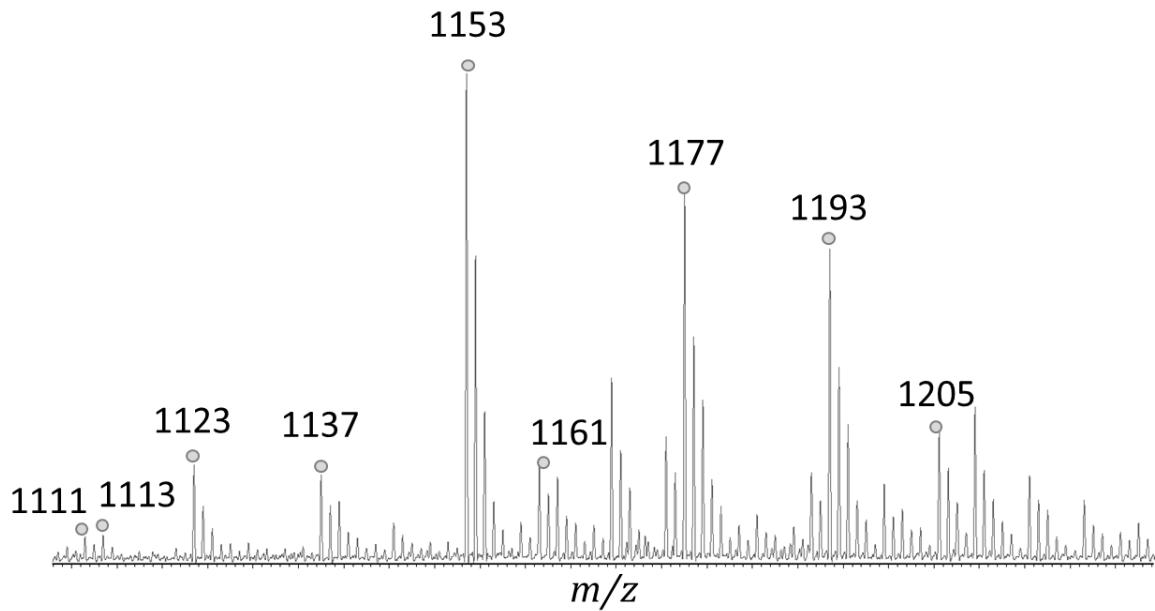
2.1.4. MALDI-ToF(+) analysis of *Aesculus hippocastanum* organs



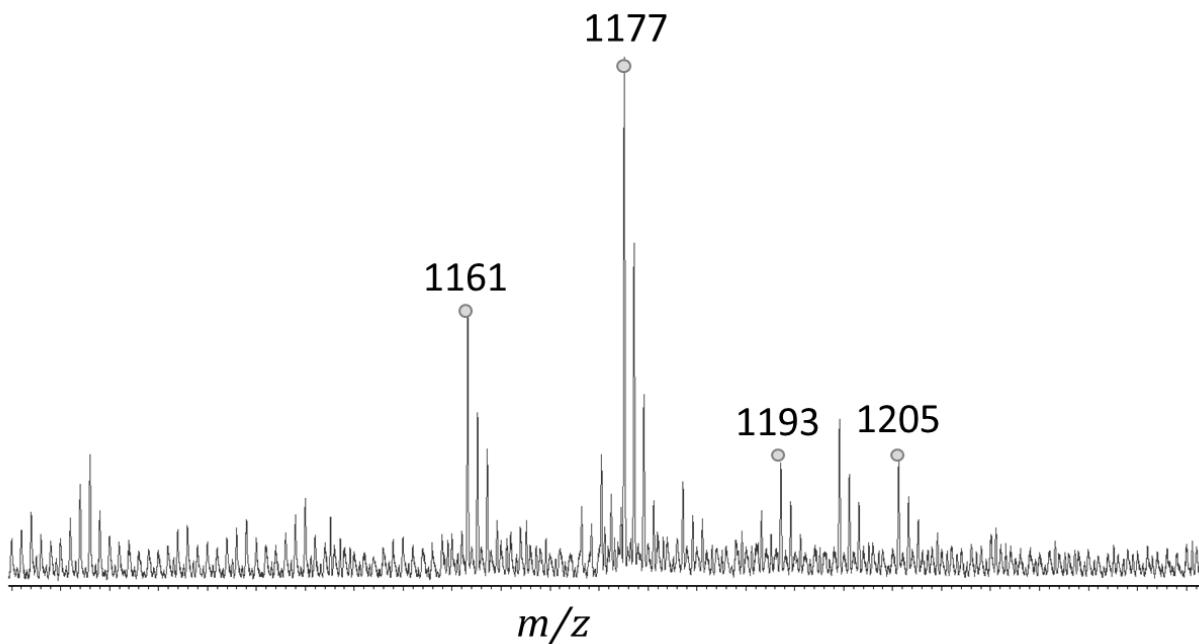
Appendix 64. MALDI-ToF(+) spectra of the shell extract from *Aesculus hippocastanum*



Appendix 65. MALDI-ToF(+) analysis of the burr extract from *Aesculus hippocastanum*

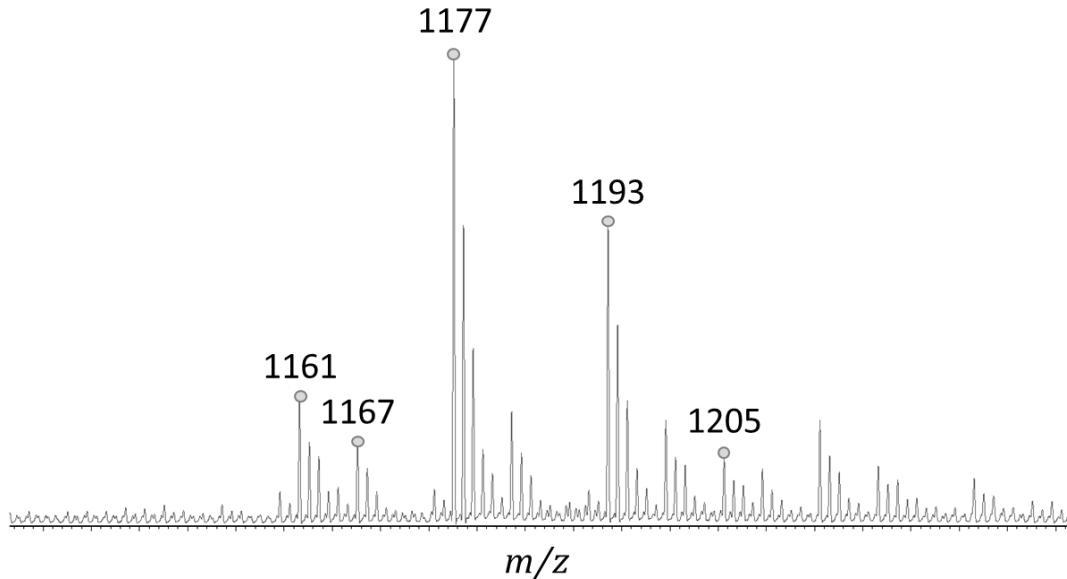


Appendix 66. MALDI-ToF(+) analysis of the root extract from *Aesculus hippocastanum*



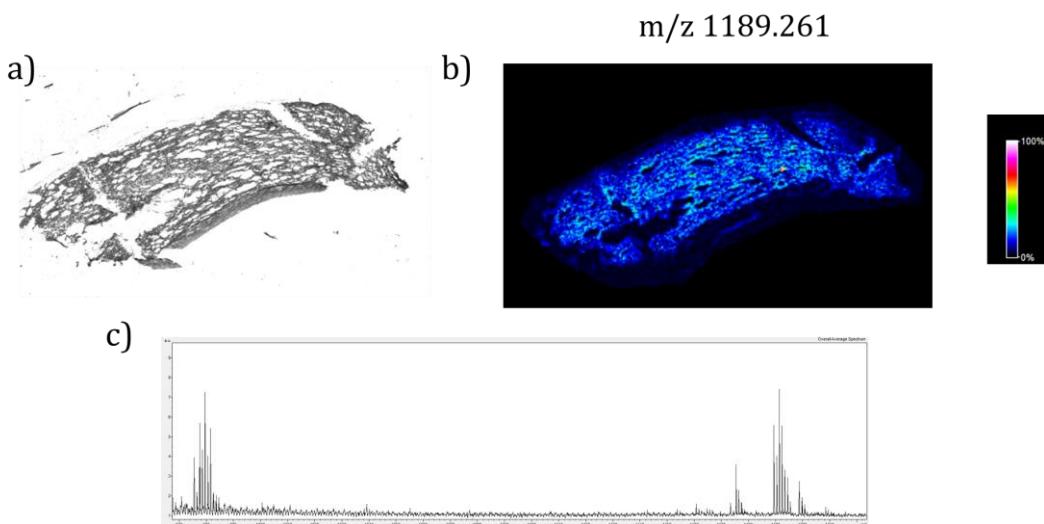
Appendix 67. MALDI-ToF(+) analysis of the flower extract from *Aesculus hippocastanum*

Appendix

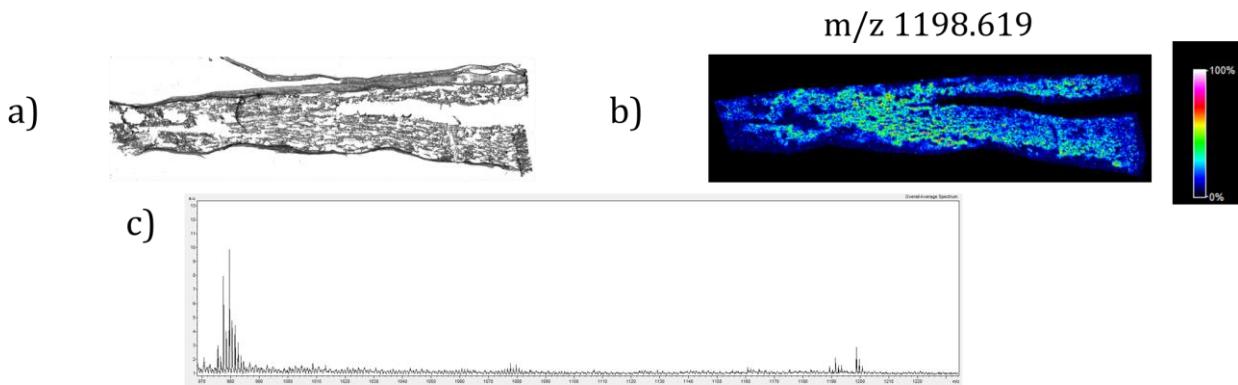


Appendix 68. MALDI-ToF(+) analysis of the stem extract from *Aesculus hippocastanum*

2.1.5. MALDI-Imaging analysis of stem and root after 4 months of germination of Horse chestnut seed

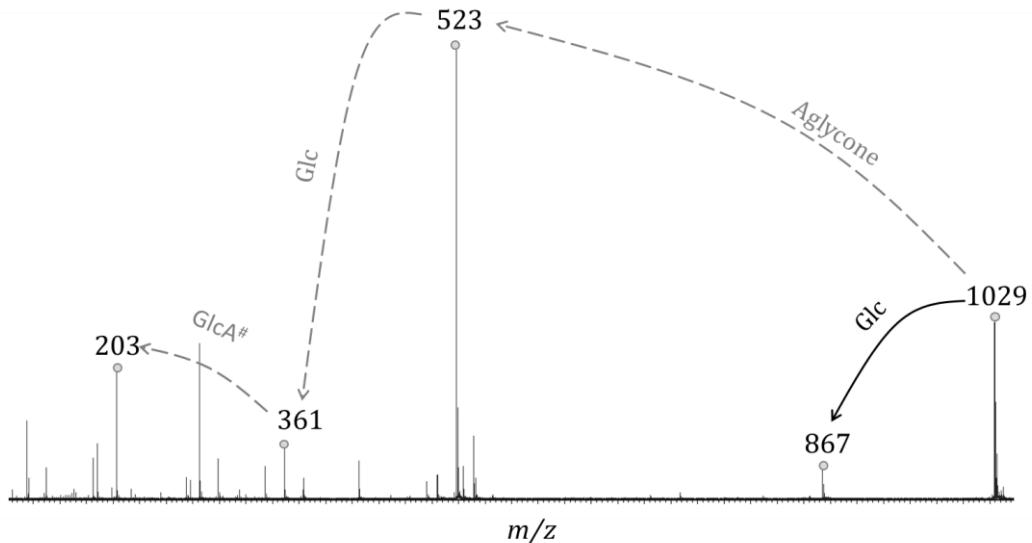


Appendix 70. MALDI-Imaging analysis of a cross section through a horse chestnut root after 4 months of germination. a) histological image of the section b) MALDI-Imaging images of m/z 1198. The color of each pixel ($2500\mu\text{m}^2$) is representative of the signal intensity. The weakest signals are indicated in blue, whereas the strongest signals are in red. c) the MALDI-Imaging (+) spectrum (B) directly acquired on a slide of the root.



