Supplementary information

Metabolic response induced by parasitic plant-fungus interactions hinder amino sugar and nucleotide sugar metabolism in the host

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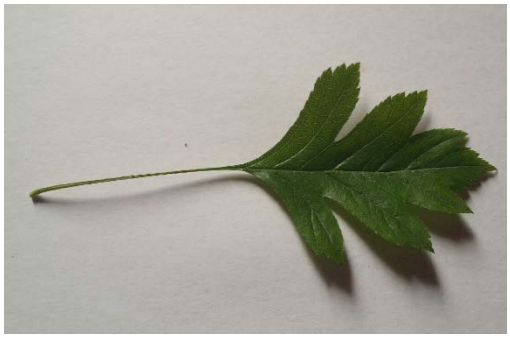
Supplementary Figure S1. Leaf samples of C*rataegus pinnatifida* (control, a; parasitized, b), *Chaenomeles sinensis* (control, c; parasitized, d) and *Pyrus pyrifolia* (control, e; parasitized, f).

a



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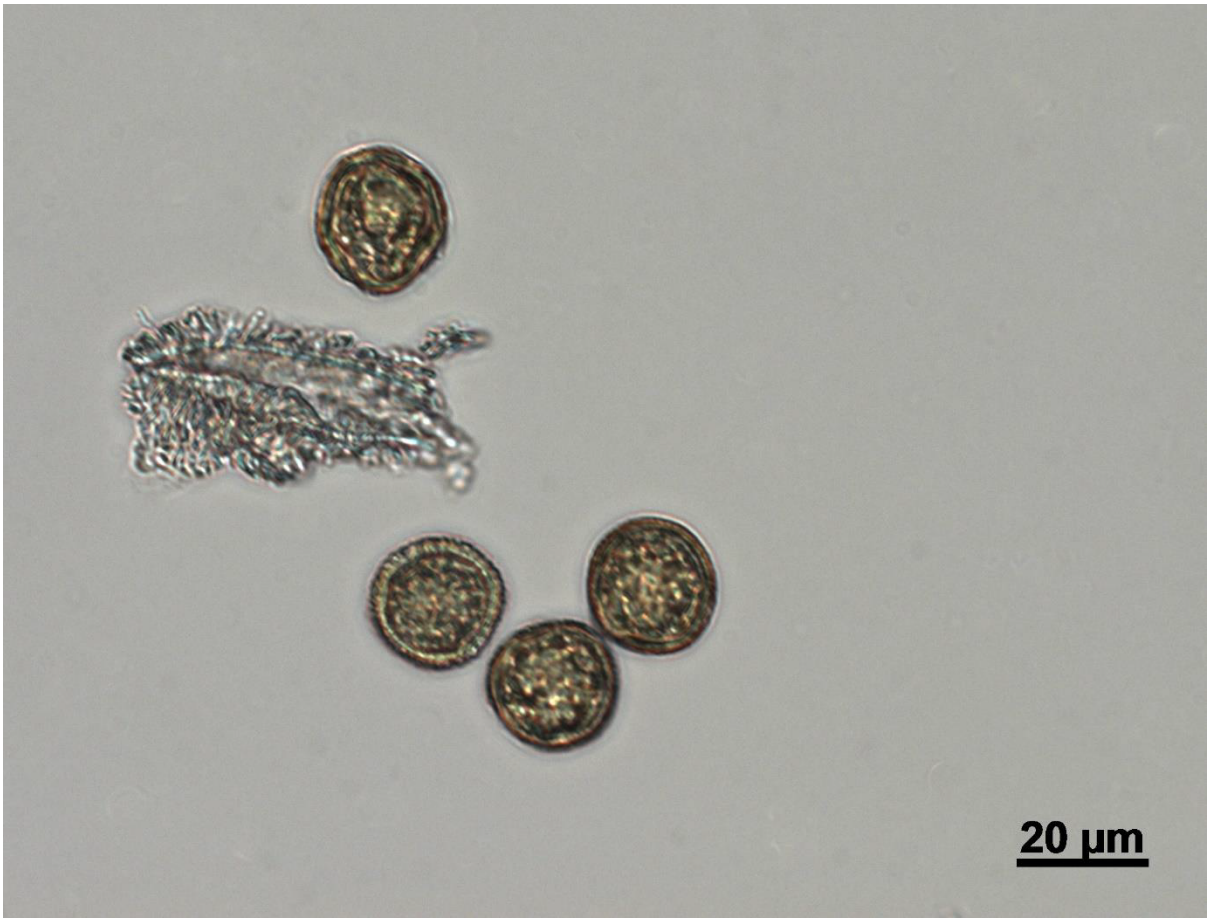
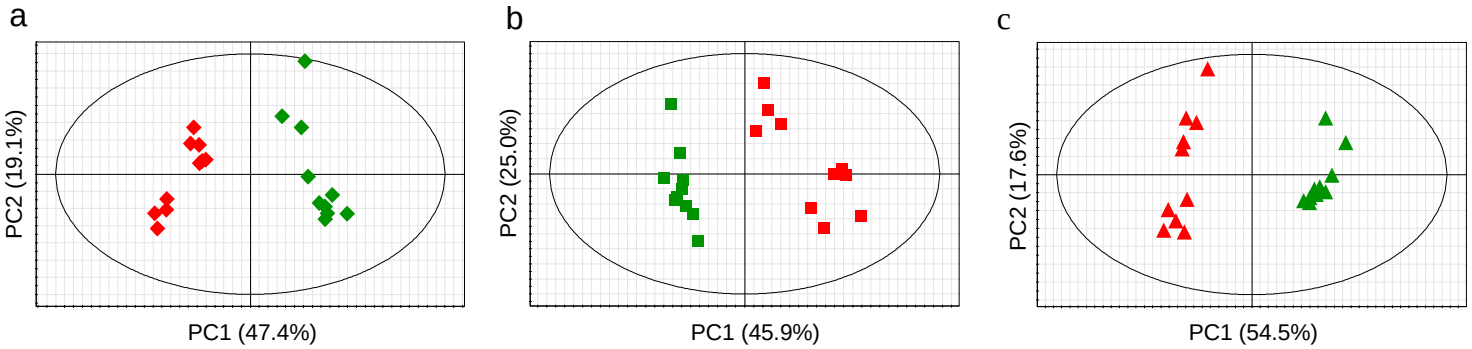
f

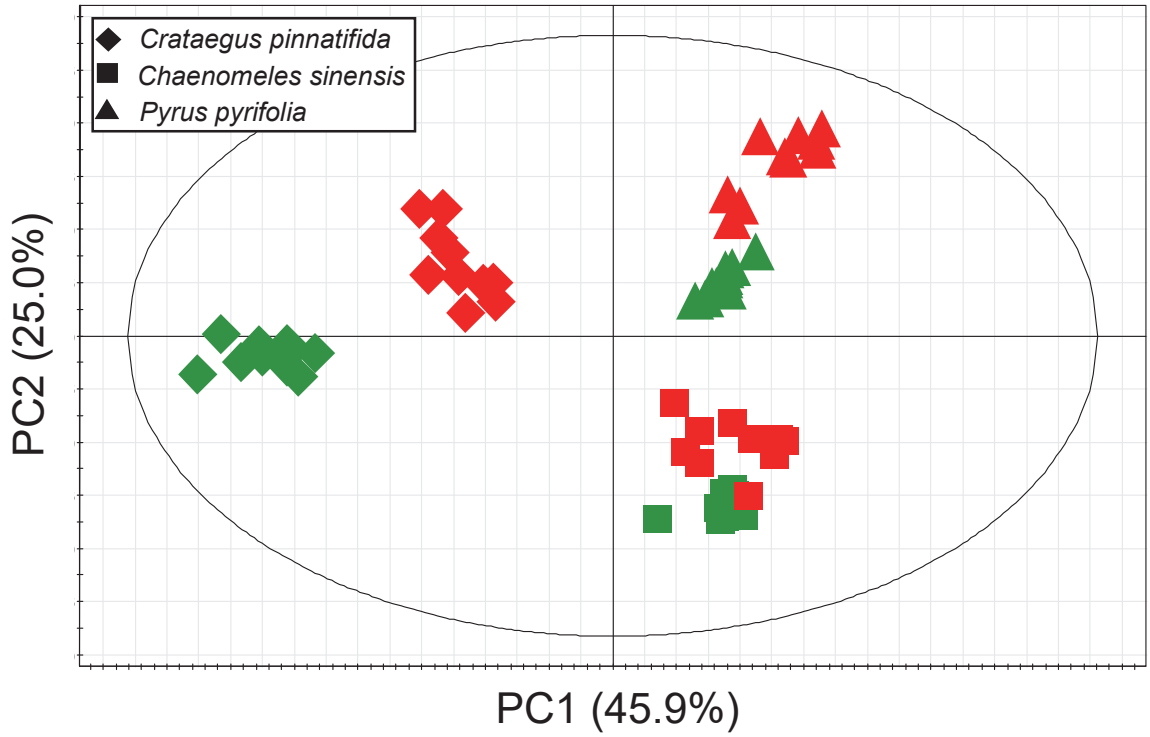
Supplementary Figure S2. Four aeciospores and a single peridial cell of Gymnosporangium asiaticum on Pyrus pyrifolia var. culta (Makino) Nakai, specimen HY2926 (×400).



