Targeted and Untargeted Metabolic Profiling of Wild Grassland Plants identifies Antibiotic and Anthelmintic Compounds Targeting Pathogen Physiology, Metabolism and Reproduction

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Supplementary Materials

Table 1 Limits of detection (LOD) and limits of quantification (LOQ) for compounds identified in single or mixed plant samples with antimicrobial and/or anthelmintic properties.

Limits of Detection and quantification			
Compound	LOQ (ng/ml)	LOD (ng/ml)	R^2
Apigenin 7-O-neohesperidoside	8	2.7	0.995
Apigenin-7-O-glucuronide	8	2.7	0.994
Benzoic acid	200	66.7	0.999
Caffeic acid	8	2.7	0.999
Catechin hydrate	40	13.3	0.999
Chicoric acid	200	66.7	0.997
Chlorogenic acid	8	2.7	0.999
Epigallocatechin gallate	200	66.7	0.998
Fisetin	8	2.7	0.999
Gallic acid	40	13.3	0.999
Genistein	1.6	0.5	0.999
Genistin	40	13.3	0.999
Gentisic acid	1.6	0.5	0.999
Hesperetin	1.6	0.5	0.999
Hispidulin	8	2.7	0.999
Hydrocinnamic acid	8	2.7	0.999
Luteolin	1.6	0.5	0.999
Luteolin-7-O-glucuronide	40	13.3	0.994
Naringenin	1.6	0.5	0.999
P-Coumaric acid	40	13.3	0.997
Quercetin	0.0128	0.004	0.978
Quinic acid	0.064	0.02	0.999
Rhamnetin	8	2.7	0.999
Rosmarinic acid	8	2.7	0.999
Rutin hydrate	1.6	0.5	0.999
Salicylic acid	40	13.3	0.996
Trans-Ferulic acid	8	0.3	0.999

Table 2 Analytical precision found using eight replicates of a Quercetin standard.

Precision	
Replicate	Peak area
1	1088734877
2	1513126643
3	1067463481
4	1323959367
5	1439017757
6	1457244393
7	1107607722