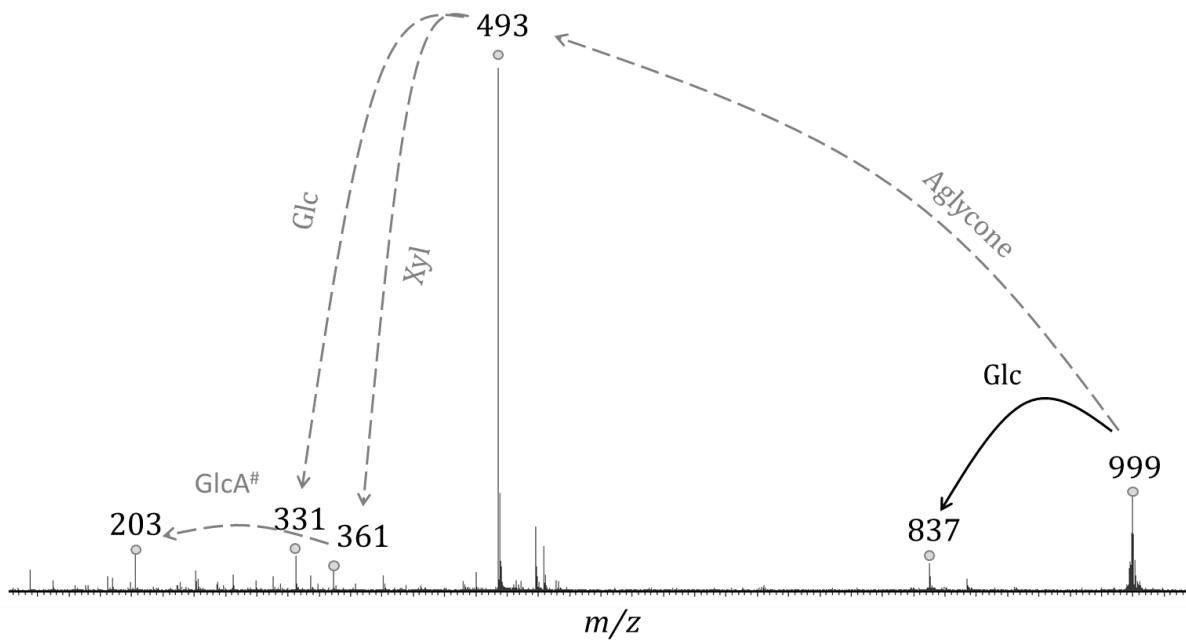
Appendix 70. MALDI-Imaging analysis of a cross section through a horse chestnut stem after 4 months of germination. a) histological image of the section b) MALDI-Imaging images of m/z 1198. The color of each pixel ($2500\mu\text{m}^2$) is representative of the signal intensity. The weakest signals are indicated in blue, whereas the strongest signals are in red. c) the MALDI-Imaging (+) spectrum (B) directly acquired on a slide of the root.

2.1.6. LC-MSMS analysis of the modified saponins from horse chestnut saponin extract

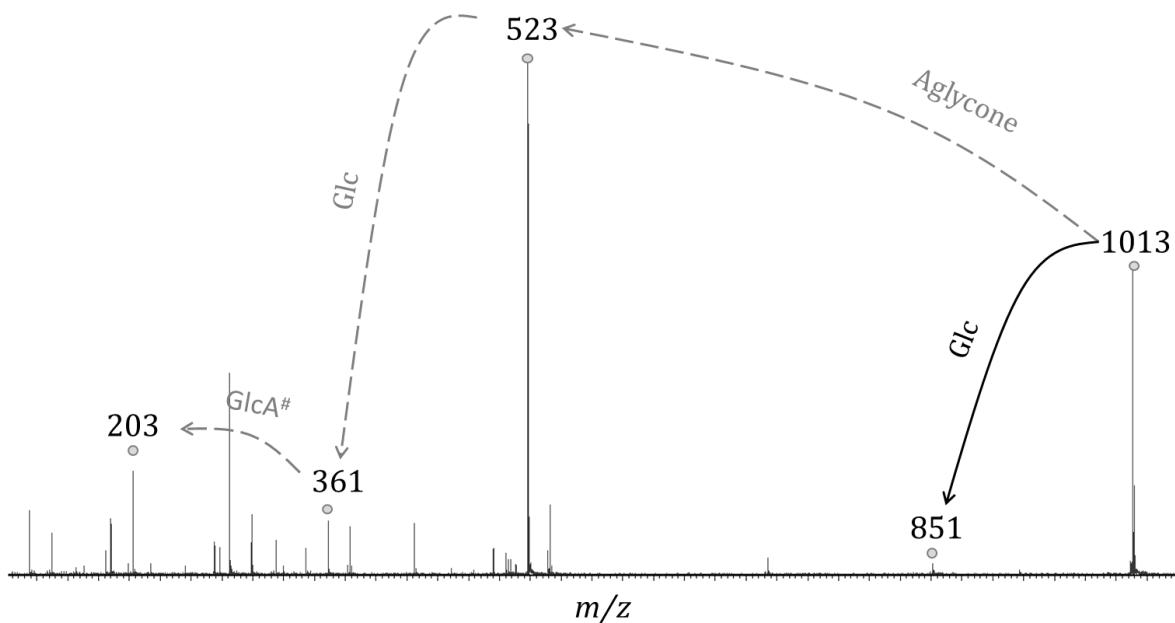


Appendix 71. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1029 precursor ions at 4.9 min retention time. The corresponding ions are assigned as $[M+\text{Na}]^+$ ions from Desacylescin 1.

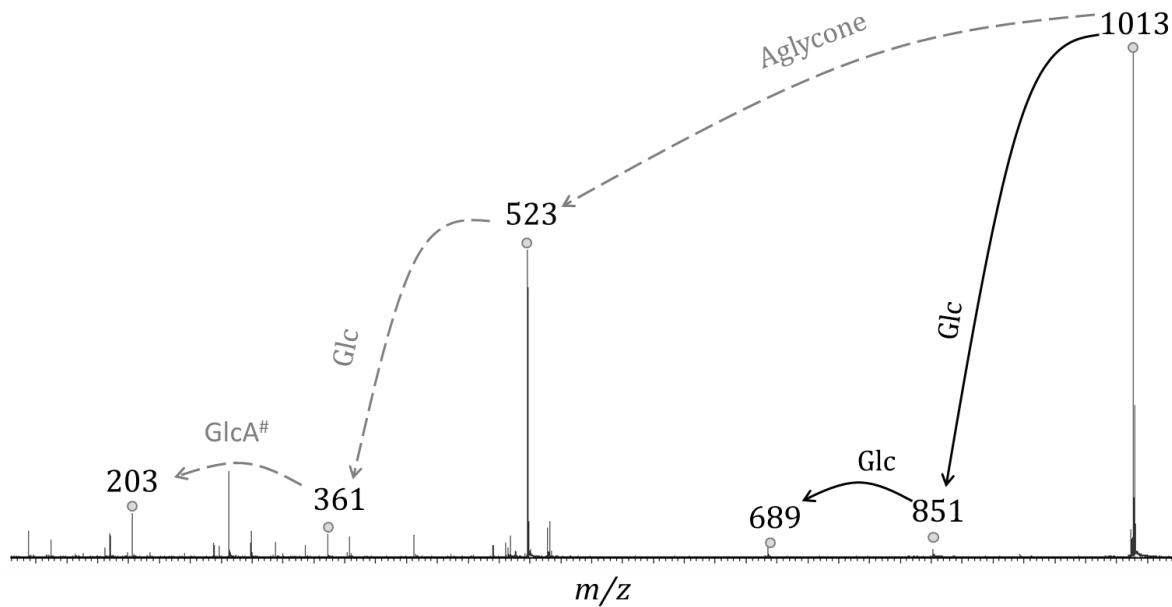
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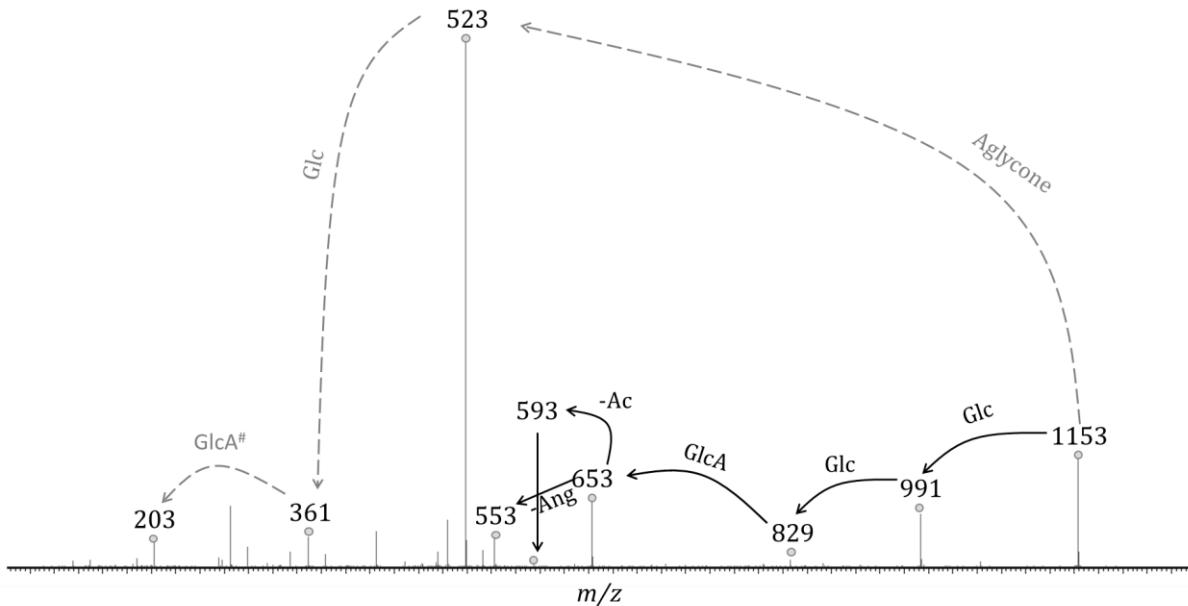
Appendix 72. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 999 precursor ions at 4.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Desacylescin 2.