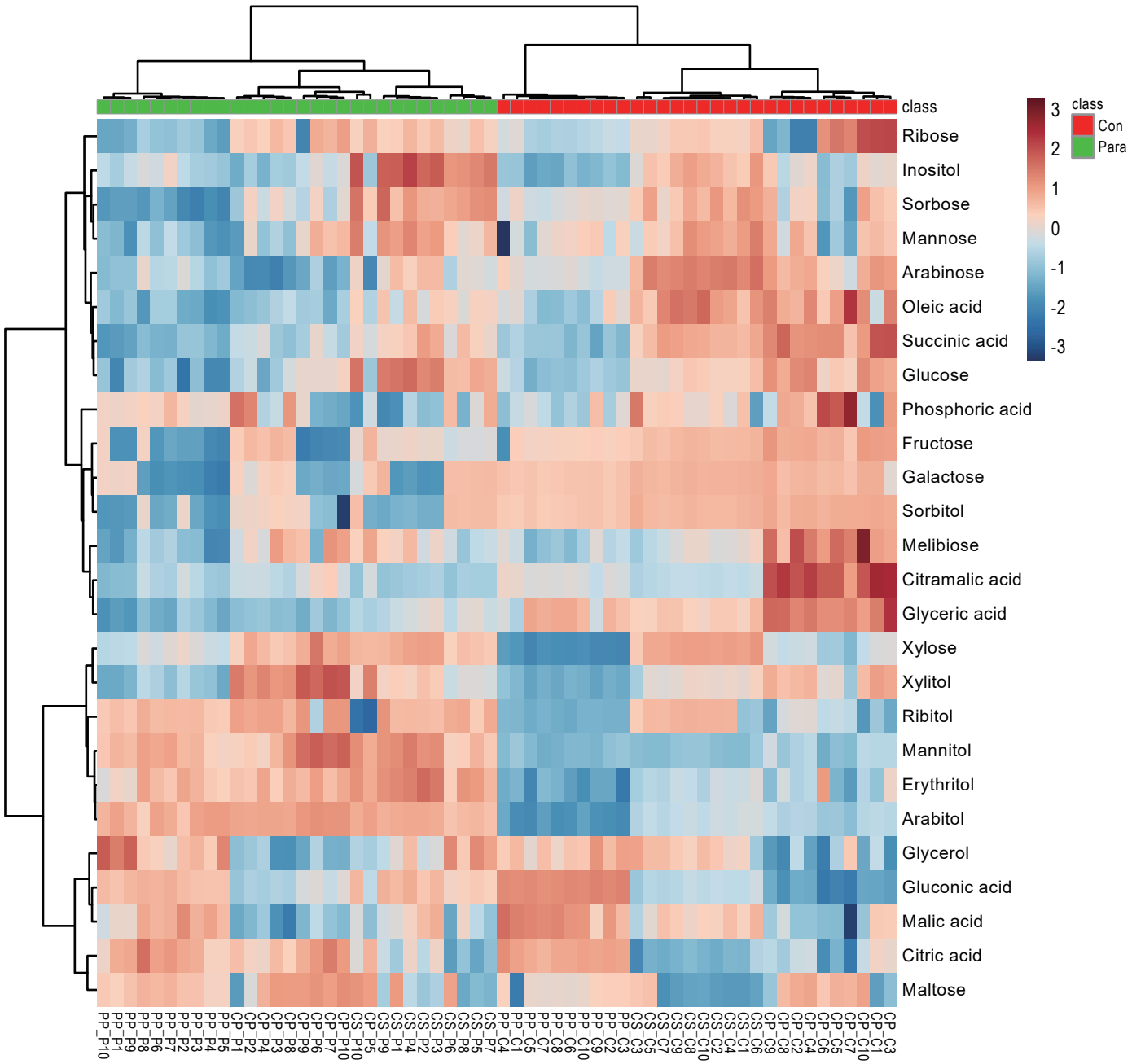
Supplementary Figure S3. PCA score scatter plots of C*rataegus pinnatifida* (n=20), *Chaenomeles sinensis* (n=20) and *Pyrus pyrifolia* (n=20) with control (green) and parasitized (red) groups.



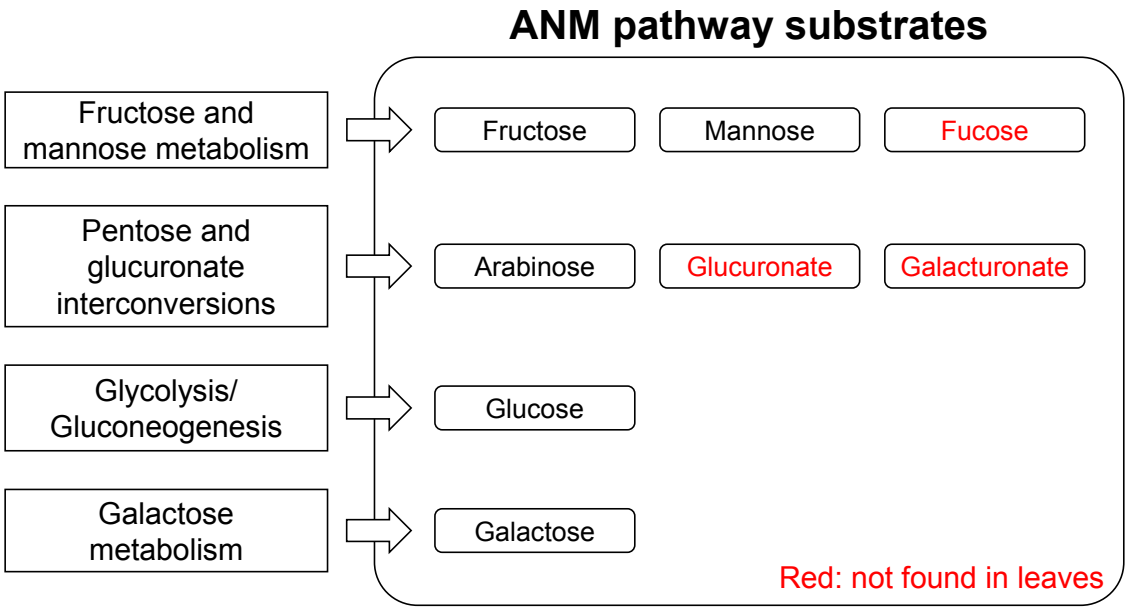
Supplementary Figure S4. PCA score scatter plot of control (green) and parasitized (red), including whole samples of Rosaceae species (n=10 for each group, total n=60)



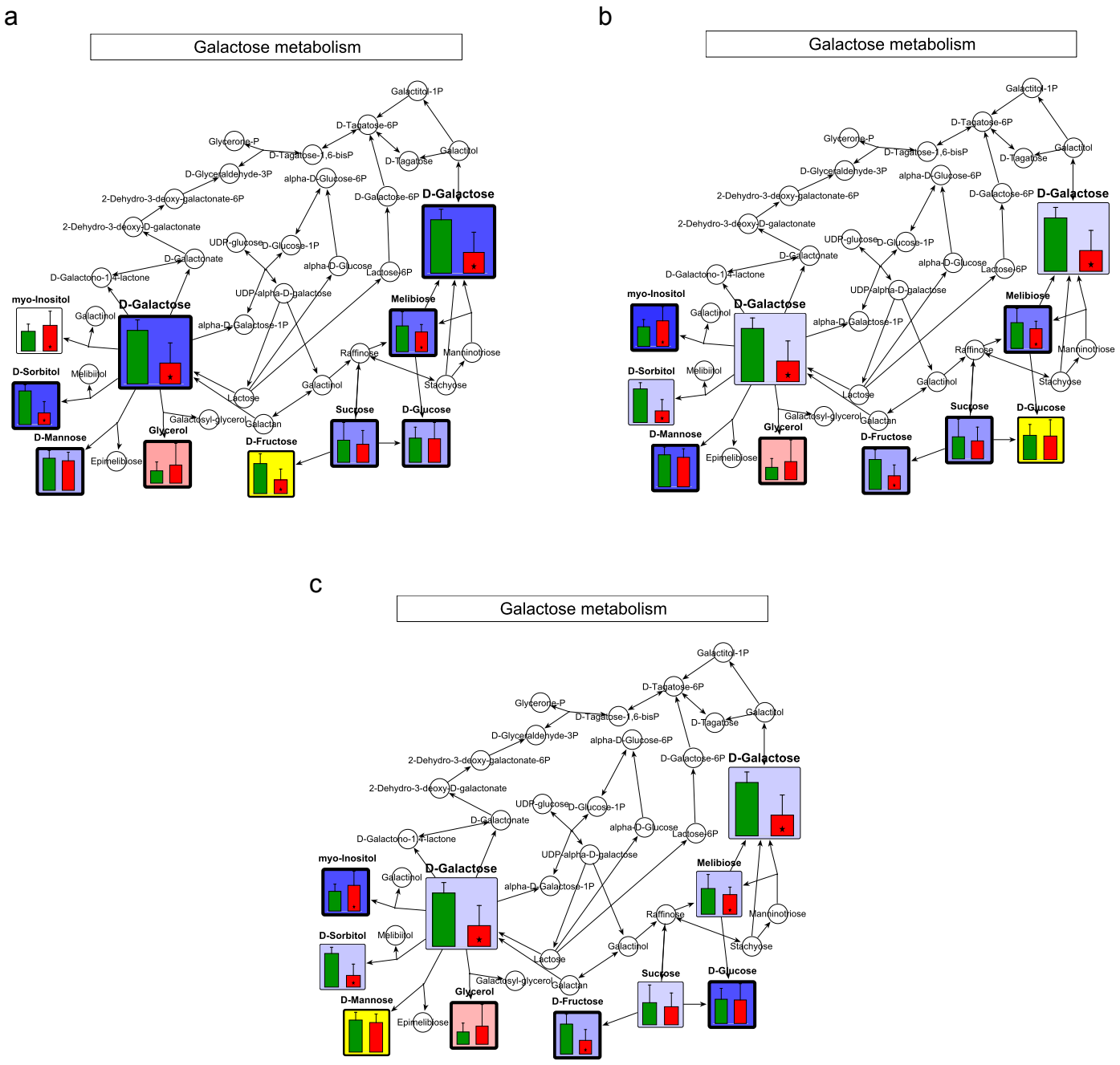
Supplementary Figure S5. Heat-map representation of unsupervised hierachical clustering analysis (HCA)