8	1193506930
Relative standard Deviation	14.2

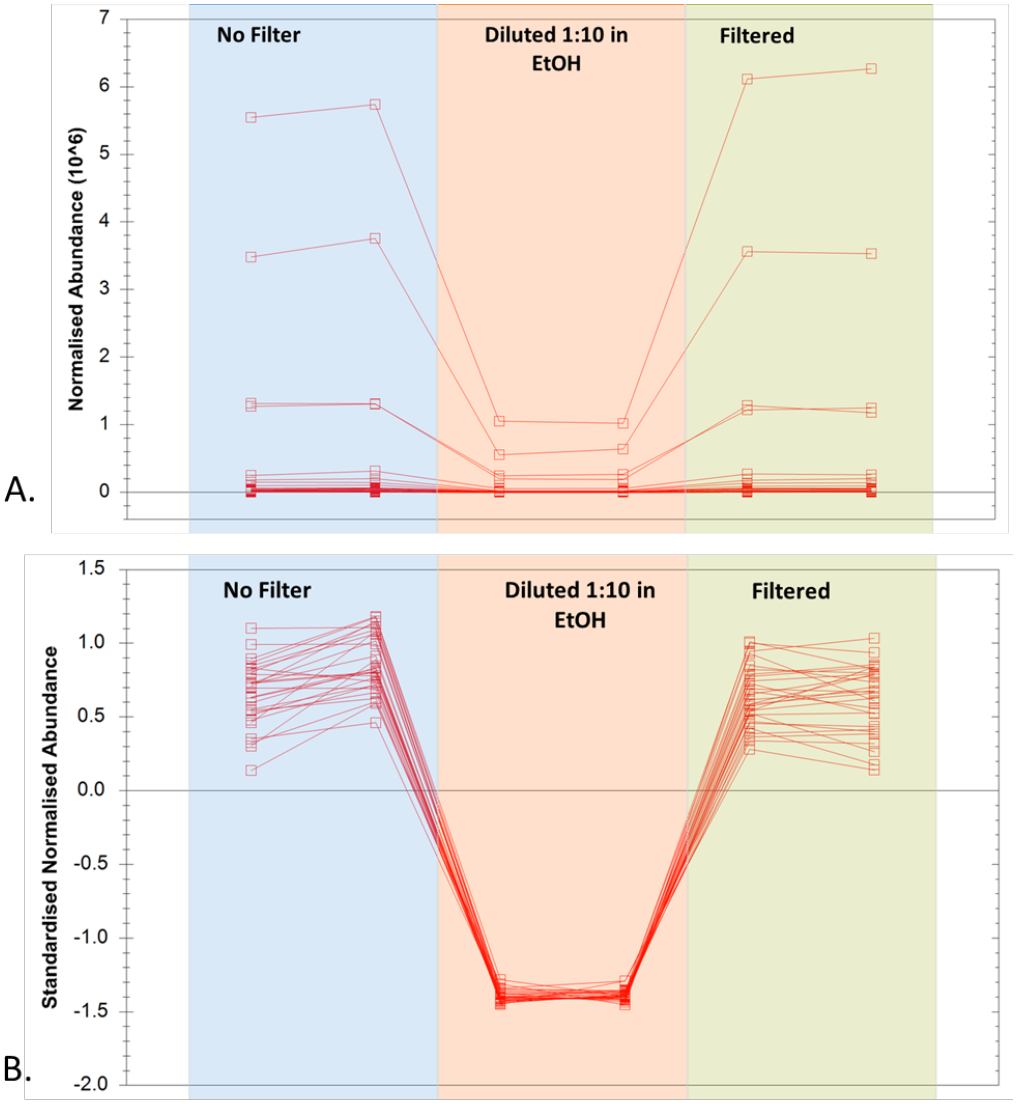

Figure 1 Effect of filtering on compound abundances. A. Normalized abundances of samples of samples without filtering, diluted 1:10 with 80% EtOH, and after filtering with a 0.45 um Millipore filter. B. Standardized normalized abundances of samples of samples without filtering, diluted 1:10 with 80% EtOH, and after filtering with a 0.45 um Millipore filter.

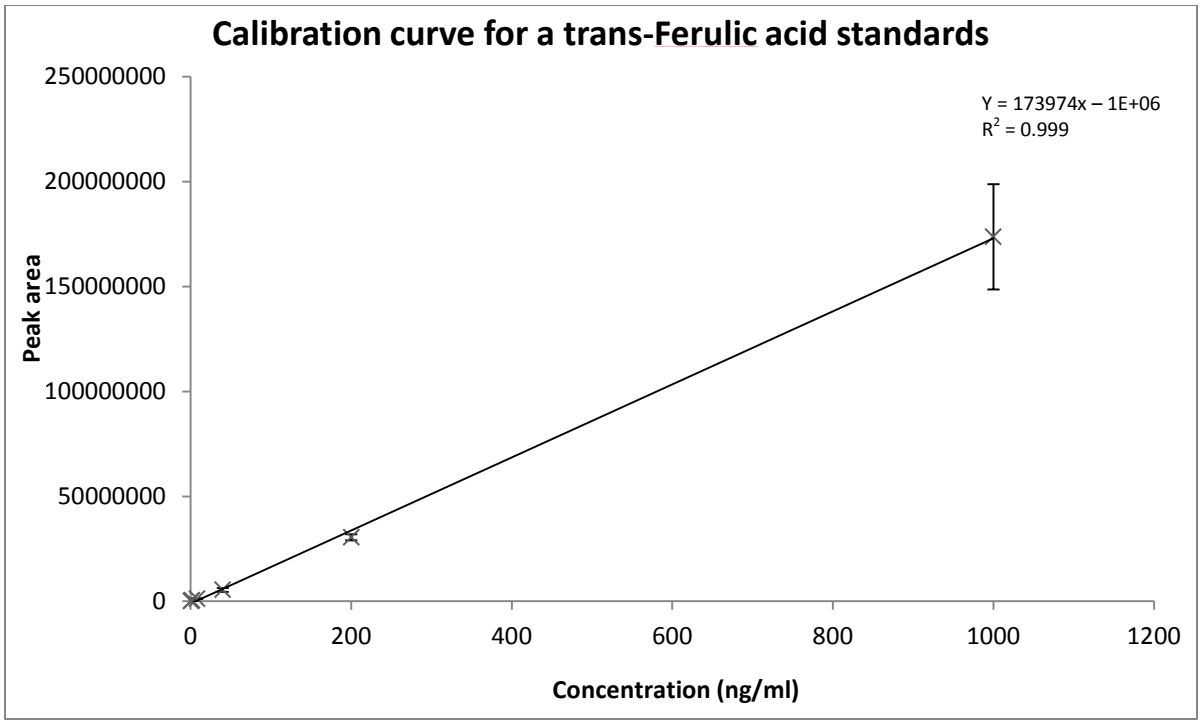

Figure 2 Calibration curve for trans-Ferulic acid authentic standard.

Table 3 Authentic standards and their database analytical properties used to identify compounds in plant extracts.