Appendix 73. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1013 precursor ions at 4.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Desacylescin 3a

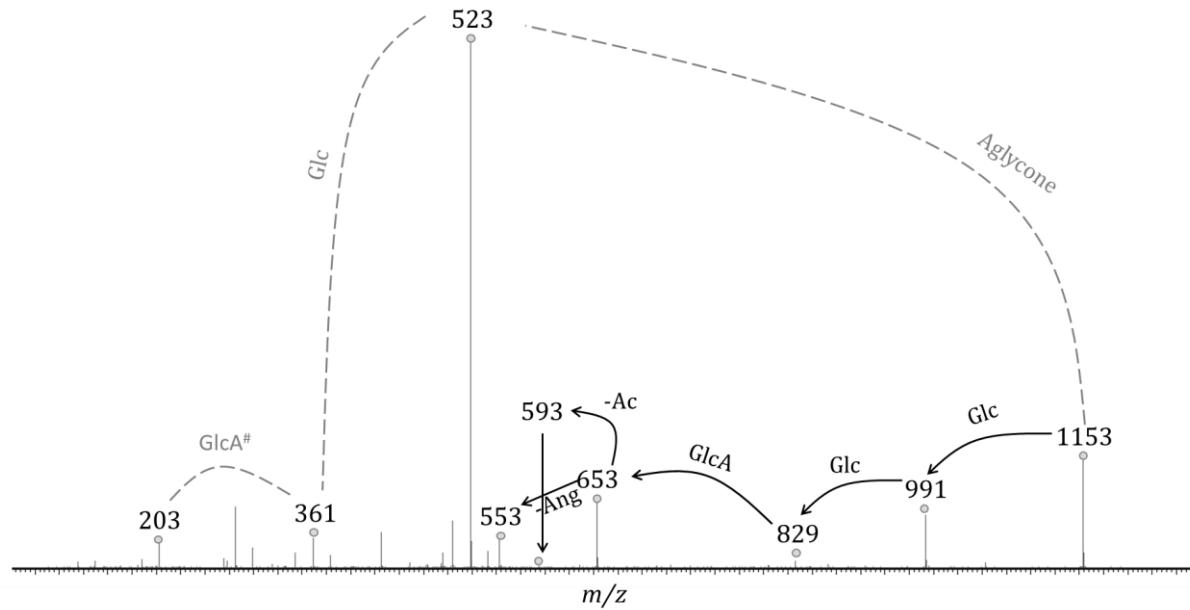


Appendix 74. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1013 precursor ions at 5.2 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Desacylescin 3b

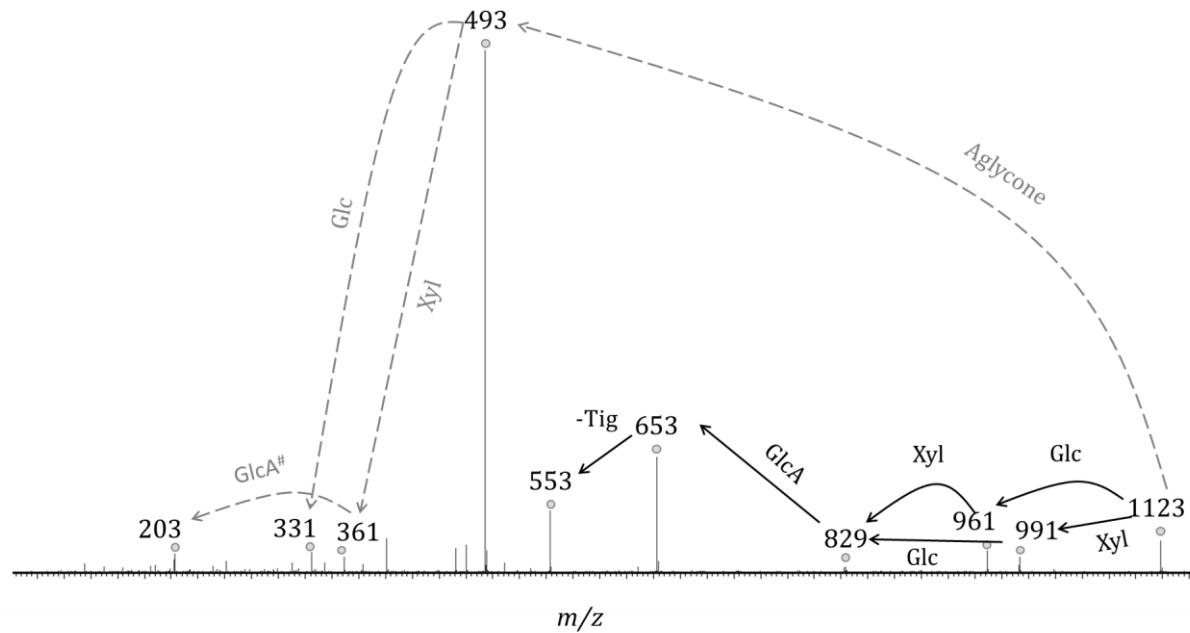


Appendix 75. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1153 precursor ions at 5.8 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 1a

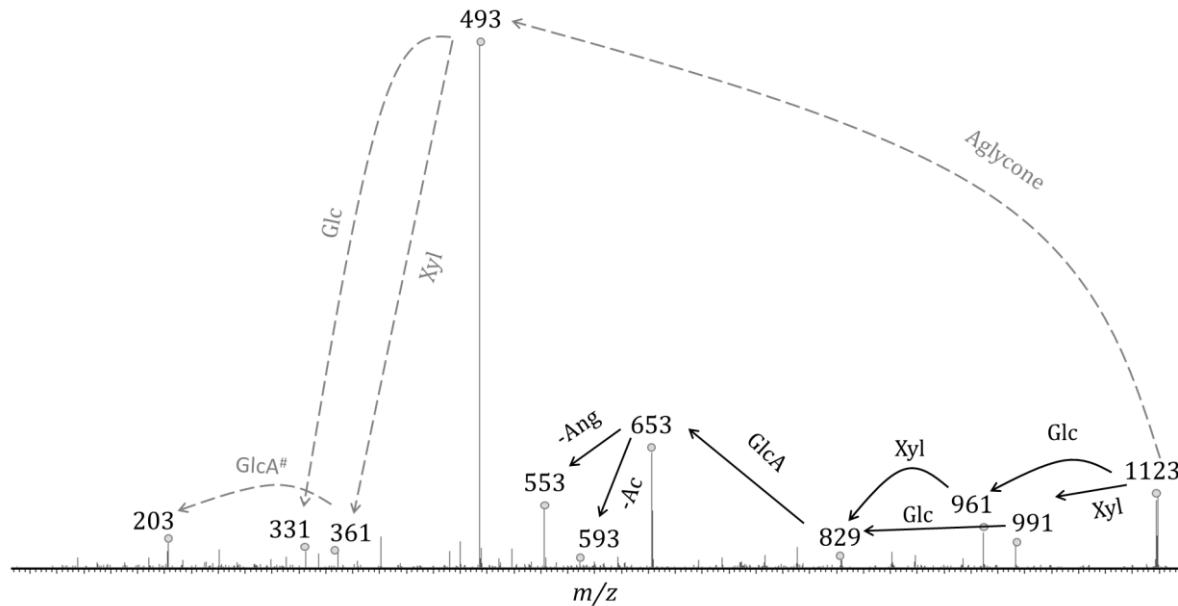
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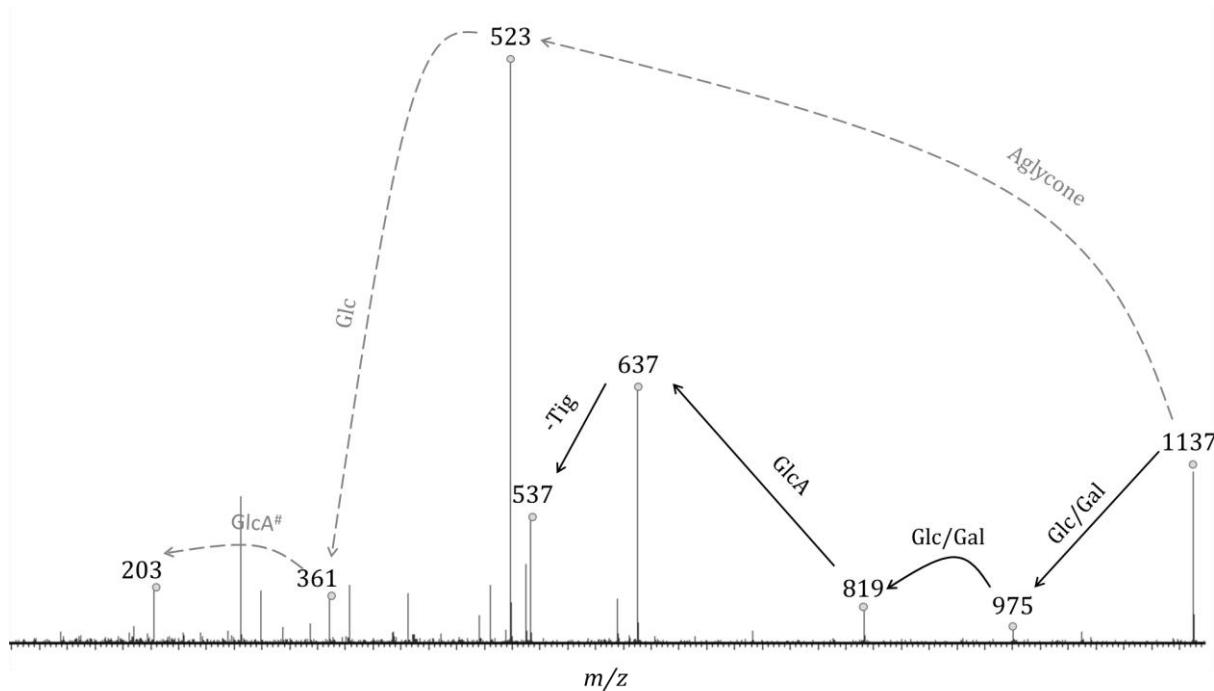
Appendix 76. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1153 precursor ions at 6.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 1b



Appendix 77. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1123 precursor ions at 5.8 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 2a

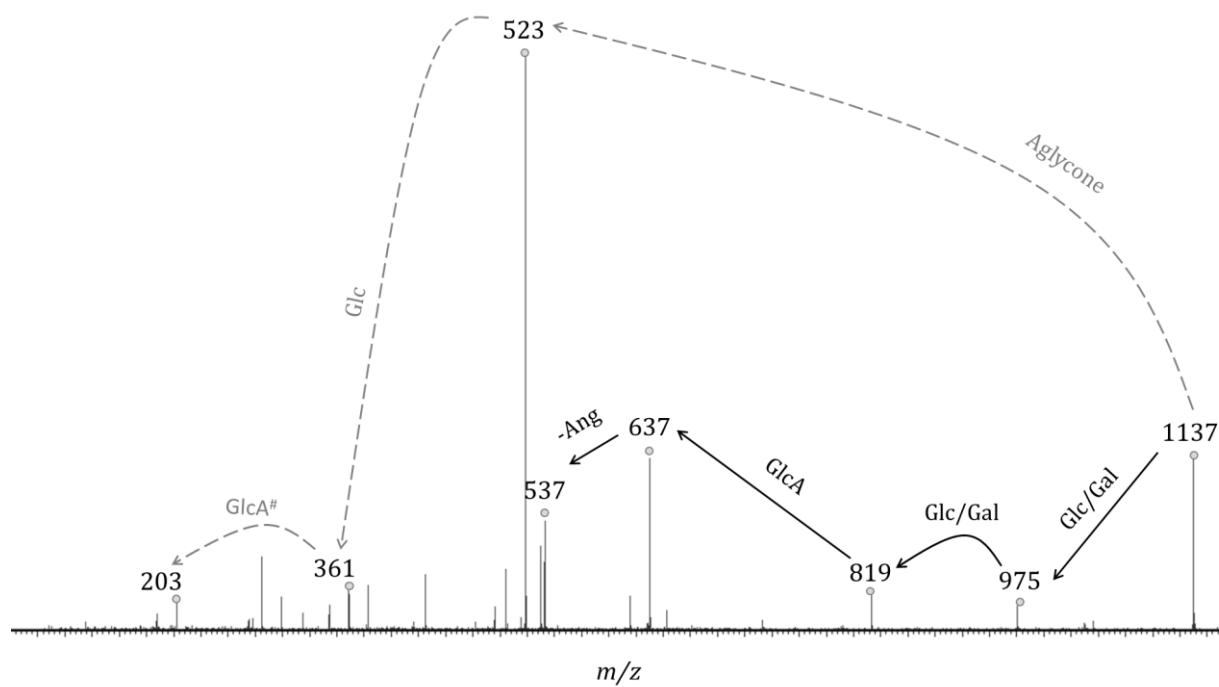


Appendix 78. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1123 precursor ions at 6.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 2b