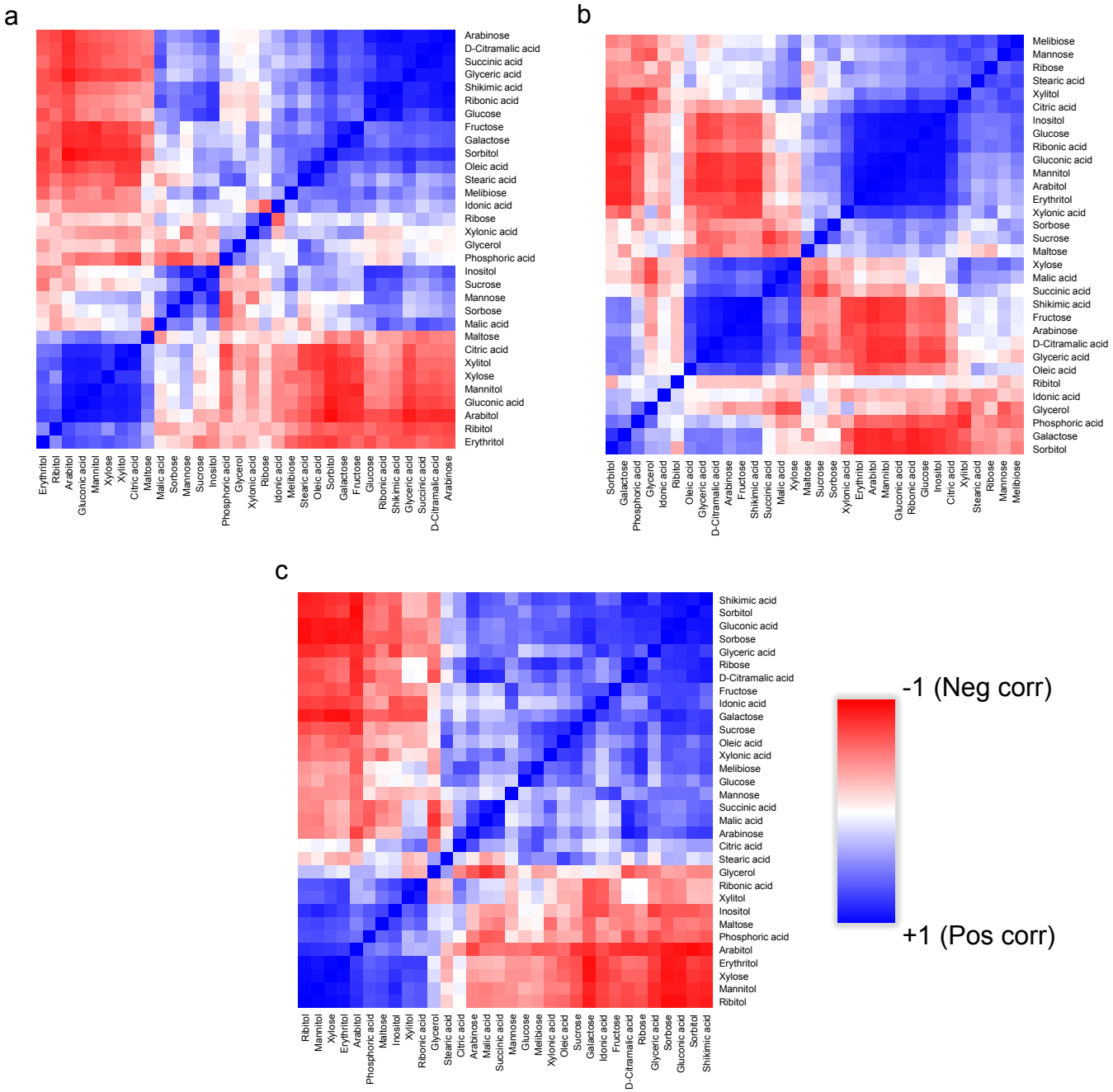
Supplementary Figure S6. Substrates of ANM pathway from other metabolic pathways



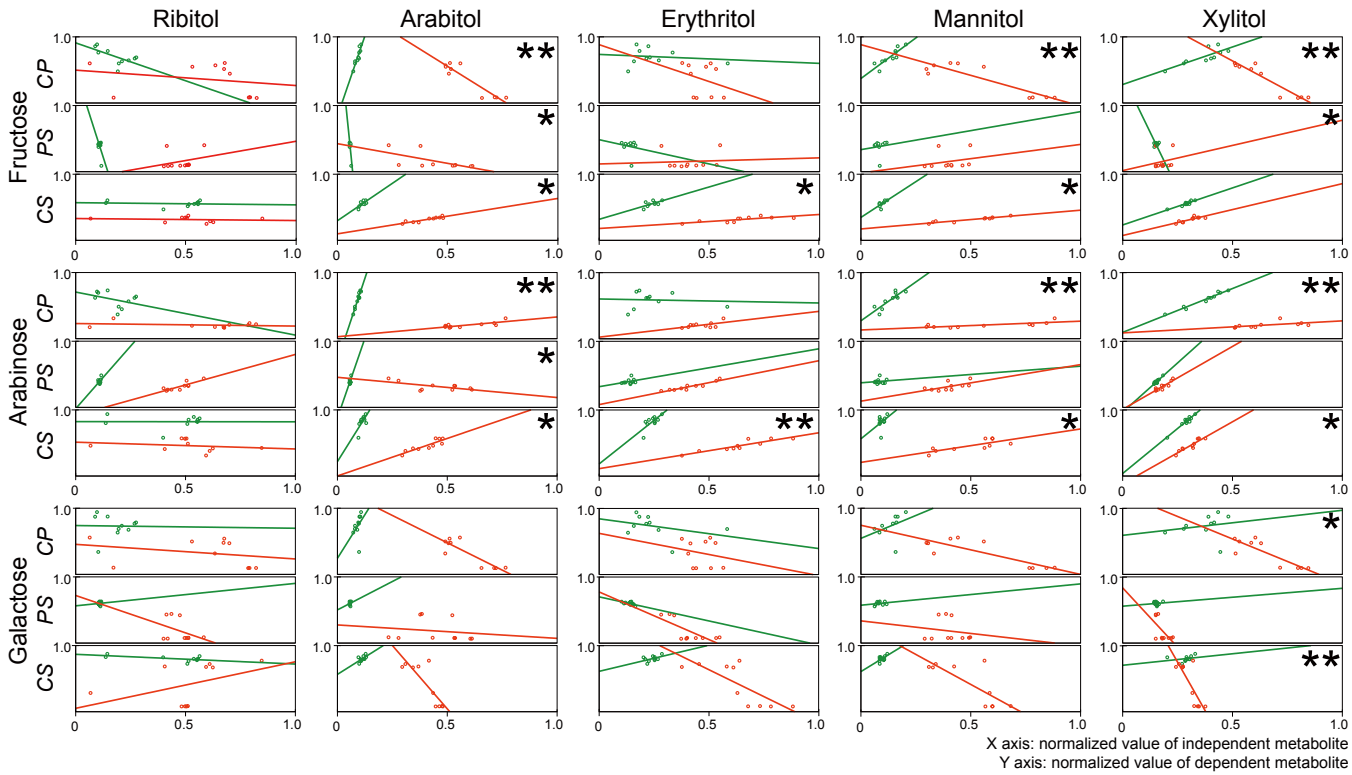
Supplementary Figure S7. Mapping of metabolic alterations on GM. Metabolites (p-value <0.05 as asterisk) between control (green) and parasitism (red) were colored based on PPMCC value from -1 (negative correlation, red) to +1 (positive correlation, blue) against fructose (a), glucose (b) and mannose (c). All values in bar graphs were mean + s.d., 30 replicates for each group.



Supplementary Figure S8. Correlation analysis (based on PPMCC) results among whole metabolic changes in C*rataegus pinnatifida* (a, n=20), *Chaenomeles sinensis* (b, n=20) and *Pyrus pyrifolia* (c, n=20). Negative correlations (maximum -1, red) and positive correlations (maximum +1, blue) were displayed on heatmaps with clustering.



Supplementary Figure S9. Interaction effect of group variable and accumulated sugar alcohols on the ANM supplies. Forty-five scatter plots (n = 15 for each species) with min-max normalized values of ANM supplies (fructose, arabinose and galactose on the y-axis) and accumulated sugar alcohols (ribitol, arabitol, erythritol, mannitol and xylitol on the x-axis). The interaction effect directions of the models were measured by the slope differences of two regression lines upon each group variable (control, green; parasitism, red). The significant models were denoted as *p < 0.05 and **p < 0.01.



Supplementary Figure S10. Mass spectra of derivatized metabolites. The list of metabolites were as follows: phosphoric acid (a), glycerol (b), succinic acid (c), glyceric acid (d), fumaric acid (e), serine (f), threonine (g), citramalic acid (h), malic acid (i), erythritol (j), aspartic acid (k), erythronic acid (l), 3-hydroxy-3-methylpentanedioic acid (m), xylonic acid (n), xylose (o), arabinose (p), ribose (q), xylitol (r), arabitol (s), ribitol (t), arabinofuranose (u), ribonic acid (v), idonic acid (w), citric acid (x), sorbose (y), mannose (z), fructose (aa), galactose (ab), mannitol (ac), sorbitol (ad), gluconic acid (ae), inositol (af), glucose (ah), linoleic acid (ah), oleic acid (ai), stearic acid (aj), sucrose (ak), maltose (al) and melibiose (am)

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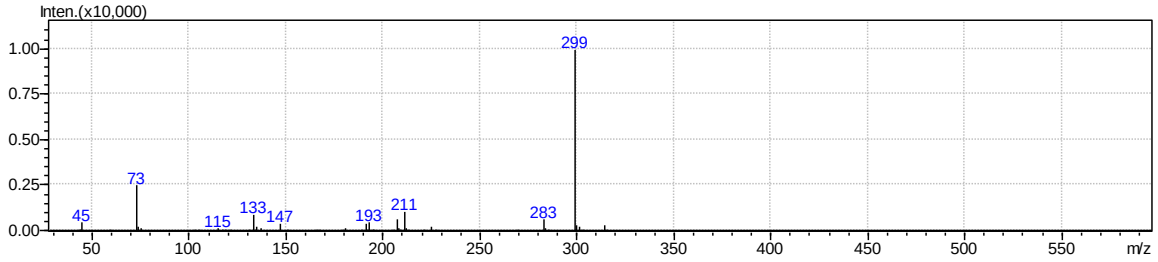