Compound ID	Neutral Mass	Adduct	Retention time (min)	Formula
2,4,6-Tris(2-pyridyl)-s-triazine	312.1123444	M+H	7.56	C18H12N6
Baicalein	270.0528233	M-H	8.65	C15H10O5
Biochanin A	284.067925	M-H	9.65	C16H12O5
Capsaicin	305.1990936	M-H	9.81	C18H27NO3
Catechin	290.079038	M-H	4.43	C15H14O6
Daidzen	254.0579087	M-H	7.30	C15H10O4
Fisetin	286.0477379	M-H	7.01	C15H10O6
Galangin	270.0528233	M-H	9.98	C15H10O5
Gallic acid	170.0215232	M-H	1.49	C7H6O5
Hesperetin	302.079038	M-H	7.90	C16H14O6
Homovanilic acid	182.0579087	M-H	4.93	C9H10O4
Luteolin	286.0477379	M-H	7.90	C15H10O6
Luteolin-7-O-β-D-Glucuronide	462.0798257	M-H	6.45	C21H18O12
Myricetin	318.0375671	M-H	6.91	C15H10O8
Naringenin	272.0684734	M-H	7.67	C15H12O5
P-Coumaric acid	164.047344	M-H	5.61	C9H8O3
Protocatechuic acid	154.0266086	M-H	2.80	C7H6O4
Pyrogallol	126.031694	M-H	1.46	C6H6O3
Quercetin	302.0426525	M-H	7.66	C15H10O7

Quinic acid	192.063388	M-H	0.77	C7H12O6
Rutin hydrate	610.1533845	M-H	6.47	C27H30O16
Salicylic acid	138.031694	M-H	6.18	C7H6O3
Sinapic acid	224.0684734	M-H	5.96	C11H12O5
Syringic acid	198.0528233	M-H	5.15	C9H10O5
Tetracycline	444.1532655	M-H	5.09	C22H24N2O8
Vanilin	152.047344	M-H	5.20	C8H8O3
Apigenin	270.0528233	M-H	8.48	C15H10O5
Chrysin	254.0579087	M-H	9.69	C15H10O4
Hispidulin	300.063388	M-H	8.54	C16H12O6
Kaempferol	286.0477379	M-H	8.37	C15H10O6
T-Ferulic acid	194.0573603	M-H	5.88	C10H10O4
Vitexin	432.1056466	M-H	6.17	C21H20O10
Caffeic acid	180.0422586	M-H	4.92	C9H8O4
Genistin	432.1056466	M+H	6.19	C21H20O10
Gentisic acid	154.0266086	M-H	3.85	C7H6O4
Hydroxytyrosol	154.0635425	M-H	2.90	C8H10O3
Lauric acid / dodecanoic acid	200.1781783	M-H	12.55	C12H24O2
Malvidin chloride	366.0511788	M+H	7.48	C17H15O7
Arachidic acid	312.3033788	M-H	15.63	C20H40O2
Berberine chloride	371.092984	M-H	4.70	C20H18NO4Cl
Caffeine	194.0809239	M-H	10.15	C8H10N4O2
Chiocoric acid	474.0798257	M-H	5.71	C22H18O12
Chlorogenic acid	354.0950819	M-H	4.70	C16H18O9
Ellagic acid	302.006267	M-H	6.67	C14H6O8
Ellaidic acid	282.2564286	M-H	14.60	C18H34O2
Epigallocatechin gallate	458.0849111	M-H	5.07	C22H18O11
Ferulic acid	194.0584571	M-H	5.88	C10H10O4
Formononetin	268.0730104	M-H	8.81	C16H12O4
Emodin	270.0533717	M-H	8.66	C15H10O5
Homovanillyl alcohol	168.0791926	M-H	13.58	C9H12O3
O-Toluic Acid	136.051881	M-H	5.84	C8H8O2
Anabasine	162.11515	M+H	16.81	C10H14N2
Apigenin-7-O-glucuronide	446.0843627	M-H	6.85	C21H18O11
Harmine	212.0944146	M-H	5.84	C13H12N2O
Quinidine	648.3670074	M+H	5.00	C40H48N4O4
Quinine	324.1837779	M+H	5.00	C20H24N2O2
Tetrandrine	622.3037385	M+H	5.76	C38H42N2O6
Theophylline	180.064177	M+H	4.10	C7H8N4O2
Castanospermine	189.0995594	M-H	0.72	C8H15NO4
Catharanthine	336.1832295	M+H	6.10	C21H24N2O2
Cyclopamine	411.313181	M+H	7.78	C27H41NO2
Pilocarpine	208.1206293	M+H	0.84	C11H16N2O2
Theobromine	180.064177	M-H	3.20	C7H8N4O2
Tomatidine	415.3444811	M+H	9.25	C27H45NO2
Aconitine	645.3143626	M+H	7.27	C34H47NO11
Cocaine	303.1465096	M+H	5.39	C17H21NO4