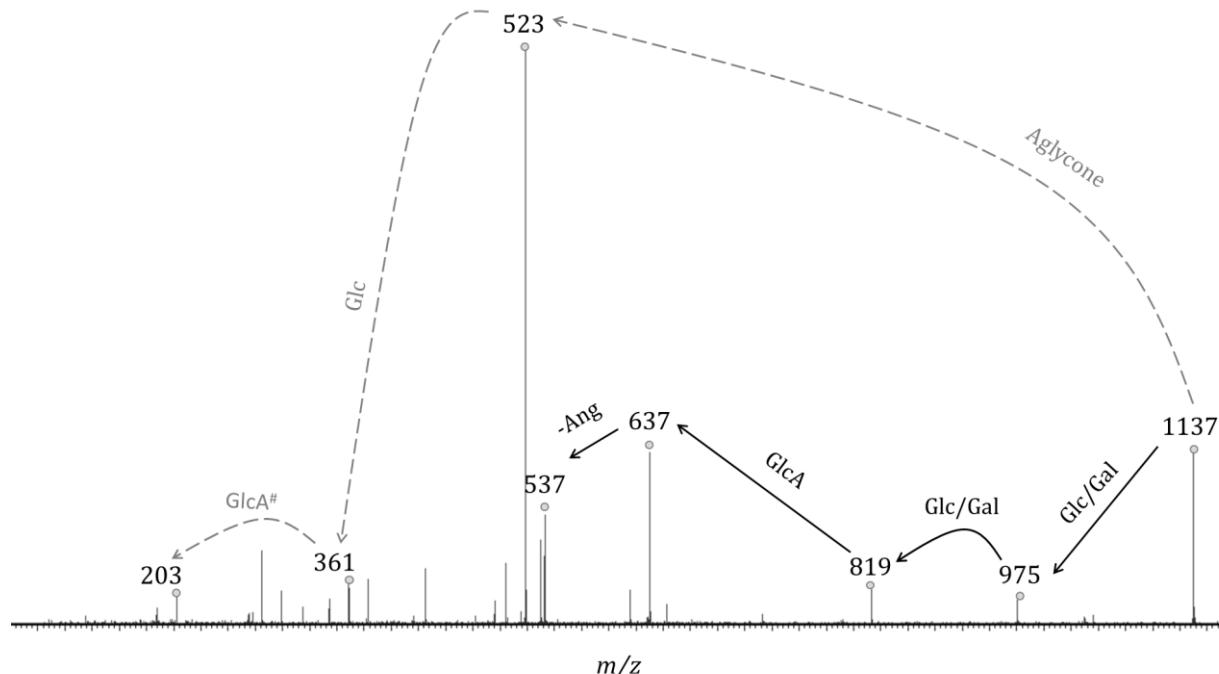


Appendix 79. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1137 precursor ions at 11.1 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Isoescin 3a

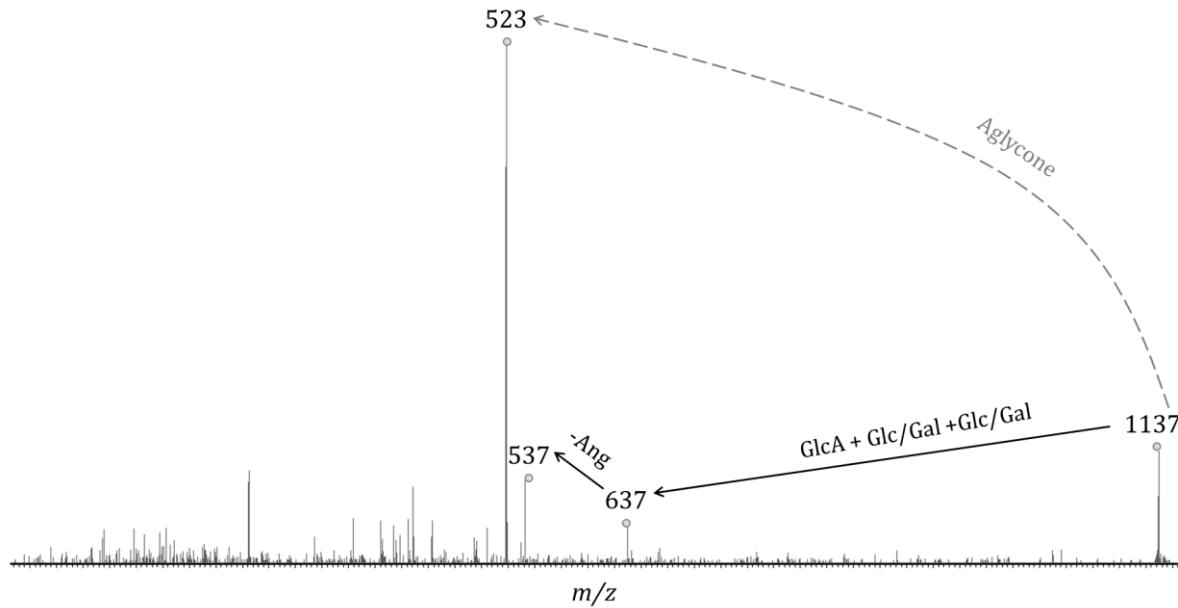
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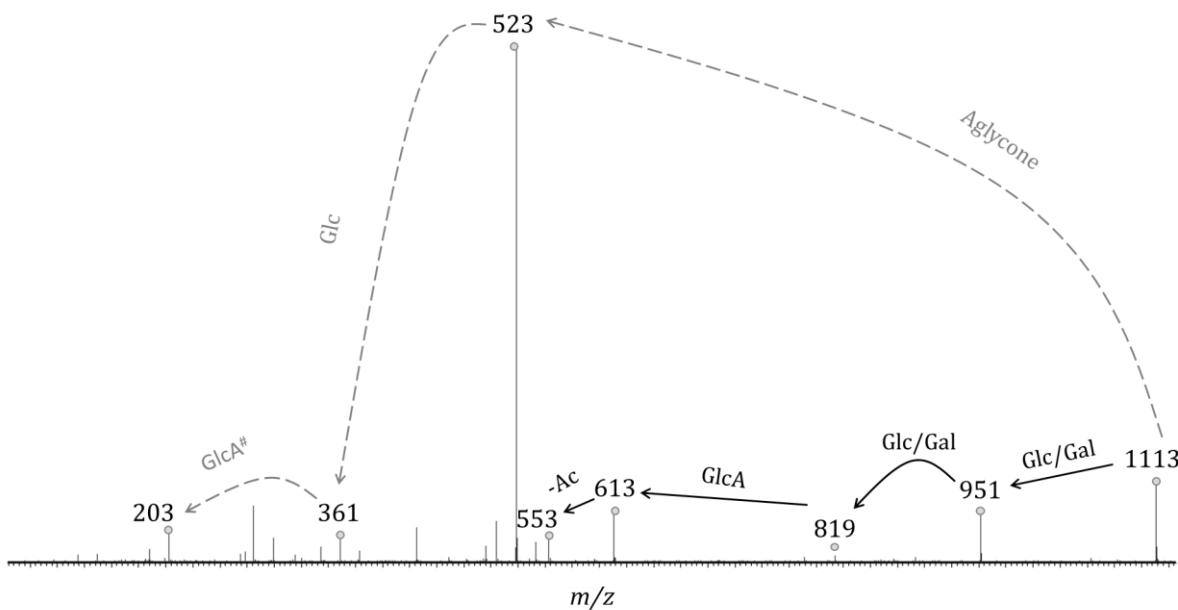
Appendix 80. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1137 precursor ions at 11.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Isoescin 3b



Appendix 81. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1137 precursor ions at 6.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 3a

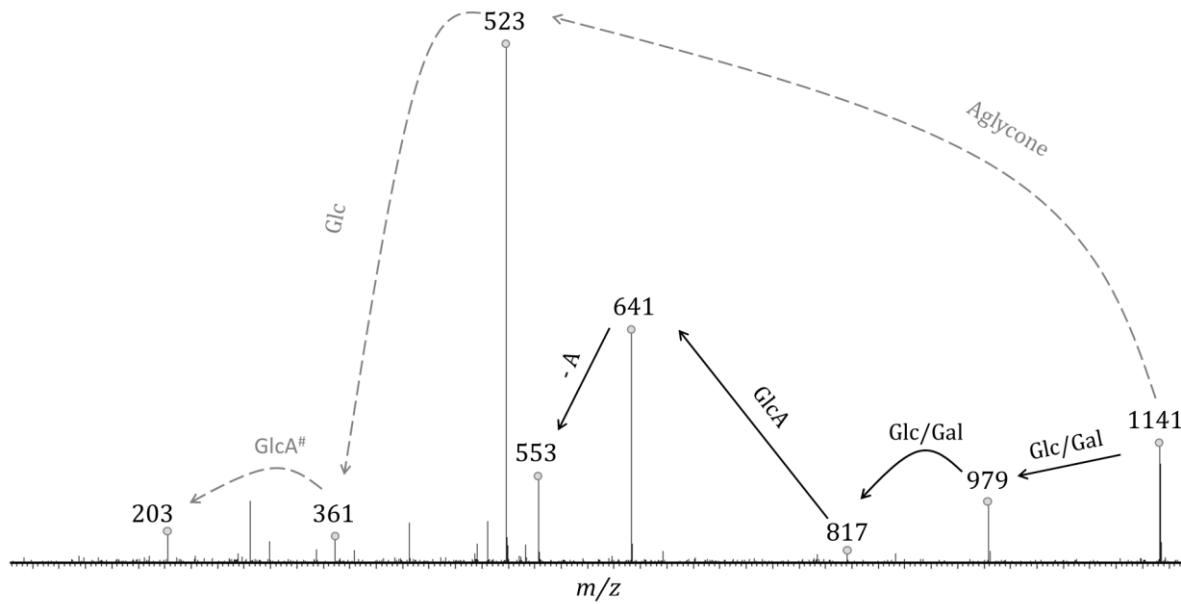


Appendix 82. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1137 precursor ions at 7.6 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 3b

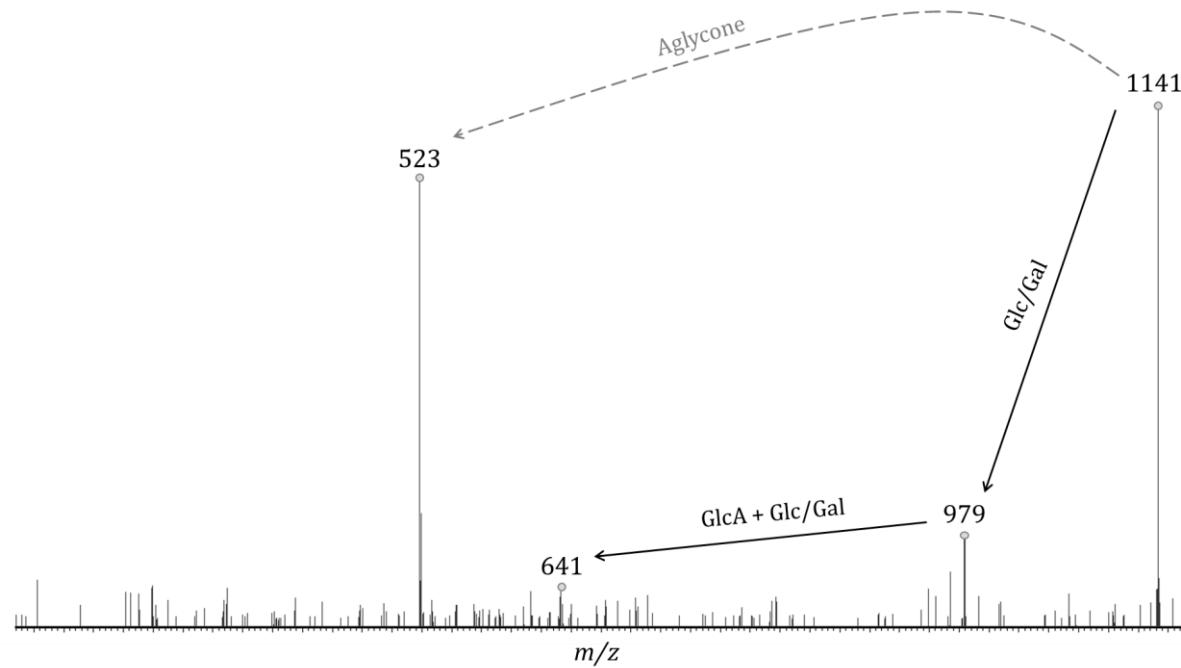


Appendix 83. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1113 precursor ions at 2.3 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 4