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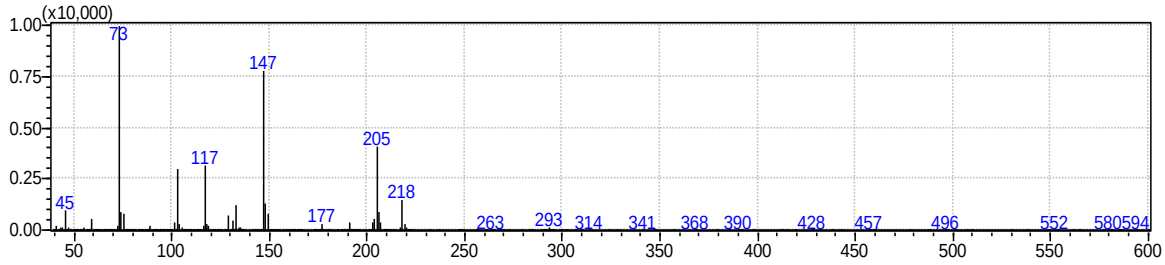

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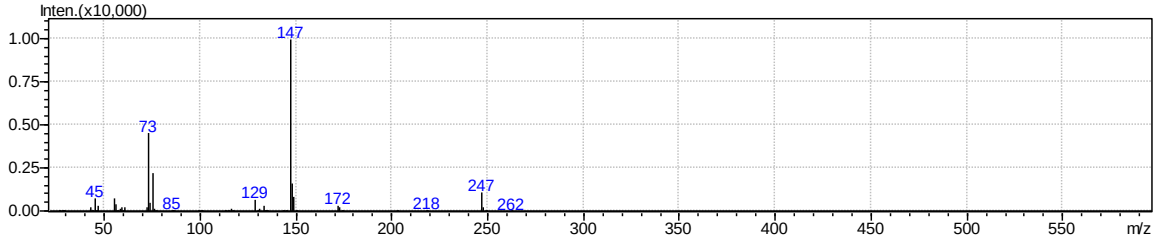

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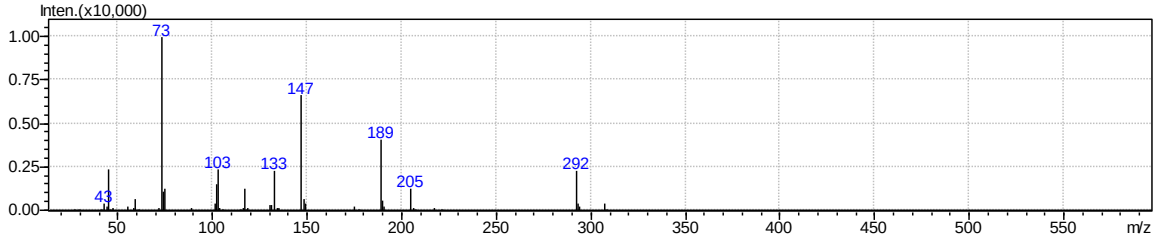

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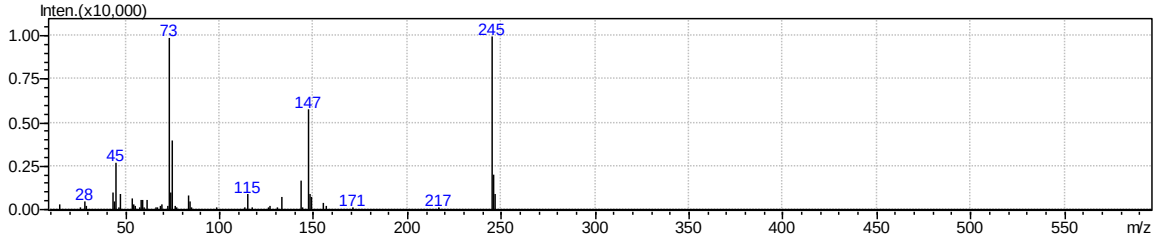