Codeine	299.151595	M+H	3.64	C18H21NO3
Galanthamine	287.151595	M+H	0.84	C17H21NO3
Mescaline hcl	211.1202949	M+H	4.29	C11H17NO3
Morphine	285.135945	M+H	0.80	C17H19NO3
Tryptamine	160.0995	M+H	3.64	C10H12N2
Tyramine	137.0835155	M+H	0.80	C8H11NO
Ajmalicine	352.1781441	M-H	6.14	C21H24N2O3
Carvacrol	150.1039166	M-H	9.41	C10H14O
Cinchonidine	294.1726649	M+H	4.92	C19H22N2O
Conessine	356.3186008	M-H	4.87	C24H40N2
Geraniol	154.1352167	M-H	8.11	C10H18O
Tubocurarine)	610.3042869	M-H	4.57	C37H42N2O6
Benzoic acid	122.0367794	M-H	6.36	C7H6O2
Genistein	270.0528233	M-H	8.08	C15H10O5
Hydrocinnamic acid	150.0680795	M-H	7.20	C9H10O2
Isorhamnetin	316.0583026	M-H	9.25	C16H12O7
Pelargonidin	271.0606483	M+H	8.11	C15H11O5
Rhamnetin	316.0583026	M-H	9.25	C16H12O7
Rosmarinic acid	360.0845173	M-H	5.78	C18H16O8
Trans-Aconitic acid	174.0164378	M-H	0.89	C6H6O6
Amentoflavone	538.0899965	M-H	9.85	C30H18O10
Apigenin-7-O-neohesperidoside	578.1635553	M-H	6.97	C27H30O14
Cyanidin chloride	322.0238672	M+H	8.00	C15H11O6
Eriodictyol	288.063388	M-H	7.15	C15H12O6
Eriodictyol-7-O-glucoside	450.1162113	M+H	6.03	C21H22O11
Isoorientin	448.1000128	M+H	5.99	C21H20O11
Orientin	448.1000128	M-H	5.99	C21H20O11
Quercetin-3-O-glucoside	464.0954758	M-H	6.63	C21H20O12
Urolithin A	228.0423	M-H	7.18	C13H8O4
Urolithin B	212.047344	M-H	8.15	C13H8O3
Urolithin C	244.0371732	M-H	6.59	C13H8O5

Table 4 Antimicrobial and anthelmintic compounds found in grassland plants. The table shows whether the compound has antimicrobial and/or anthelmintic properties and whether the biosynthetic pathway of the compound is known (designated by 'x').

Compound	Antimicrobial	Anthelmintic	Pathway	References
Agropyrene	x			Williamson and Evans, 1989
Apigenin-7-O-glucuronide	x		x	Duke, 1992; Newall et al., 1996; Recio et al., 1989
Apigenin-7-O-neohesperidoside	x		x	Duke, 1992; Newall et al., 1996; Recio et al., 1989
Benzoic acid	x		x	Duke 1992; Park 2001
Biochanin A	x		x	Flesar et al. 2009; Sklenickova et al. 2010
Caffeic acid		x	x	Cowan, 1999
Carvacrol	x	x		Baxter et al., 1998; Duke, 1992; Lagouri et al., 1993; Leung and Foster, 2003;

				Russell, 1986
Catechin hydrate		x		Cowan, 1999
Chicoric acid		x		Street et al. 2013
Chlorogenic acid	x		x	Lou et al. 2011; Kabir et al. 2014
Chrysin	x		x	Duke 1992
Coumarin	x		x	Baxter et al., 1998; Buchbauer et al., 1996; Newall et al., 1996
Epigallocatechin gallate		x	x	Cowan, 1999
Fisetin	x			Duke 1992
Gallic acid	x		x	Cowan, 1999
Genistein	x		x	Duke 1992
Genistin	x		x	Duke 1992
Gentisic acid	x		x	Duke 1992
Geraniol	x	x	x	Duke, 1992; Fleisher and Fleisher, 1991; Madaus, 1976; Muroi and Kubo, 1993
Hesperetin	x		x	Duke 1992
Hispidulin	x			Duke 1992
Hydrocinnamic acid	x		x	Duke 1992
Luteolin	x		x	Duke 1992; Joung et al. 2016
Luteolin-7-O-D-glucuronide	x		x	Duke 1992
Malvidin chloride	x			Duke 1992
Mannitol		x	x	Duke, 1992
Myricetin	x		x	Duke 1992
Naringenin	x		x	Duke 1992
p-Coumaric acid	x		x	Duke 1992
Quercetin	x		x	Baxter et al., 1998; Duke, 1992; Newall et al., 1996
Quinic acid	x		x	Duke 1992
Rhamnetin	x		x	Duke 1992
Rosmarinic acid	x		x	Duke 1992
Rutin hydrate	x			Duke 1992
Salicylic acid	x	x	x	Cowan, 1999
t-Ferulic acid	x		x	Duke 1992
Thymol	x	x		Duke, 1992; Sezik et al., 1993; Williamson and Evans, 1989