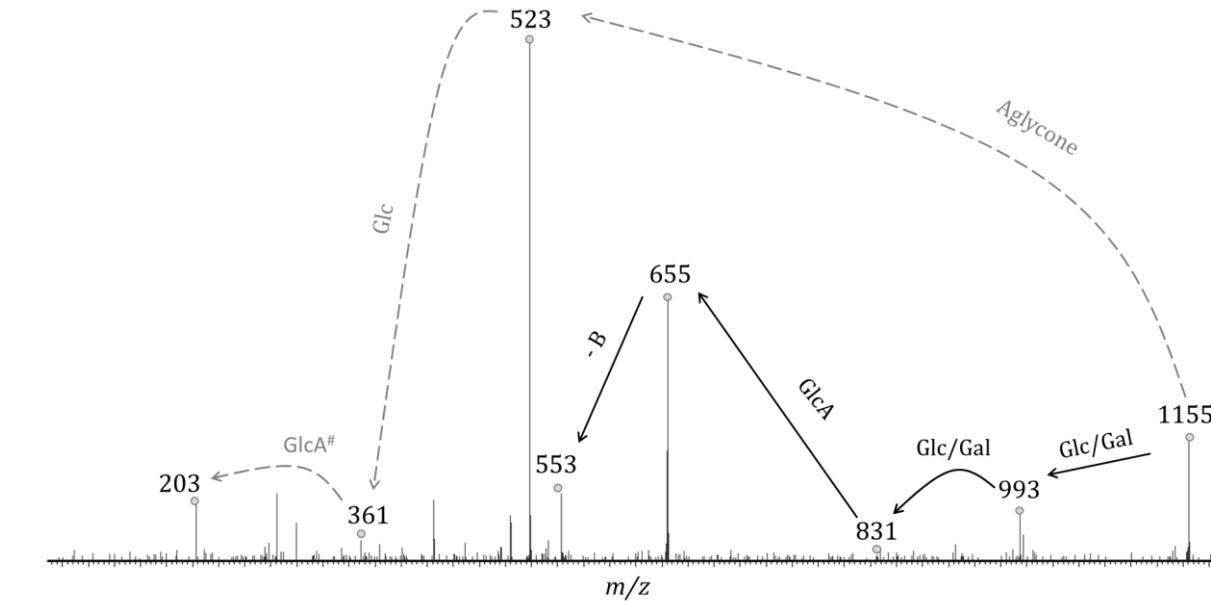
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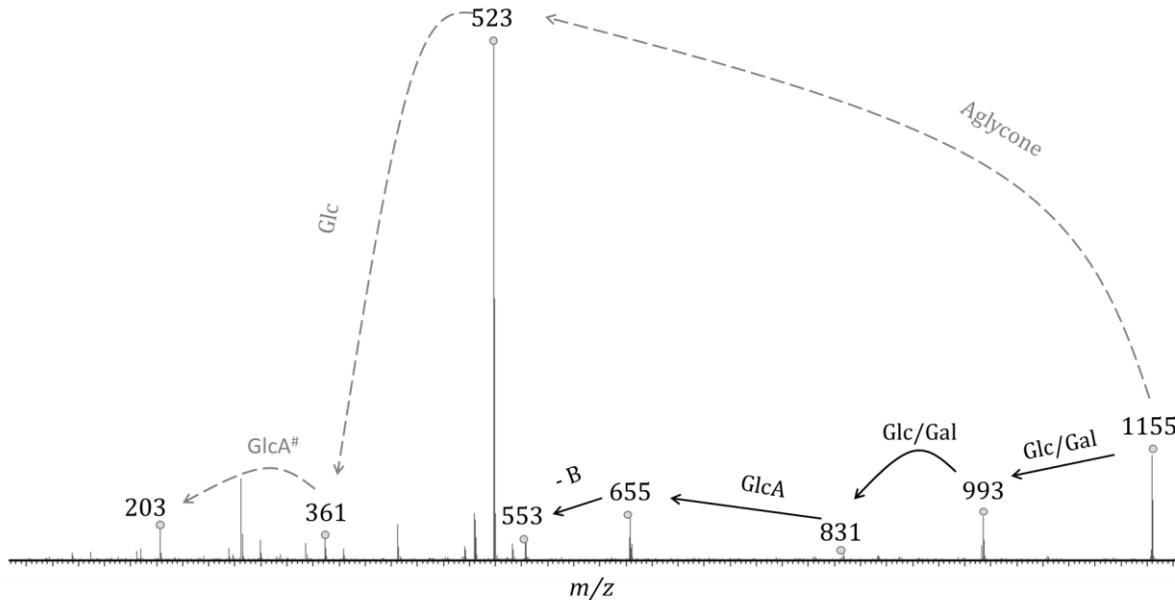
Appendix 84. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1141 precursor ions at 9.4 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Isoescin 5



Appendix 85. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1141 precursor ions at 4.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 5

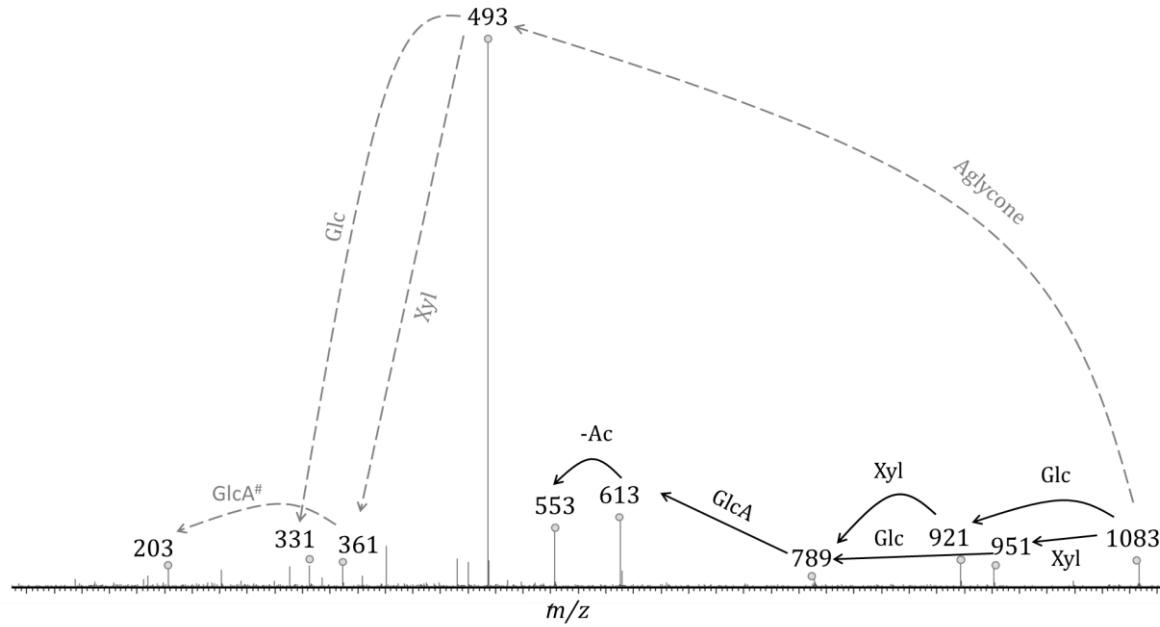


Appendix 86. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1155 precursor ions at 11.1 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Isoescin 6

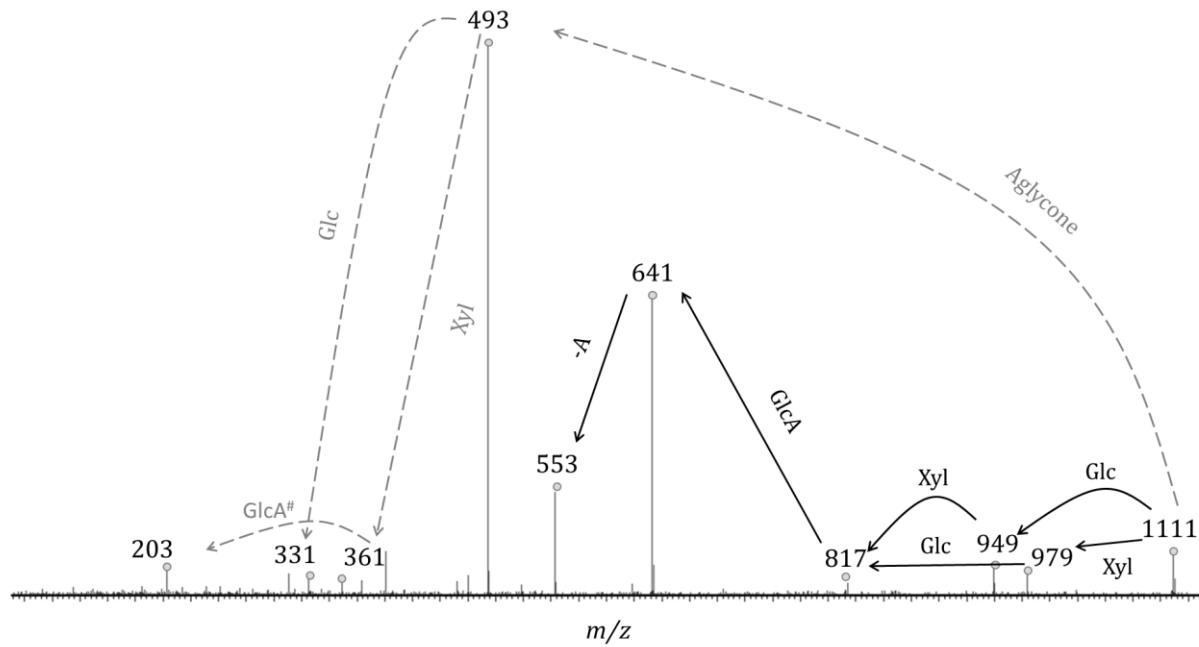


Appendix 87. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1155 precursor ions at 6.6 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 6

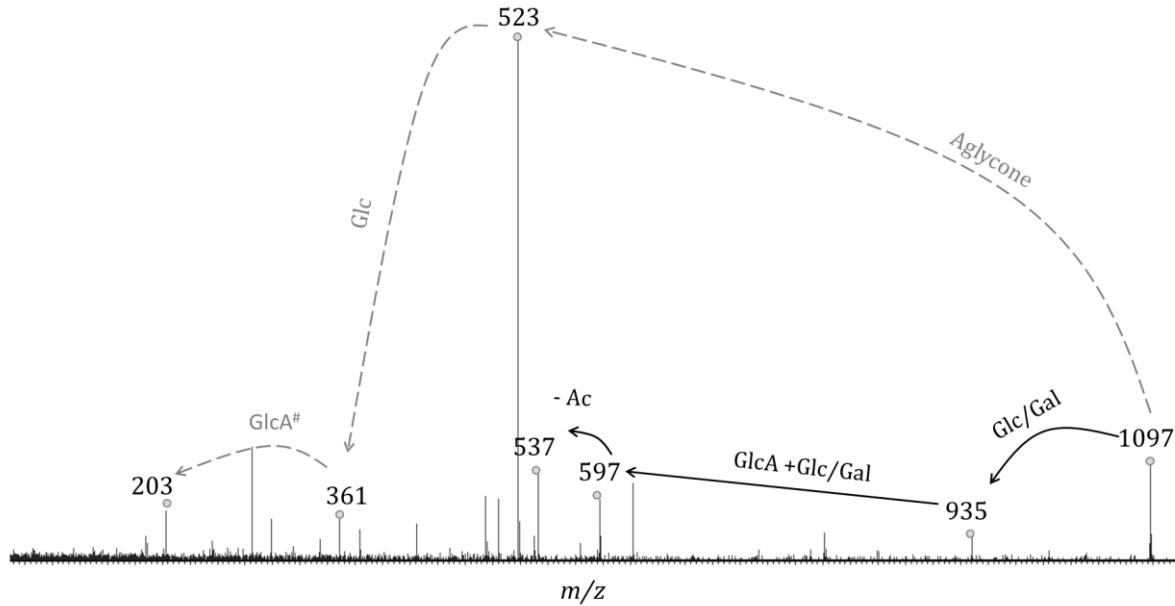
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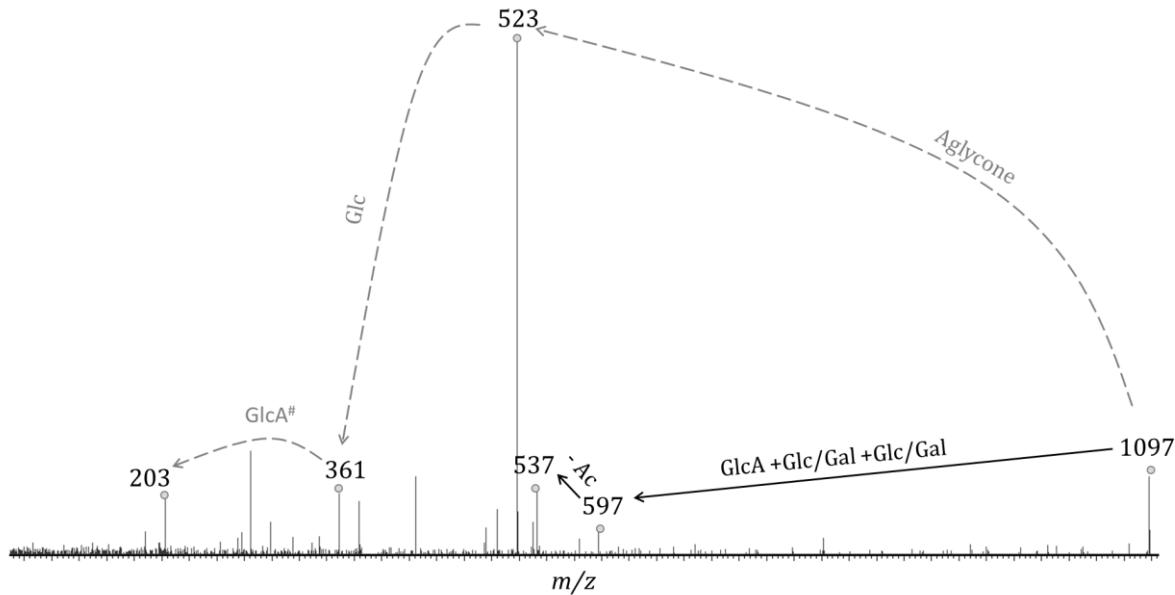
Appendix 88. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1083 precursor ions at 2.3 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 7