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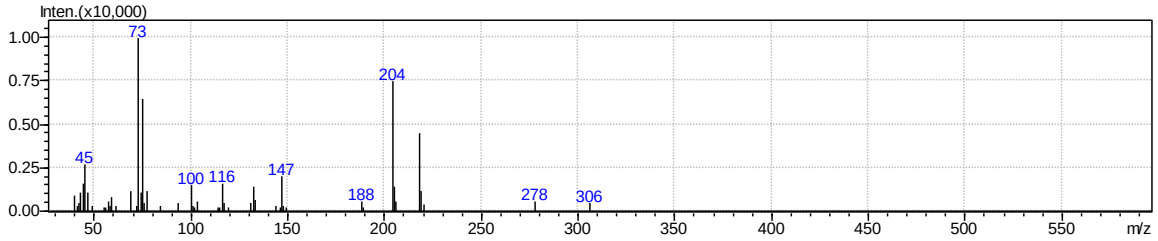
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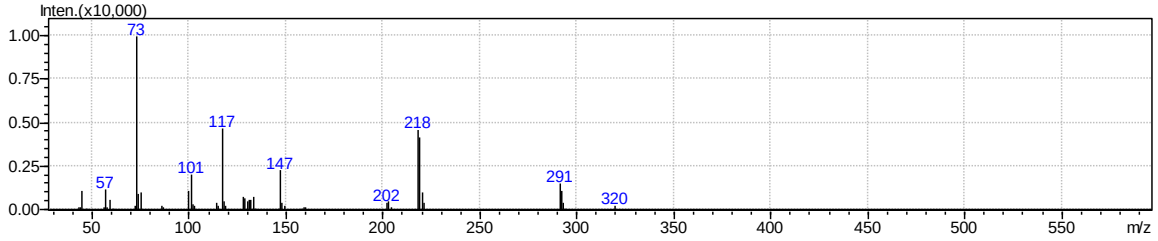
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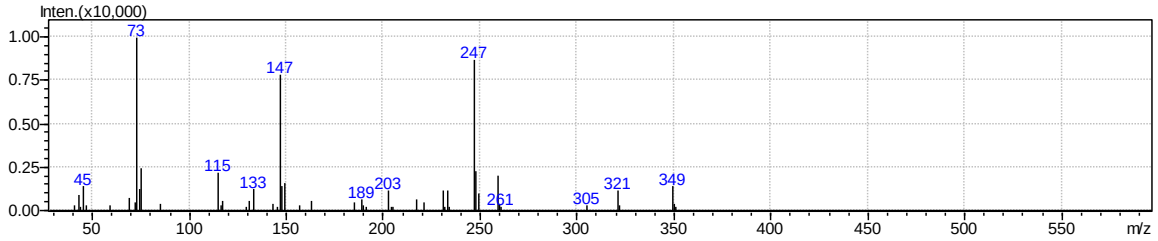
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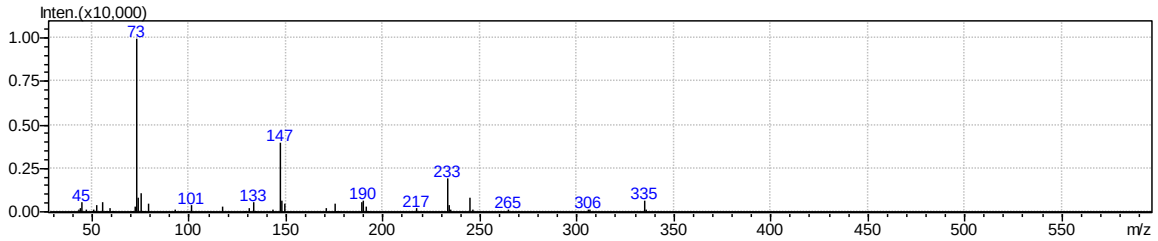
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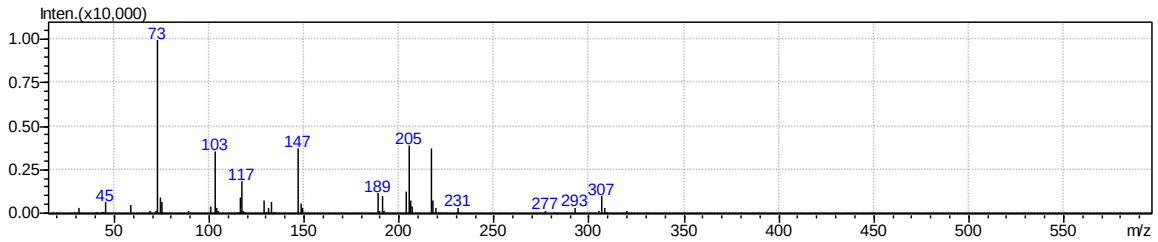
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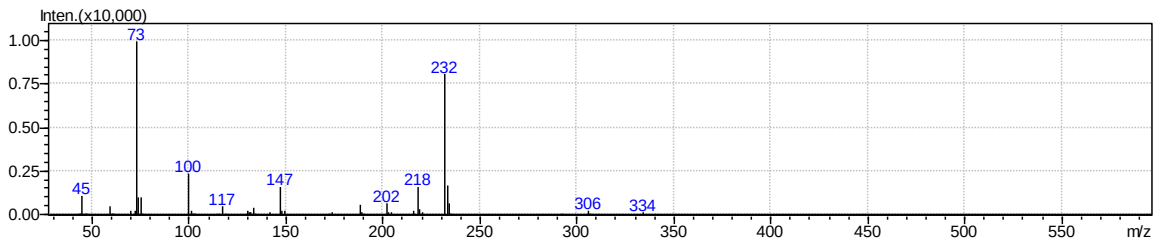
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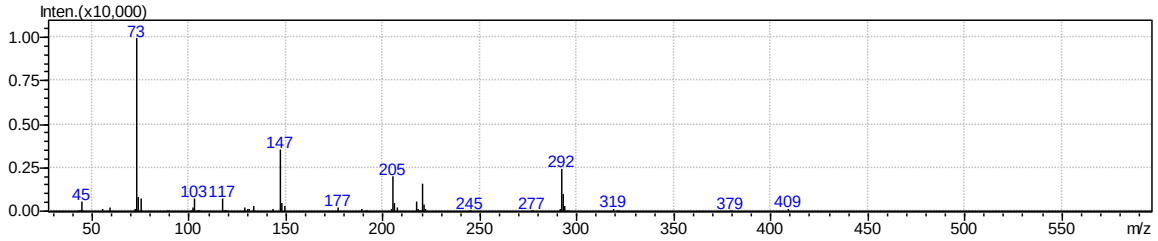
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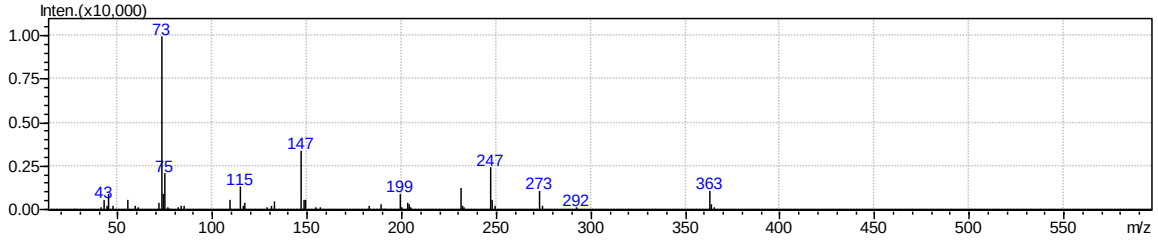
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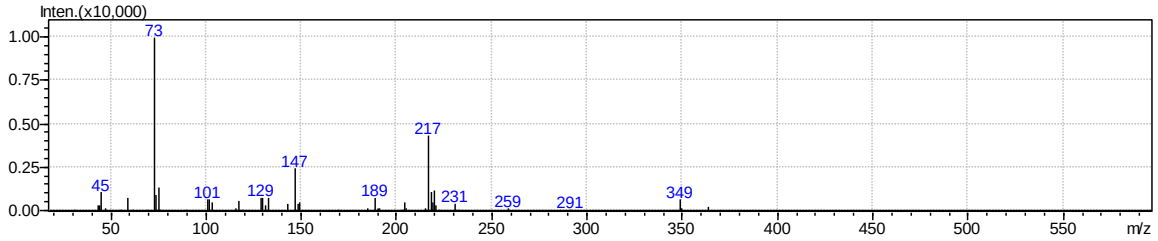
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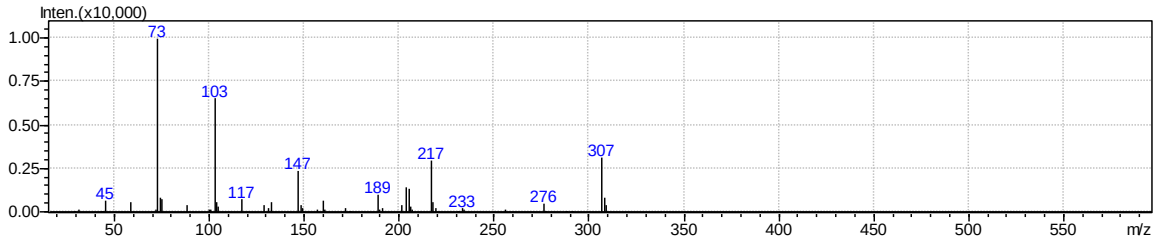
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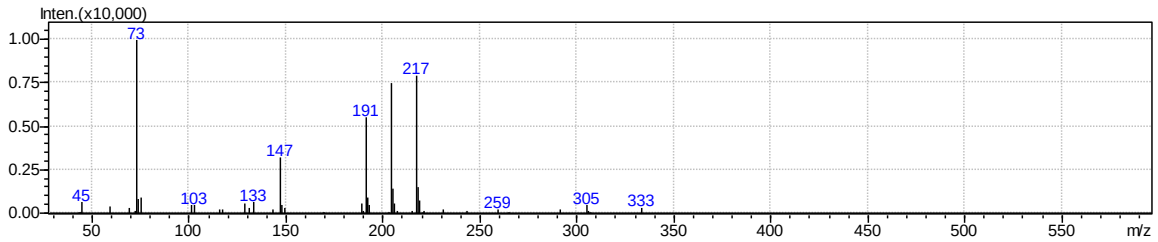
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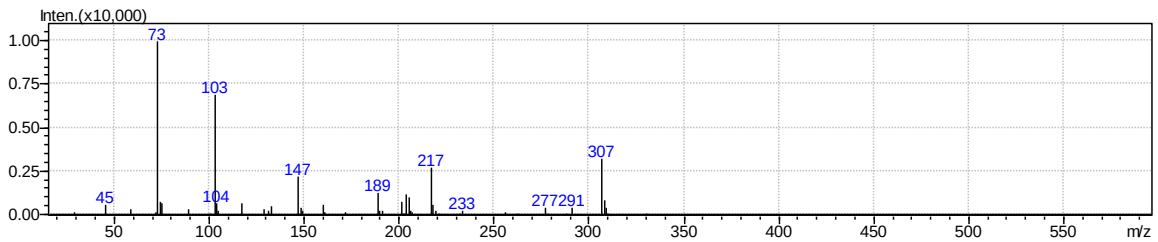
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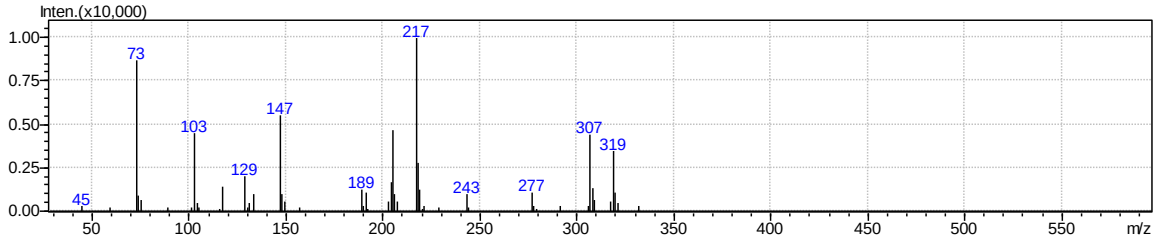
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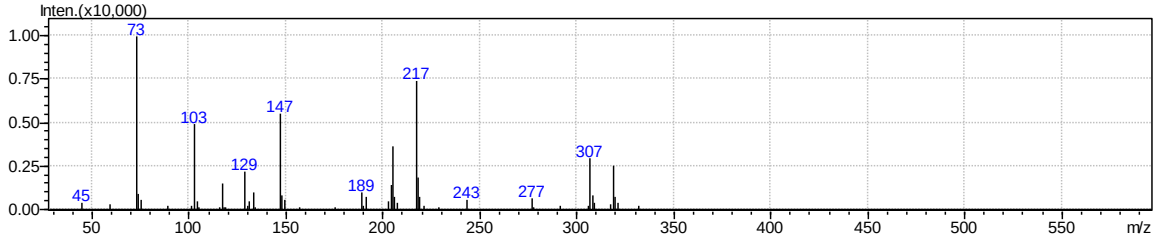
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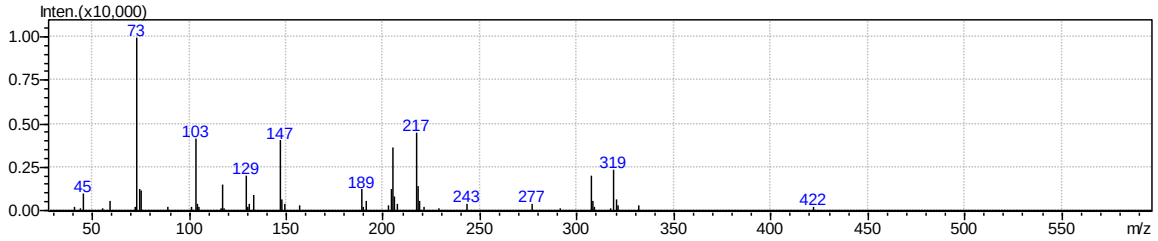
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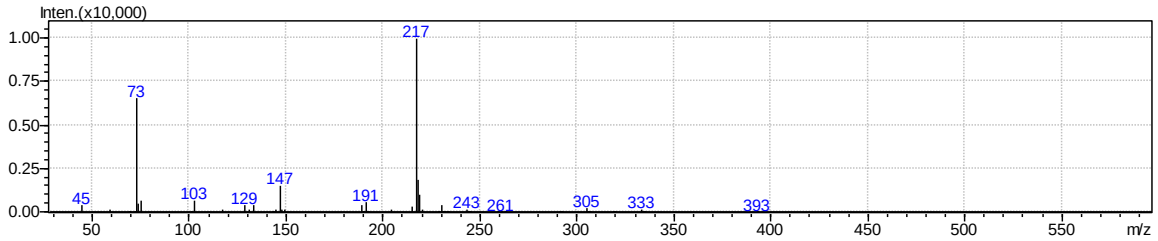
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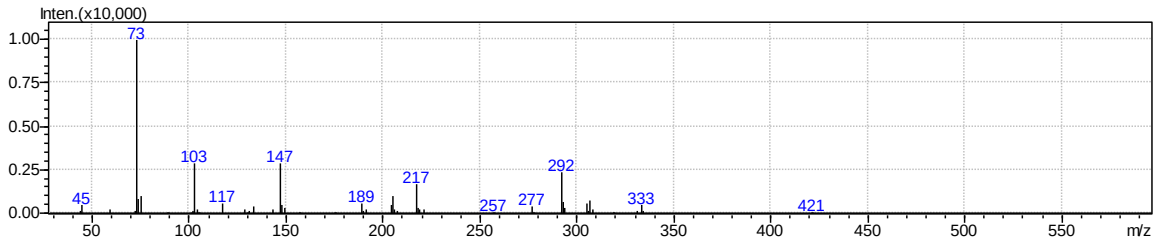
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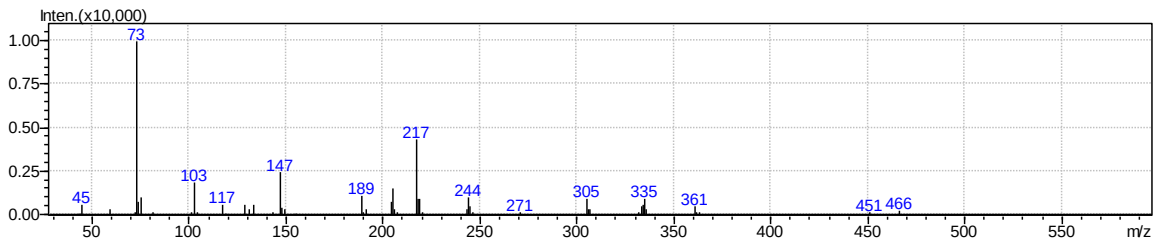
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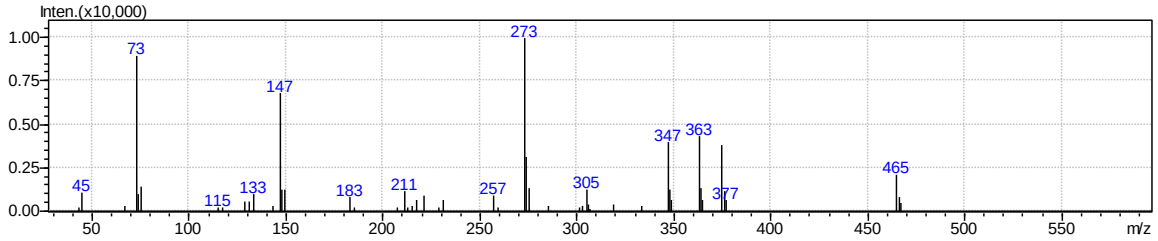
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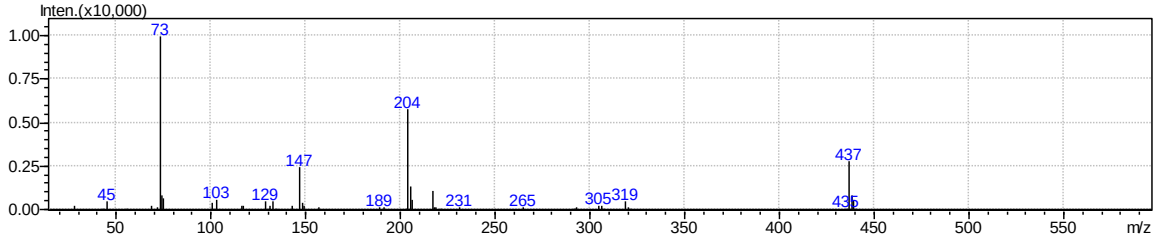
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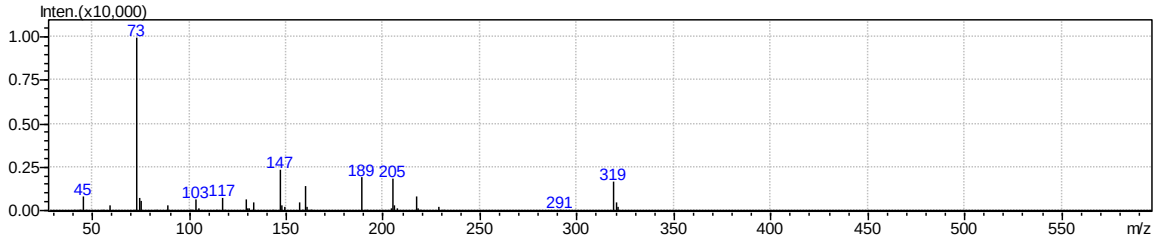
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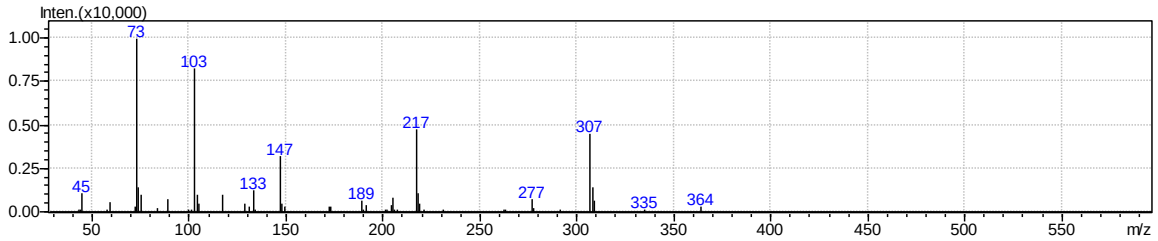
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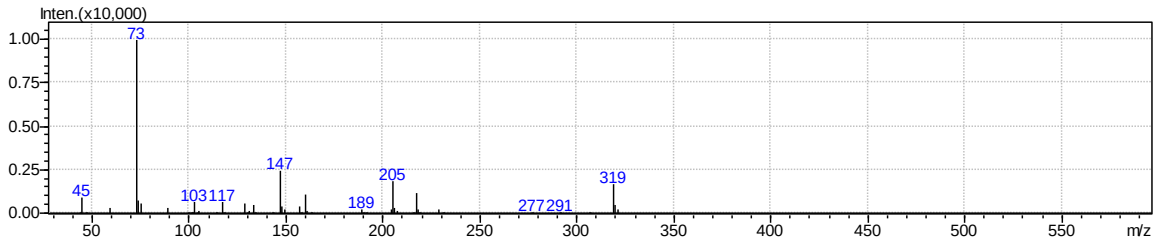
(ab)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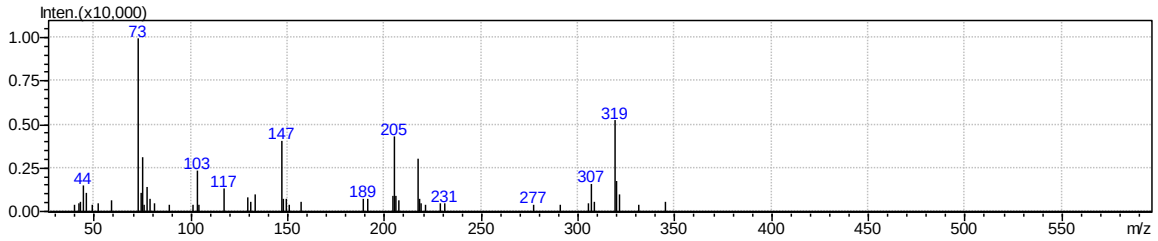
(ac)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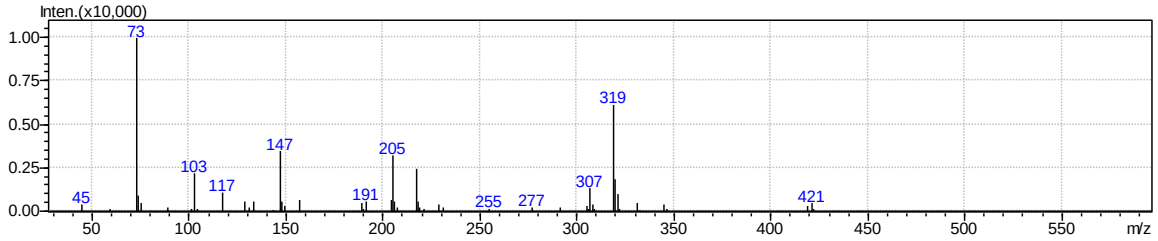