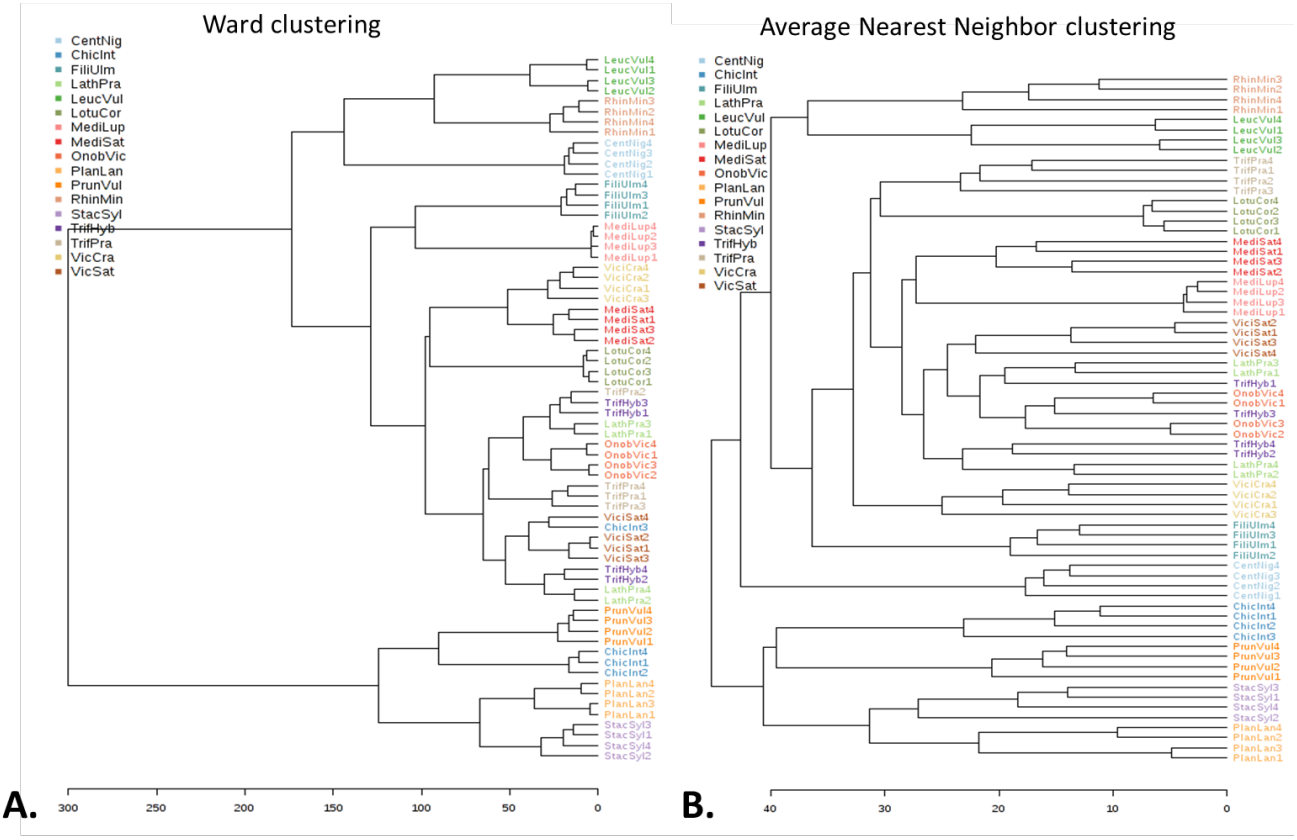

Figure 3 Chemotaxonomy of 17 grassland plants based on 51 compounds using Euclidean distances and (A) Ward clustering and (B) Average Nearest Neighbor.