Appendix 89. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1111 precursor ions at 9.5 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Isoescin 8



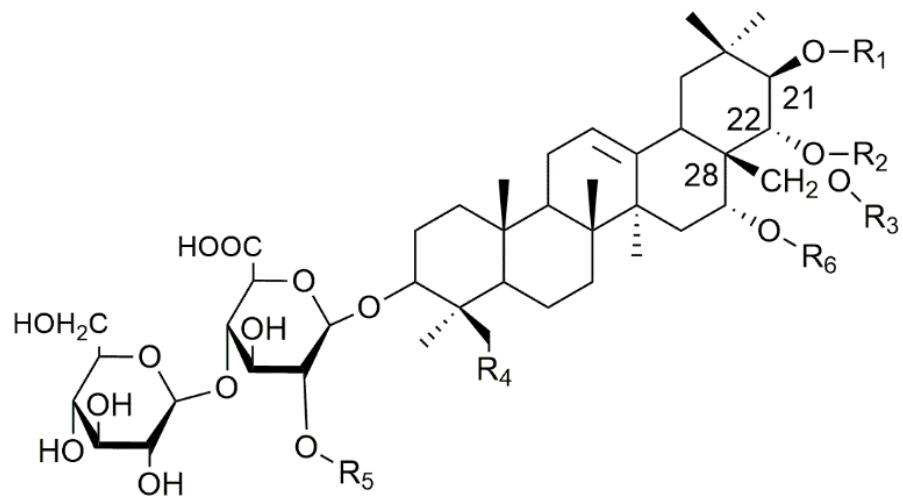
Appendix 90. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1097 precursor ions at 6.3 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from Isoescin 9



Appendix 91. LC-MSMS analysis of the horse chestnut saponin extract: CID spectrum recorded for the m/z 1097 precursor ions at 2.9 min retention time. The corresponding ions are assigned as $[M+Na]^+$ ions from γ -Escin 9

Appendix

Appendix 92. Table of Microwave-assisted of intramolecular transesterification (4min, pH 7, 120°C) of HC saponin extract: identification by MS-based methods of the saponin content. RT stands for Retention Time in LC-MS experiments., * : designation of compounds based on mass to charge ratio (no CID experiments performed



Appendix

	Composition	m/z [M+Na ⁺]	R ₁	R ₂	R ₃	R ₆	R ₄	R ₅	RT (min)
Escin 1a	$C_{55}H_{86}O_{24}$ 1153.5407 (1.8)		- Tig	- Ac	- H	- H	- OH	- Glc	9.1
Escin 1b			- Ang	- Ac	- H	- H	- OH	- Glc	9.6
Isoescin 1a			- Tig	- H	- Ac	- H	- OH	- Glc	10.3
Isoescin 1b			- Ang	- H	- Ac	- H	- OH	- Glc	10.7
γ -Escin 1a			- Tig	- H	- H	- Ac	- OH	- Glc	5.8
γ -Escin 1b			- Ang	- H	- H	- Ac	- OH	- Glc	6.5
Escin 2a	$C_{54}H_{84}O_{23}$ 1123.5301 (0.9)		- Tig	- Ac	- H	- H	- OH	- Xyl	9.1
Escin 2b			- Ang	- Ac	- H	- H	- OH	- Xyl	9.7
Isoescin 2a			- Tig	- H	- Ac	- H	- OH	- Xyl	10.4
Isoescin 2b			- Ang	- H	- Ac	- H	- OH	- Xyl	10.8
γ -Escin 2a			- Tig	- H	- H	- Ac	- OH	- Xyl	5.8
γ -Escin 2b			- Ang	- H	- H	- Ac	- OH	- Xyl	6.5
Escin 3a	$C_{55}H_{86}O_{23}$ 1137.5458 (1.4)		- Tig	- Ac	- H	- H	- H	- Gal	9.9
Escin 3b			- Ang	- Ac	- H	- H	- H	- Gal	10.6
Isoescin 3a			- Tig	- H	- Ac	- H	- H	- Gal	11.1
Isoescin 3b			- Ang	- H	- Ac	- H	- H	- Gal	11.5
γ -Escin 3a			- Tig	- H	- H	- Ac	- H	- Gal	6.9
γ -Escin 3b			- Ang	- H	- H	- Ac	- H	- Gal	7.6
Escin 4	$C_{52}H_{82}O_{24}$ 1113.5094 (1.3)		- Ac	- Ac	- H	- H	- OH	- Glc	4.4
Isoescin 4			- Ac	- H	- Ac	- H	- OH	- Glc/Gal	5.6
γ -Escin 4			- Ac	- H	- H	- Ac	- OH	- Glc/Gal	2.3
Escin 5	$C_{54}H_{86}O_{24}$ 1141.5407 (2.2)		- A	- Ac	- H	- H	- OH	- Glc	8.2
Isoescin 5			- A	- H	- Ac	- H	- OH	- Glc/Gal	9.4
γ -Escin 5			- A	- H	- H	- Ac	- OH	- Glc/Gal	4.9
Escin 6	$C_{55}H_{88}O_{24}$ 1155.5411 (4.1)		- B	- Ac	- H	- H	- OH	- Glc	9.9
Isoescin 6			- B	- H	- Ac	- H	- OH	- Glc/Gal	11.1
γ -Escin 6			- B	- H	- H	- Ac	- OH	- Glc/Gal	6.6
Escin 7	$C_{51}H_{80}O_{23}$ 1083.4988 (2.3)		- Ac	- Ac	- H	- H	- OH	- Xyl	4.4
Isoescin 7			- Ac	- H	- Ac	- H	- OH	- Xyl	5.7
γ -Escin 7			- Ac	- H	- H	- Ac	- OH	- Xyl	2.3
Escin 8	$C_{53}H_{84}O_{23}$ 1111.5301 (0.3)		- A	- Ac	- H	- H	- OH	- Xyl	8.1
Isoescin 8			- A	- H	- Ac	- H	- OH	- Xyl	9.5
γ -Escin 8 *			- A	- H	- H	- Ac	- OH	- Xyl	4.9
Escin 9	$C_{52}H_{82}O_{23}$ 1097.5145 (0.5)		- Ac	- Ac	- H	- H	- H	- Glc/Gal	5.2
Isoescin 9			- Ac	- H	- Ac	- H	- H	- Glc/Gal	6.3
γ -Escin 9			- Ac	- H	- H	- Ac	- H	- Glc/Gal	2.9

Appendix

2.1. Biological data of original and modified saponins from *Chenopodium quinoa*

2.2.1. Statistical data of antifungal classic assays

Concentration ($\mu\text{g.mL}^{-1}$)	5	10	20	50	100	200	500
Natural extract saponins	ns	ns	ns	ns	**	**	**
Hydrolyzed saponins	ns	ns	ns	ns	**	*	ns
Transesterified saponins	ns	ns	ns	ns	ns	*	**

Appendix 93. Statistical data of survival rate of *C. albicans* with HC saponins (Natural extract, hydrolyzed and transesterified) where ns = non-significant. P-value in green = inhibitory effect and P-value in red = amplification effect. P-value : * <0.05 ; **<0.01;***<0,001.

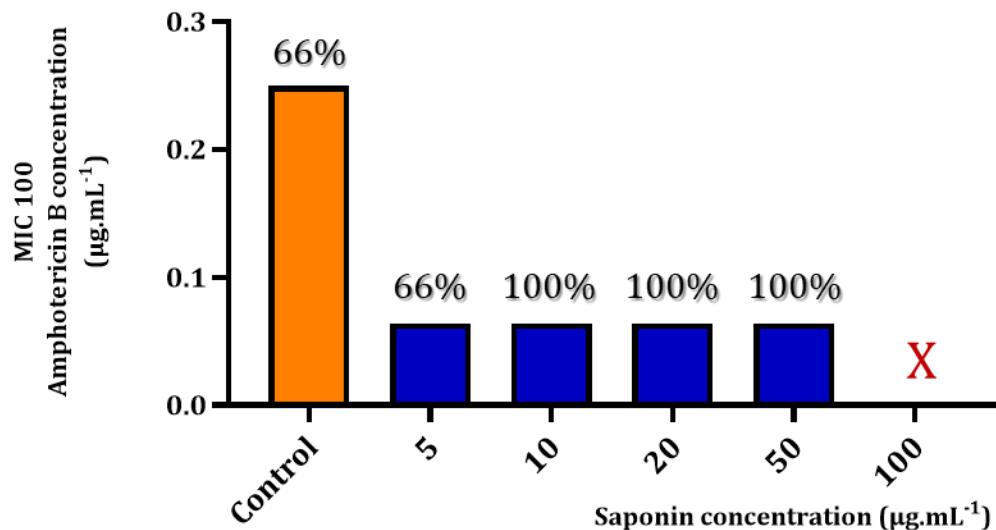
Concentration ($\mu\text{g.mL}^{-1}$)	5	10	20	50	100	200	500
Natural extract saponins	ns	ns	*	ns	ns	ns	ns
Hydrolyzed saponins	ns	ns	***	**	***	ns	*
Transesterified saponins	ns	ns	ns	ns	ns	ns	ns

Appendix 94. Statistical data of survival rate of *A. fumigatus* with HC saponins (Natural extract, hydrolyzed and transesterified) where ns = non-significant. P-value in green = inhibitory effect and P-value in red = amplification effect. P-value : * <0.05 ; **<0.01;***<0,001.