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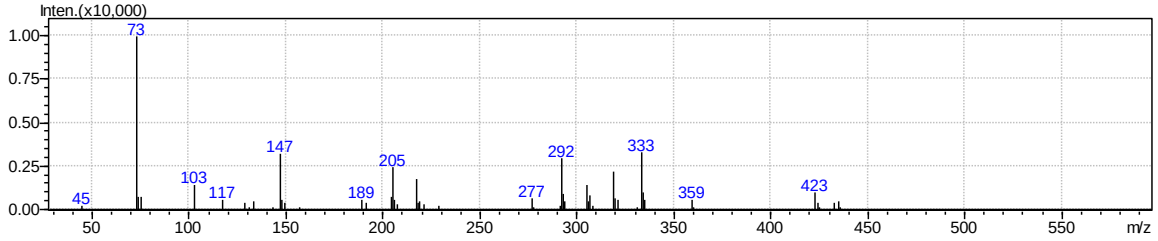

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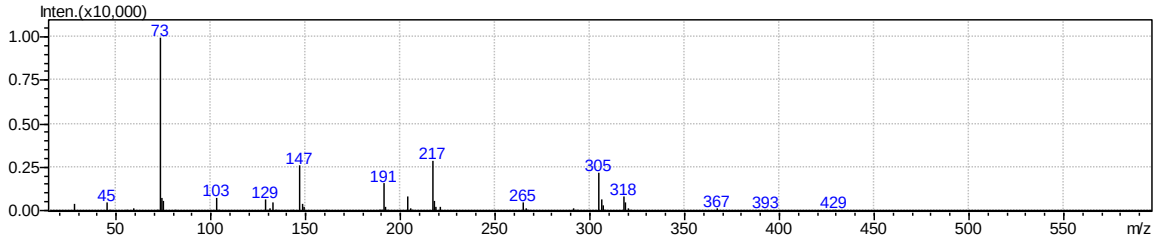