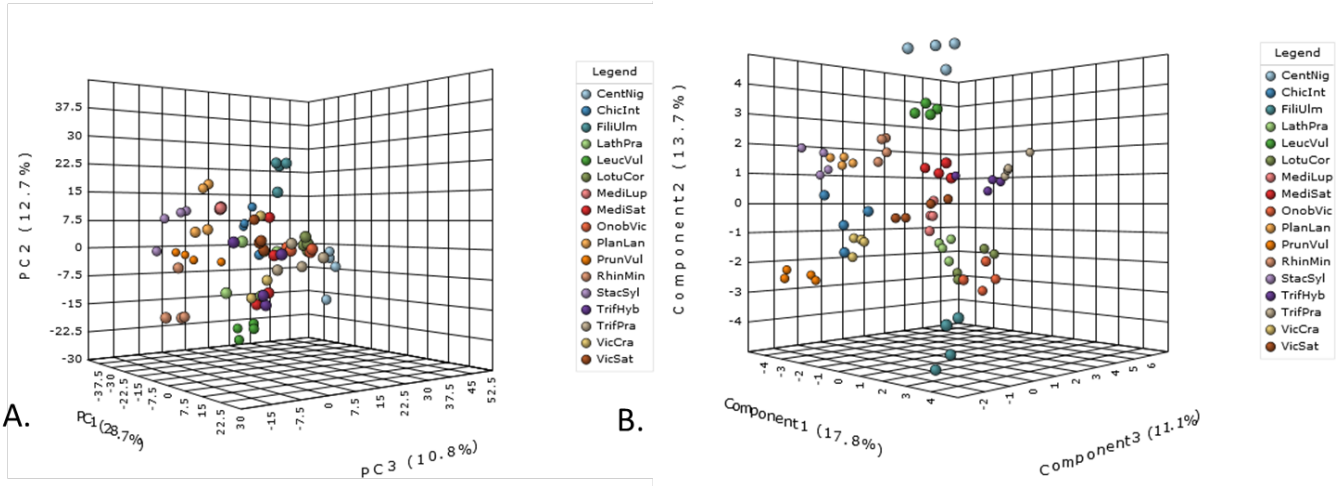

Figure 4 PCA (A.) and PLSDA (B.) showing the similarities and differences in metabolomic composition of 17 grassland plants. In all figures, samples from each plant are identified as the first four letters of the genus and first three letters of the species according to Linnean classification.