Concentration ($\mu\text{g.mL}^{-1}$)	5	10	20	50	100	200	500
Natural extract saponins	ns	ns	ns	ns	ns	ns	*
Hydrolyzed saponins	ns	ns	ns	ns	ns	ns	ns
Transesterified saponins	ns	ns	ns	ns	ns	ns	*

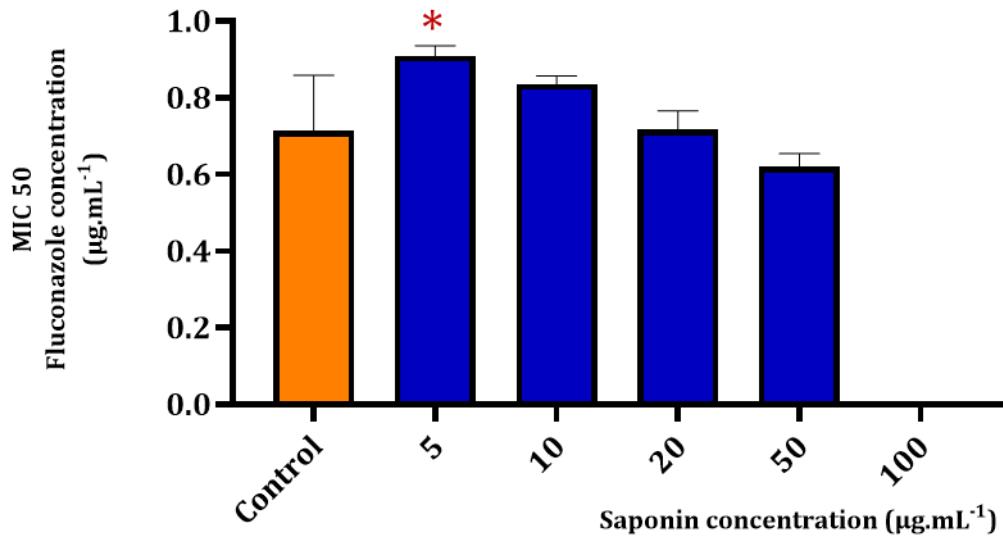
Appendix 95. Statistical data of survival rate of *T. interdigitale* with HC saponins (Natural extract, hydrolyzed and transesterified) where ns = non-significant. P_{value} in green = inhibitory effect and P_{value} in red = amplification effect. $P_{\text{value}} : * < 0.05 ; ** < 0.01 ; * < 0.001$.**

2.2.2. Additive assays

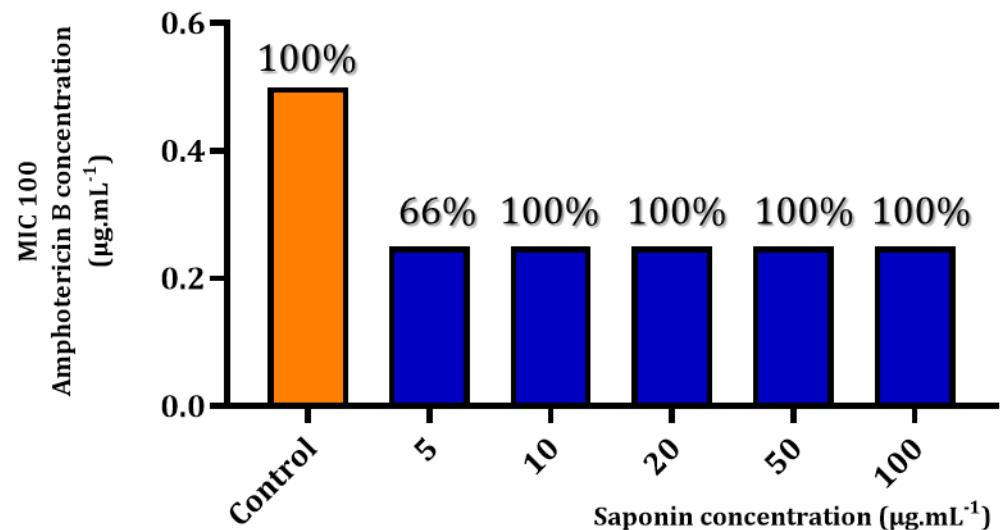


Appendix 96. Estimation of the antifungal activity of the natural extract saponins from *Aesculus hippocastanum* combined with Amphotericin B on *C. albicans*. An additive effect is already observed at a saponin concentration of $5 \mu\text{g.mL}^{-1}$. The value above the column represents the reproducibility of the analysis (based on three assays).

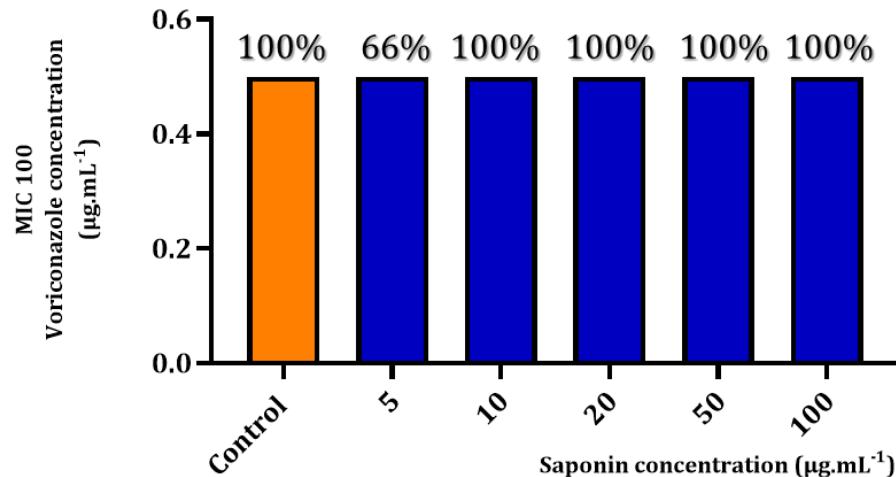
Appendix



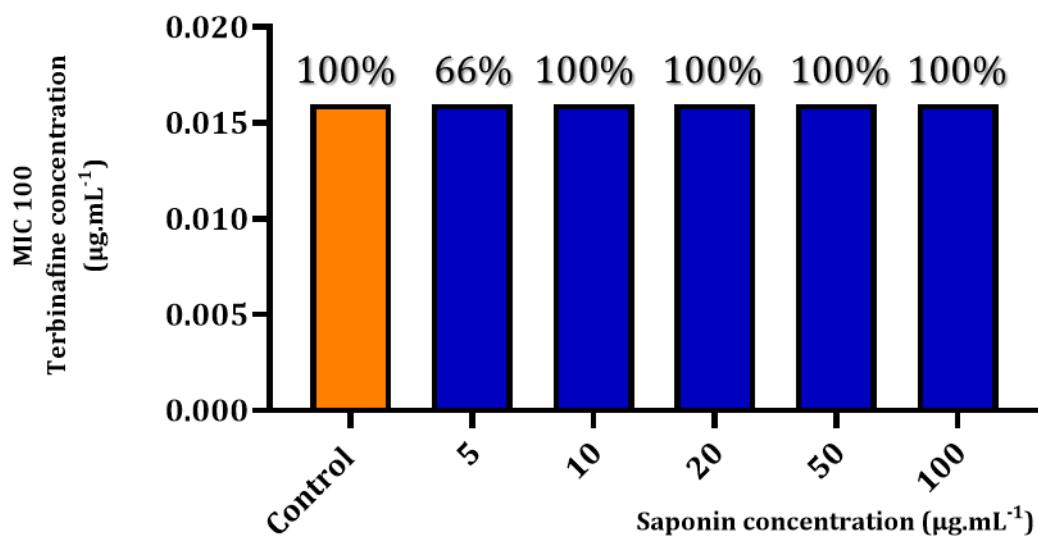
Appendix 97. Estimation of the antifungal activity of the natural extract saponins *Aesculus hippocastanum* combined with Fluconazole on *C. albicans*. No additive effect is observed.



Appendix 98. Estimation of the antifungal activity of the natural extract saponins from *Aesculus hippocastanum* combined with Amphotericin B on *A. fumigatus*. An additive effect is already observed at a saponin concentration of 5 µg.mL⁻¹. The value above the column represents the reproducibility of the analysis (based on three assays).

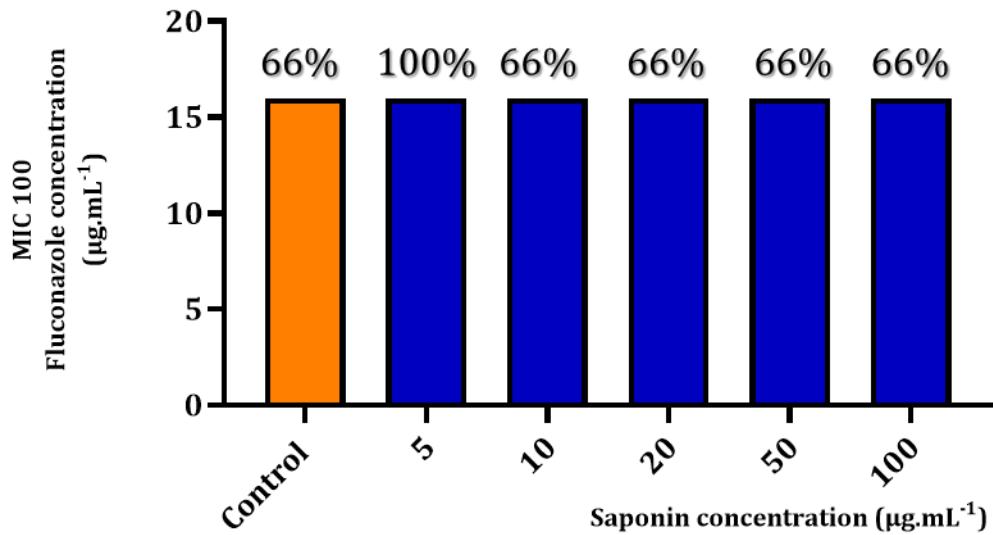


Appendix 99. *Estimation of the antifungal activity of the natural extract saponins from Aesculus hippocastanum combined with Voriconazole on A. fumigatus. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays).*

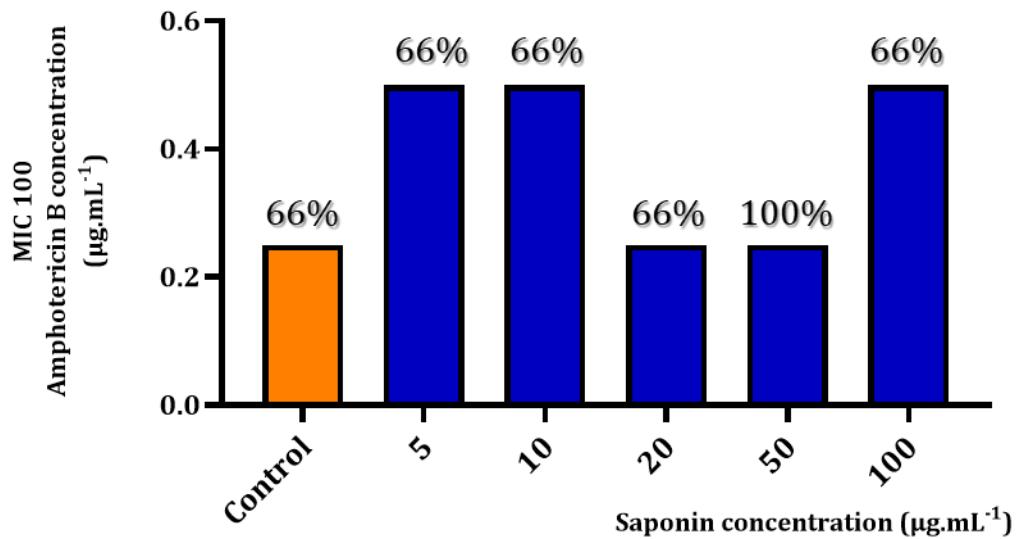


Appendix 100. *Estimation of the antifungal activity of the natural extract saponins from Aesculus hippocastanum combined with Terbinafine on T. interdigitale. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays).*