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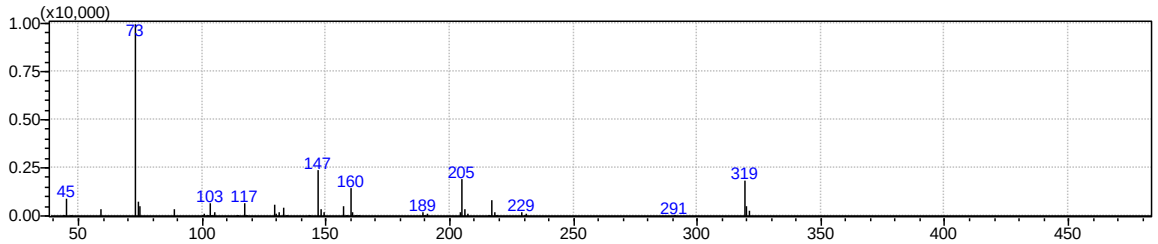

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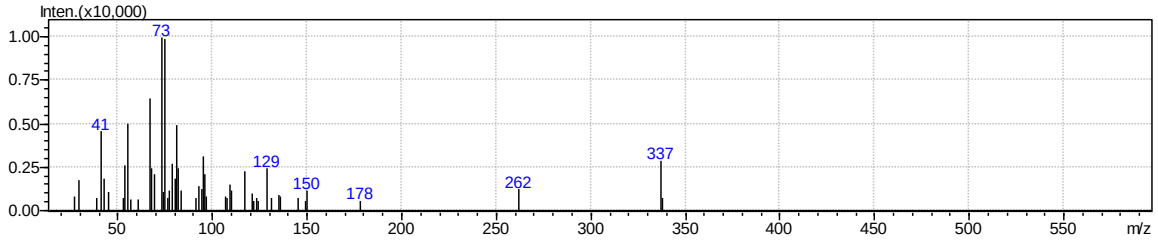

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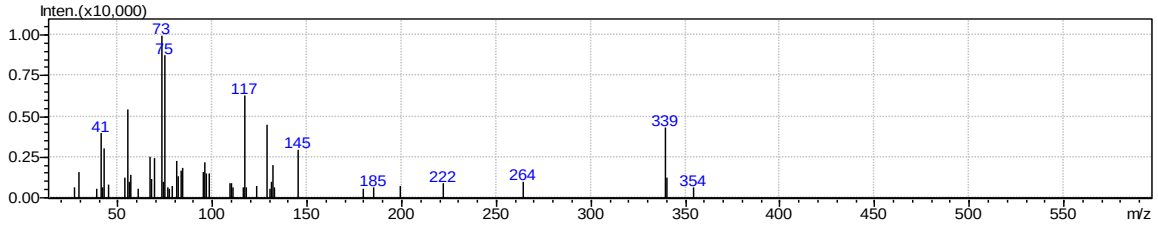

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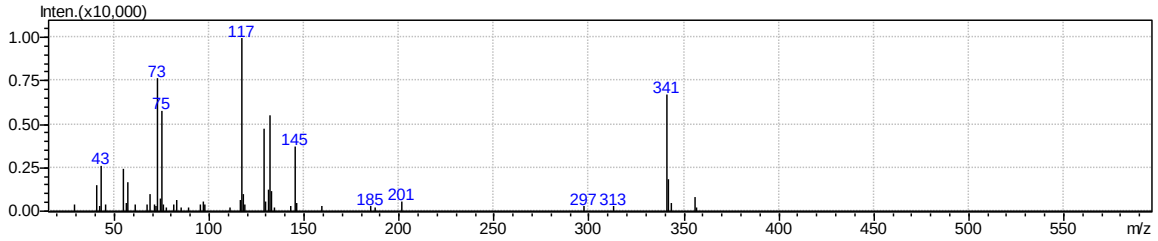
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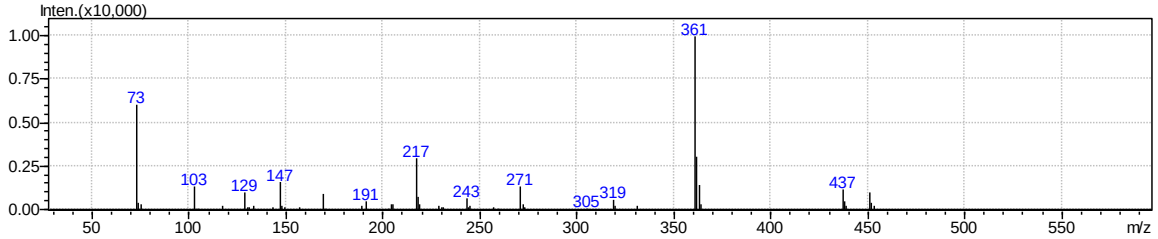
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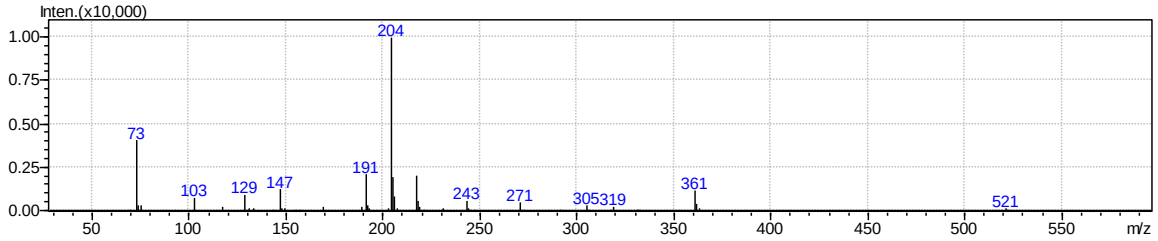
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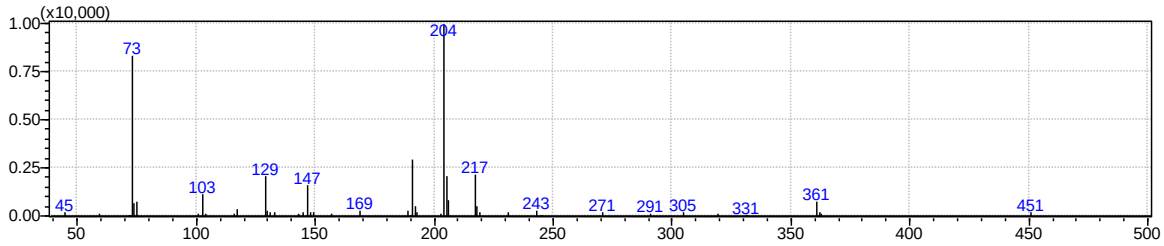
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Supplementary Table S1. List of identified metabolites and the differences between control and parasitized leaves.

Retention time (min)	Compound name	Fragment ion (derivatized compound)[a]	p-value	Fold change	Identification NIST	Identification STD
8.04	Phosphoric acid, 3TMS	298(100), 73(60), 313(18), 45(11)	0.13	0.73	○	
8.08	Glycerol, 3TMS	73(100), 147(78), 205(43), 117(32), 103(31)	0.18	1.47	○	○
8.73	Succinic acid, 2TMS	147(100), 73(52), 75(19), 148(16), 246(10)	3.0×10^{-4}	0.54	○	
9.25	Glyceric acid, 3TMS	73(100), 147(61), 189(31), 292(23), 102(17)	6.5×10^{-13}	0.53	○	
9.41	Fumaric acid, 2TMS	245(100), 73(57), 147(41), 143(23)	NM		○	
9.84	Serine, 3TMS	73(100), 204(95), 217(53), 100(27), 147(20)	NM		○	
10.43	Threonine, 3TMS	73(100), 117(48), 218(41), 57(22), 147(17)	NM		○	
12.16	Citramalic acid, 3TMS	73(100), 147(53), 247(41), 75(23), 115(15)	2.3×10^{-4}	0.33	○	
12.47	Malic acid, 2TMS	73(100), 147(53), 233(18)	0.05	0.87	○	
13.04	Erythritol, 4TMS	73(100), 147(57), 216(51), 103(34), 205(28)	1.3×10^{-13}	2.85	○	○
13.10	Aspartic acid, 3TMS	232(100), 73(98), 100(32), 147(22), 218(16)	NM		○	
13.73	Erythronic acid, 4TMS	73(100), 147(51), 292(24), 117(14), 103(11)	NM		○	
14.69	3-H-3-MP acid, 3TMS[b]	73(100), 147(47), 247(31), 115(18), 231(18)	NM		○	
15.27	Xylonic acid, 3TMS	73(100), 217(29), 147(27), 129(18), 102(11)	0.93	0.99	○	
16.00	Xylose, 4TMS, 1 MO	73(100), 103(77), 217(49), 307(32), 147(28)	0.018	1.50	○	
16.12	Arabinose, 4TMS	73(100), 103(88), 217(43), 307(29), 147(25)	3.0×10^{-8}	0.50	○	○
16.40	Ribose, 4TMS, 1 MO	73(100), 103(80), 217(44), 307(28), 147(24)	0.25	0.88	○	○
17.00	Xylitol, 5TMS	73(100), 217(64), 103(42), 147(40), 205(21)	0.015	1.49	○	○
17.21	Arabitol, 5TMS	73(100), 217(63), 103(44), 147(41), 205(22)	3.9×10^{-23}	11.39	○	○
17.29	Ribitol, 5TMS	73(100), 217(57), 147(43), 103(40), 205(27)	2.5×10^{-7}	2.43	○	○
17.92	Arabinofuranose, 4TMS	217(100), 73(92), 147(31), 232(10)	NM		○	○
18.01	Ribonic acid, 5TMS	73(100), 292(45), 147(36), 103(30), 217(29)	0.64	0.93	○	
18.23	Idonic acid, 4TMS	73(100), 147(36), 217(28), 103(24), 436(20)	8.2×10^{-4}	0.51	○	
18.82	Citric acid, 4TMS	73(100), 273(71), 147(59), 347(17), 375(14)	0.017	1.18	○	
19.91	Sorbose, 5TMS	73(100), 103(76), 217(52), 307(32), 147(24)	0.17	0.92	○	○
20.09	Mannose, 5TMS, 1 MO	73(100), 319(48), 205(41), 147(39), 160(26)	0.24	0.92	○	○
20.37	Fructose, 5TMS, 1 MO	73(100), 103(73), 217(53), 307(33), 147(24)	9.0×10^{-8}	0.46	○	○
20.65	Galactose, 5TMS, 1 MO	73(100), 319(49), 205(40), 147(39), 217(21)	1.0×10^{-10}	0.39	○	○
20.93	Mannitol, 6TMS	73(100), 319(56), 147(43), 205(39), 217(35)	1.7×10^{-17}	2.86	○	○
21.07	Sorbitol, 6TMS	73(100), 319(53), 147(42), 205(36), 103(29)	3.6×10^{-13}	0.35	○	○
22.10	Gluconic acid, 6TMS	73(100), 147(36), 333(28), 292(25), 103(15)	0.79	0.94	○	
23.39	myo-Inositol, 6TMS	73(100), 217(60), 305(58), 147(44), 318(31)	0.044	1.30	○	○
23.79	Glucose, 5TMS, 1 MO	73(100), 319(63), 205(38), 147(36), 103(21)	0.84	0.97	○	○
24.61	Linoleic acid, 1TMS	75(100), 73(97), 67(77), 81(70), 55(54)	NM		○	
24.70	Oleic acid, 1TMS	73(100), 117(94), 75(90), 129(64), 55(56)	1.54×10^{-6}	0.61	○	
25.06	Stearic acid, 1TMS	117(100), 73(75), 75(56), 341(55), 132(54)	0.061	0.81	○	
31.10	Sucrose, 8TMS	73(100), 361(88), 216(37), 147(25), 103(22)	0.32	0.82	○	○
32.84	Maltose, 8TMS	73(100), 361(86), 204(59), 147(34), 217(31)	0.007	1.70	○	○
33.97	Melibiose, 8TMS	204(100), 73(66), 217(29), 205(28), 361(27)	0.041	0.76	○	○