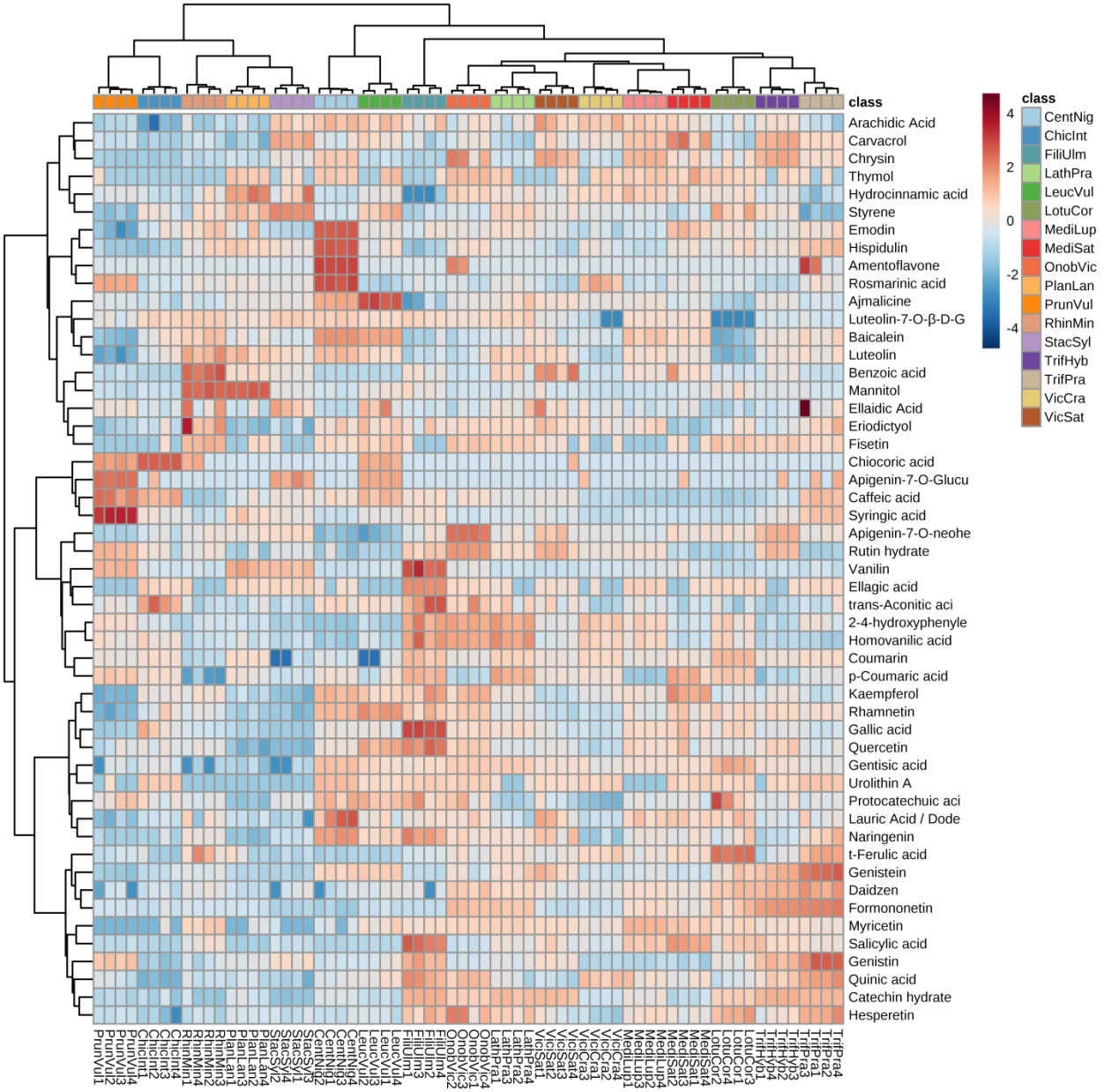

Figure 5 Heat map of 51 compounds found in 17 grassland plants based on Euclidean distances and Ward clustering. Samples are color coded by species labeled according to the first four letters of the genus and first three letters of the species.

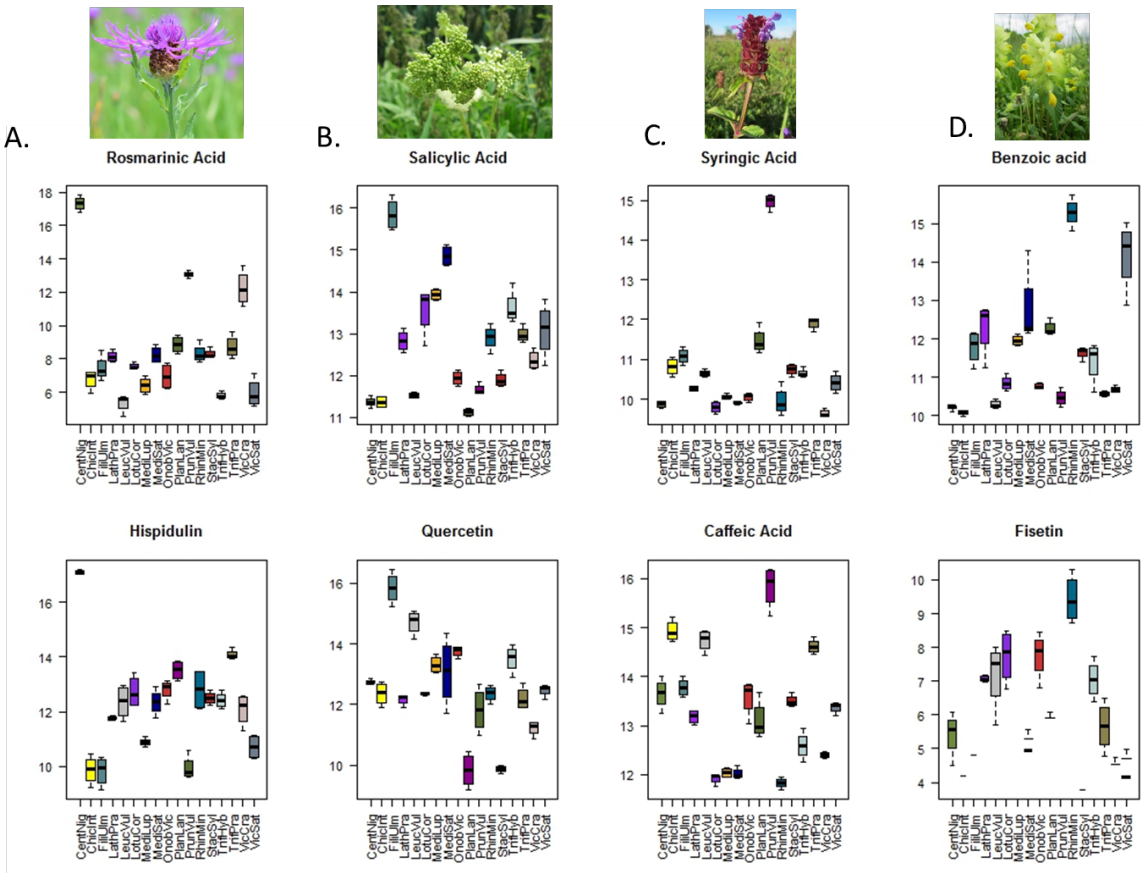

Figure 6 Concentration of antimicrobial and anthelmintic compounds in *Centaurea nigra* (A), *Filipendula ulmaria* (B), *Prunella vulgaris* (C) and *Rhinanthus minor* (D). Samples from each plant are identified as the first four letters of the genus and first three letters of the species according to Linnean classification. Compound concentrations are represented on a log scale.