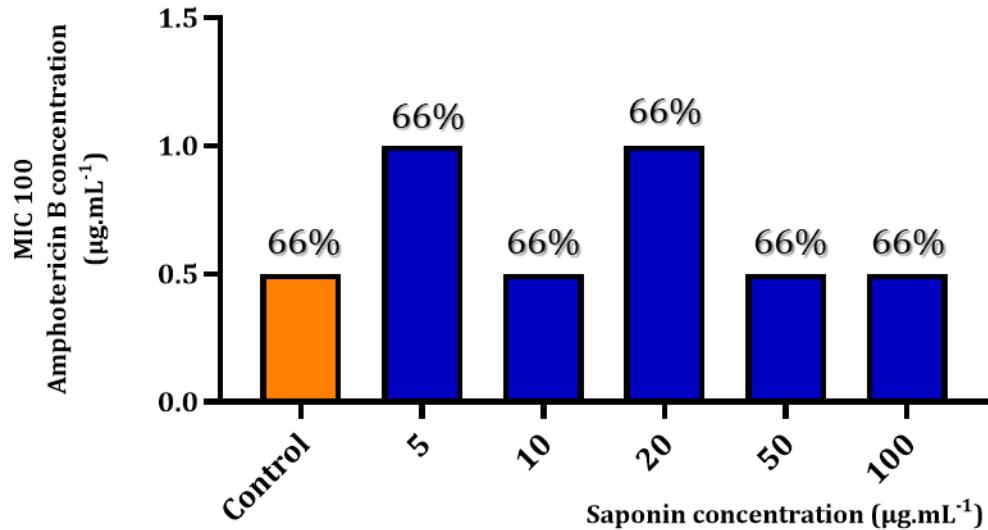
Appendix



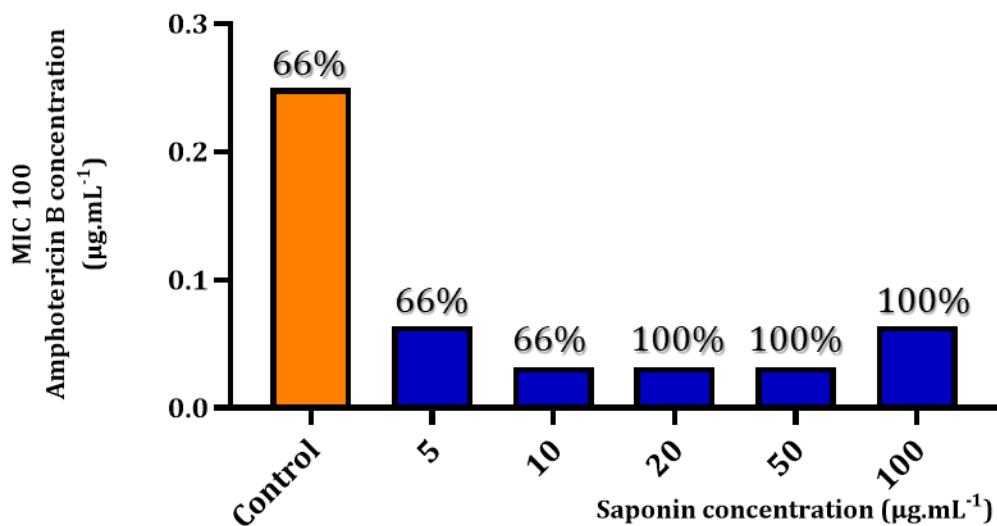
Appendix 101. Estimation of the antifungal activity of the natural extract saponins from *Aesculus hippocastanum* combined with Fluconazole on *T. interdigitale*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays).



Appendix 102. Estimation of the antifungal activity of the hydrolyzed saponins from *Aesculus hippocastanum* combined with Amphotericin B on *C. albicans*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays)

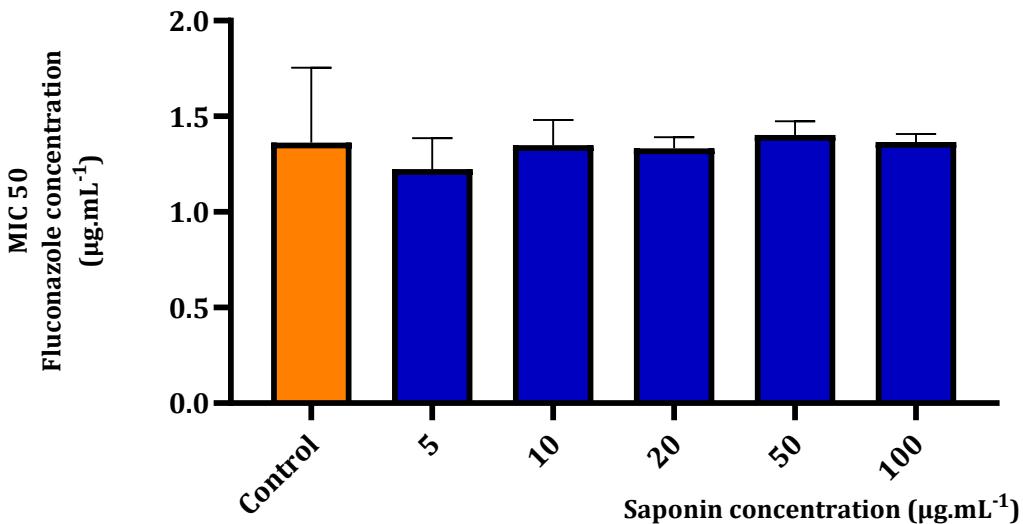


Appendix 103. Estimation of the antifungal activity of the hydrolyzed saponins from *Aesculus hippocastanum* combined with Amphotericin B on *A. fumigatus*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays).

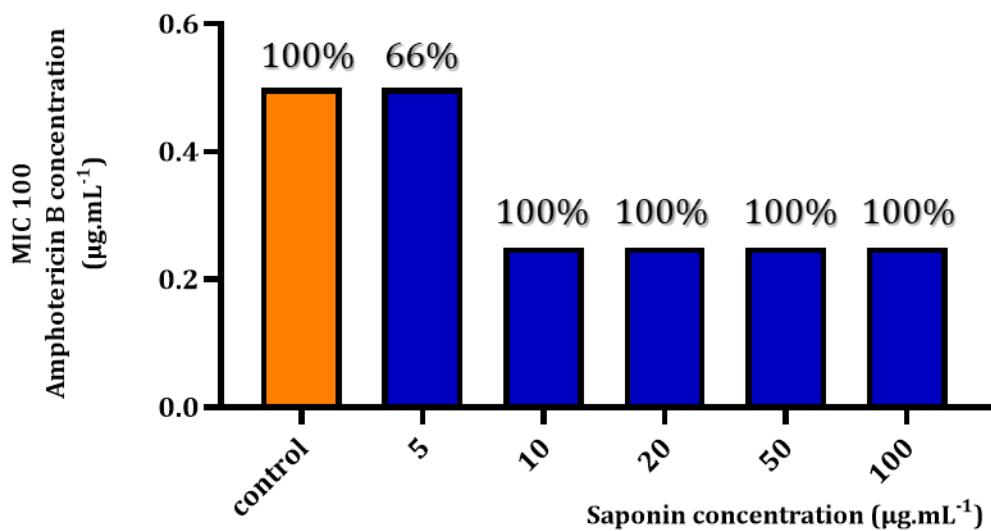


Appendix 104. Estimation of the antifungal activity of the transesterified saponins from *Aesculus hippocastanum* combined with Amphotericin B on *C. albicans*. An additive effect is already observed at a saponin concentration of 5 µg.mL⁻¹. The value above the column represents the reproducibility of the analysis (based on three assays).

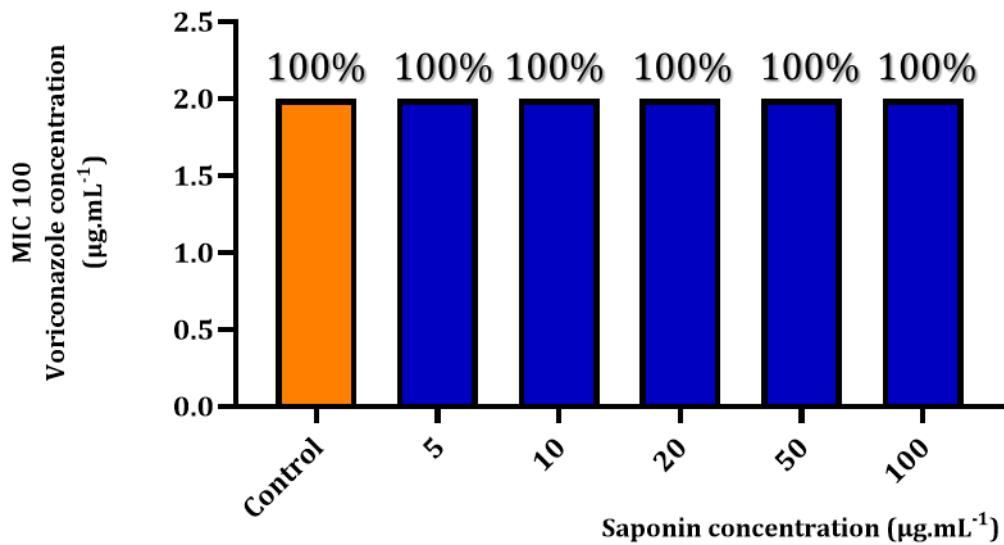
Appendix



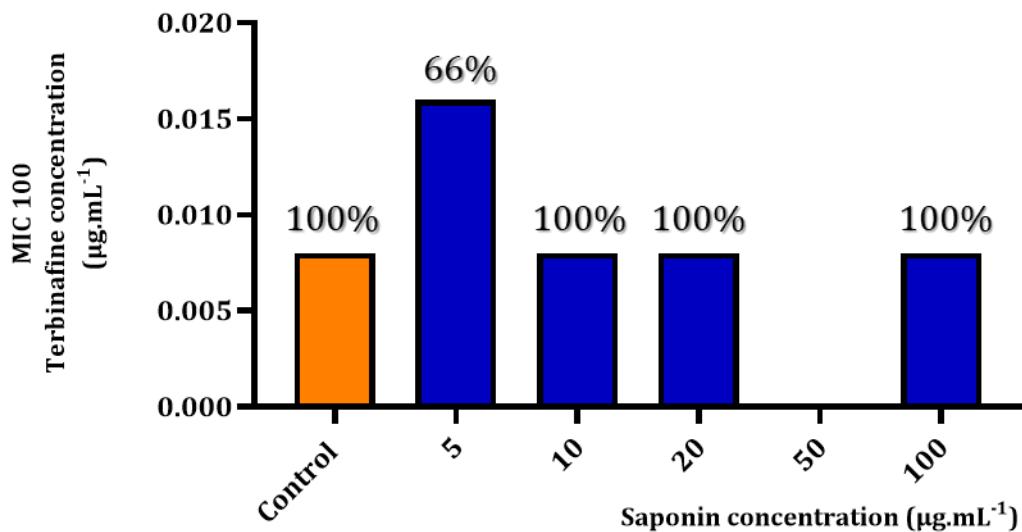
Appendix 105. Estimation of the antifungal activity of the transesterified saponins from Aesculus hippocastanum combined with Fluconazole on C. albicans. No additive effect is observed.



Appendix 106. Estimation of the antifungal activity of the transesterified saponins from Aesculus hippocastanum combined with Amphotericin B on A. fumigatus. An additive effect is already observed at a saponin concentration of 10 µg.mL⁻¹. The value above the column represents the reproducibility of the analysis (based on three assays).

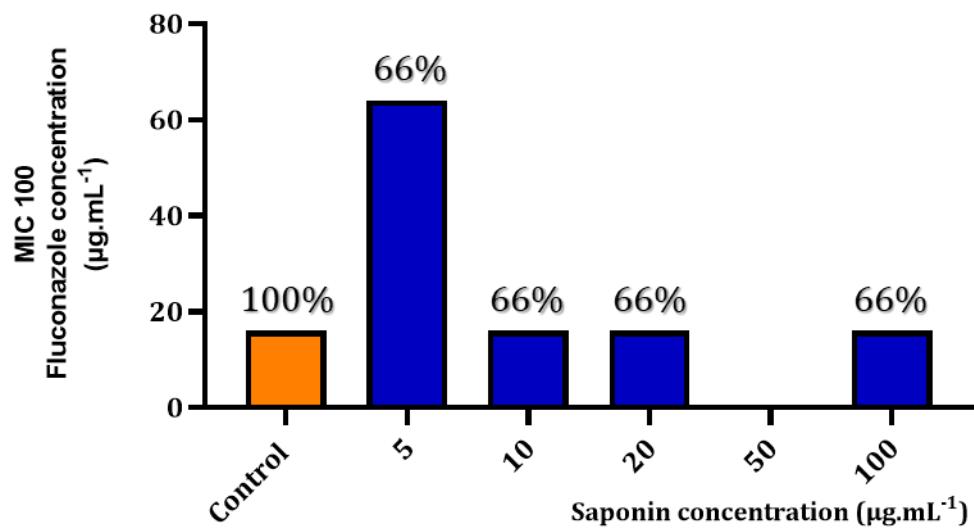


Appendix 107. Estimation of the antifungal activity of the transesterified saponins from *Aesculus hippocastanum* combined with Voriconazole on *A. fumigatus*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays).



Appendix 108. Estimation of the antifungal activity of the transesterified saponins from *Aesculus hippocastanum* combined with Terbinafine on *T. interdigitale*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays). 7

Appendix



Appendix 109. Estimation of the antifungal activity of the transesterified saponins from *Aesculus hippocastanum* combined with Fluconazole on *T. interdigitale*. No additive effect is observed. The value above the column represents the reproducibility of the analysis (based on three assays).