NIST: putatively identified using NIST database (match score over 80/100)
STD: identified using standard compound

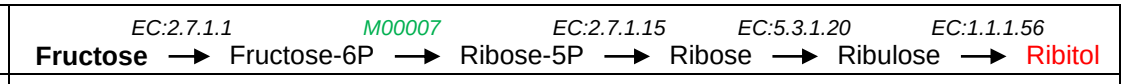
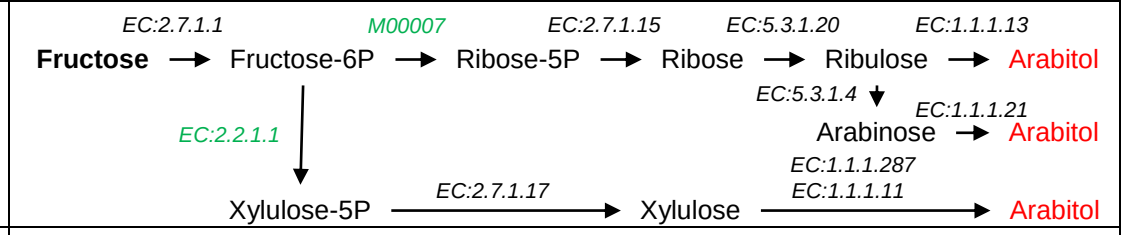
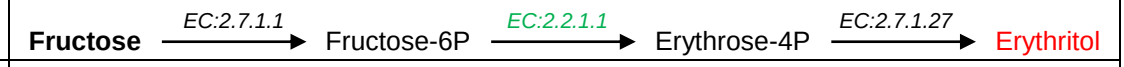
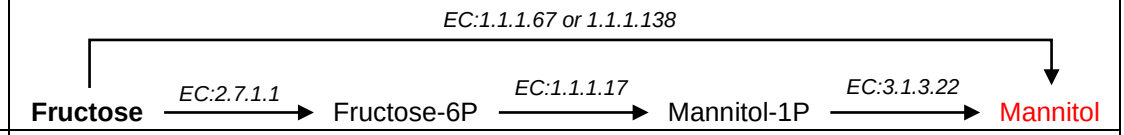
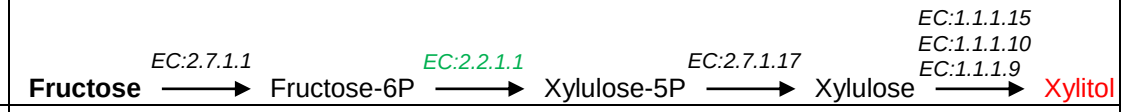
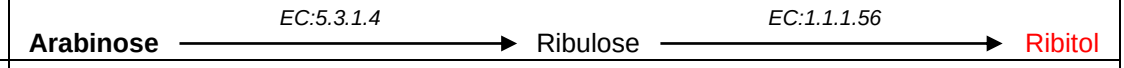
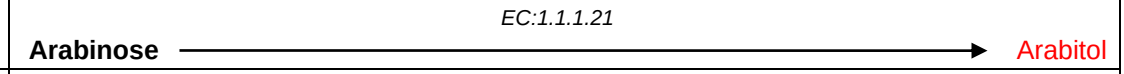
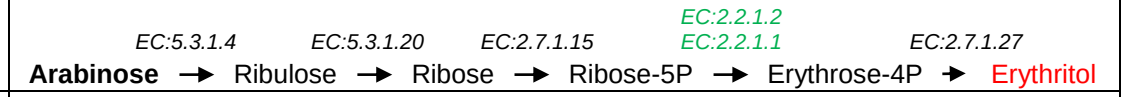
Supplementary Table S2. Multivariate (VIP value > 1.00) and univariate (p-value < 0.05) statistical parameters of 26 marker metabolites with whole data (n=60) implying importance for discriminating control and parasitized groups.

Metabolites	VIP value	p-value
Erythritol	2.35	1.3×10^{-13}
Arabitol	2.34	3.9×10^{-23}
Galactose	1.89	1.0×10^{-10}
Citric acid	1.65	0.017
Maltose	1.50	0.007
myo-Inositol	1.47	0.044
Mannitol	1.45	1.7×10^{-17}
Arabinose	1.40	3.0×10^{-8}
Xylitol	1.40	0.015
Malic acid	1.39	0.05
Glucose	1.37	0.84
Ribitol	1.33	2.5×10^{-7}
Glycerol	1.28	0.18
Gluconic acid	1.26	0.79
Phosphoric acid	1.25	0.13
Fructose	1.22	9.0×10^{-8}
Mannose	1.08	0.24
Ribose	1.03	0.25
Sorbitol	1.01	3.6×10^{-13}
Sorbose	1.00	0.17
Succinic acid	<1.00	3.0×10^{-4}
Glyceric acid	<1.00	6.5×10^{-13}
Citramalic acid	<1.00	2.3×10^{-4}
Oleic acid	<1.00	1.54×10^{-6}
Melibiose	<1.00	0.041
Xylose	<1.00	0.018

Supplementary Table S3. Metabolic pathway enrichment results.

Metabolic pathway	Adjusted p-value	Total metabolites included	Matched metabolites	Matched/Total (%)
Pentose and glucuronate interconversions	2.08×10^{-04}	53	5	27.8
Galactose metabolism	9.06×10^{-04}	41	4	22.2
Fructose and mannose metabolism	0.015	48	3	16.7
Starch and sucrose metabolism	0.015	50	3	16.7
Glyoxylate and dicarboxylate metabolism	0.015	44	3	16.7
Biosynthesis of plant hormones	0.026	68	3	16.7
Citrate cycle (TCA cycle)	0.026	20	2	11.1
Biosynthesis of alkaloids derived from histidine and purine	0.056	35	2	11.1
Biosynthesis of phenylpropanoids	0.056	97	3	16.7
Biosynthesis of alkaloids derived from terpenoid and polyketide	0.084	48	2	11.1
Ascorbate and aldarate metabolism	0.084	47	2	11.1
Biosynthesis of alkaloids derived from shikimate pathway	0.102	138	3	16.7
Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	0.122	67	2	11.1
Amino sugar and nucleotide sugar metabolism	0.181	87	2	11.1
Biosynthesis of terpenoids and steroids	0.208	98	2	11.1

Supplementary Table S4. Enzymatic pathways about metabolic flows from AMN supplies (fructose, arabinose and galactose) to five sugar alcohols. Enzymes were designated as enzyme entry in KEGG pathway and modules. Enzymes and modules in PPP were colored as green.

Fructose	Ribitol	
	Arabitol	
	Erythritol	
	Mannitol	
	Xylitol	
Arabinose	Ribitol	
	Arabitol	
	Erythritol	
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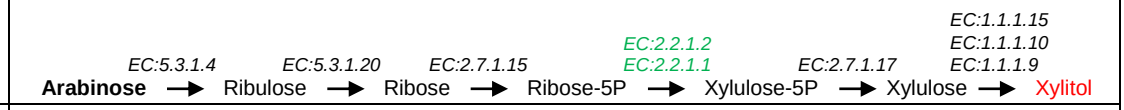
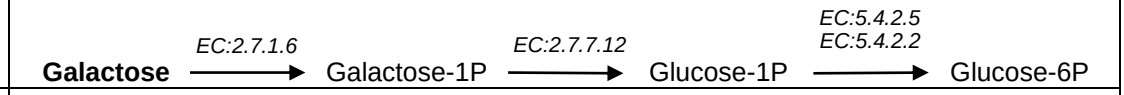
	Xylitol	
Galactose	*Leloir pathway*	
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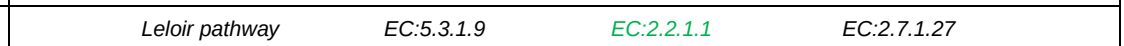
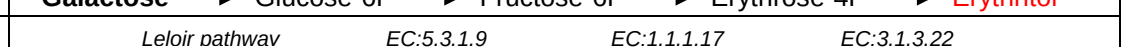
	Arabitol	
	Erythritol	
	Mannitol	
	Xylitol