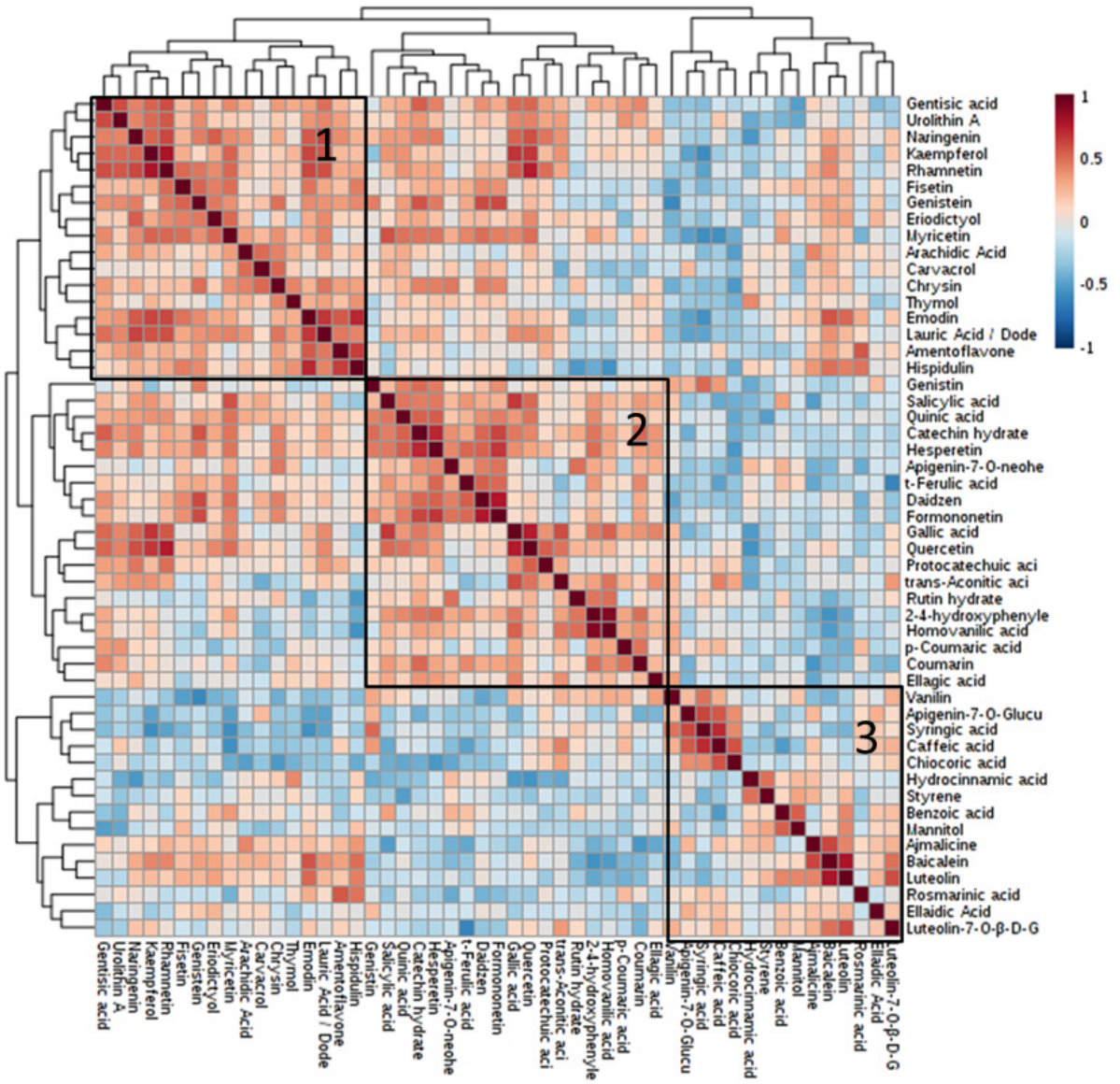

Figure 7 Heat map showing the correlations among compounds present in 17 grassland species. Pearson correlation (r) is used as the distance measure. Compounds fell into three main groups. The colors refer to the pair-wise correlation coefficient ranging from 1 (red) to -1 (blue).

Figure 8 Metabolic map showing the three groups of highly correlated compounds and the pathways they belong to using *Arabidopsis* as a model. The map can be accessed at: http://pmn.plantcyc.org/overviewsWeb/celOv.shtml?zoomlevel=0&lon=1560&lat=29768&orgid=ARA&xnids=gentisic%20acid%0Aurolithin%20A%0Anarigenin%0Akaempferol%0Arhamnetin%0Afisetin%0Amyricetin%0Agenistein%0Aerodictol%0Aarachidic%20acid%0Ahispidulin%0Achrysin% .
Not all of the compounds have known pathways (e.g. thymol) and are not shown on the map.