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Heterologous gene expression system for the production of hydrolyzable tannin intermediates in herbaceous model plants

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Table S1 Accession numbers of DQD/SDHs shown in Fig. 2

Species	Protein name	Accession	Reference
Arabidopsis thaliana (At)	DQD/SDH	AT3G06350	Singh and Christendat 2006
Camellia sinensis (Cs)	DQD/SDHa	AYP64306	Huang et al. 2019
Camellia sinensis (Cs)	DQD/SDHb	AYP64307	Huang et al. 2019
Camellia sinensis (Cs)	DQD/SDHc	AYP64308	Huang et al. 2019
Camellia sinensis (Cs)	DQD/SDHd	AYP64309	Huang et al. 2019
Eucalyptus camaldulensis (Ec)	DQD/SDH1	BBL52470	Tahara et al. 2021
Eucalyptus camaldulensis (Ec)	DQD/SDH2	BBL52471	Tahara et al. 2021
Eucalyptus camaldulensis (Ec)	DQD/SDH3	BBL52472	Tahara et al. 2021
Eucalyptus camaldulensis (Ec)	DQD/SDH4 (QDH)	BBL52473	Tahara et al. 2021
Juglans regia (Jr)	SDH	AAW65140	Muir et al. 2011
Nicotiana benthamiana (Nb)		Niben101Scf00247g04007	This study
Nicotiana benthamiana (Nb)		Niben101Scf01642g01015	This study
Nicotiana benthamiana (Nb)		Niben101Scf01740g12012	This study
Nicotiana benthamiana (Nb)		Niben101Scf02709g04001	This study
Nicotiana benthamiana (Nb)		Niben101Scf04146g01023	This study
Nicotiana benthamiana (Nb)		Niben101Scf04216g05005	This study
Nicotiana benthamiana (Nb)		Niben101Scf06613g05001	This study
Nicotiana tabacum (Nt)	DQD/SDH1	AAS90325	Ding et al. 2007
Nicotiana tabacum (Nt)	DQD/SDH2	AAS90324	Ding et al. 2007
Pinus taeda (Pit)	SDH		Carrington et al. 2018
Pinus taeda (Pit)	QDH		Carrington et al. 2018
Populus trichocarpa (Pot)	DQD/SDH1 (Poptr1)	Potri.010G019000.2	Guo et al. 2014
Populus trichocarpa (Pot)	DQD/SDH2 (Poptr5)	Potri.013G029800.1	Guo et al. 2014
Populus trichocarpa (Pot)	QDH1 (Poptr2)	Potri.013G029900.2	Guo et al. 2014
Populus trichocarpa (Pot)	QDH2 (Poptr3)	Potri.005G043400.1	Guo et al. 2014
Populus trichocarpa (Pot)	QDH3 (Poptr4)	Potri.014G135500.3	Guo et al. 2014
Solanum lycopersicum (Sl)	DQD/SDH1	AAC17991	Bischoff et al. 2001
Solanum lycopersicum (Sl)	QDH	Solyc10g038080.1.1	Gritsunov et al. 2018
Vitis vinifera (Vv)	SDH1	KU163040	Bontpart et al. 2016
Vitis vinifera (Vv)	SDH2	KU163041	Bontpart et al. 2016
Vitis vinifera (Vv)	SDH3	KU163042	Bontpart et al. 2016
Vitis vinifera (At)	SDH4	KU163043	Bontpart et al. 2016

Table S2 Accession numbers of UGT84As and UGT72s shown in Fig. S1.

Species	Protein name	Accession	Reference
Arabidopsis thaliana (At)	UGT72B1	AT4G01070	Yang et al. 2018
Arabidopsis thaliana (At)	UGT72B2	AT1G01390	This study
Arabidopsis thaliana (At)	UGT72B3	AT1G01420	Yang et al. 2018
Arabidopsis thaliana (At)	UGT72C1	AT4G36770	Yang et al. 2018
Arabidopsis thaliana (At)	UGT72D1	AT2G18570	Yang et al. 2018
Arabidopsis thaliana (At)	UGT72D2	AT2G18560	This study
Arabidopsis thaliana (At)	UGT72E1	AT3G50740	Yang et al. 2018
Arabidopsis thaliana (At)	UGT72E2	AT5G66690	Yang et al. 2018
Arabidopsis thaliana (At)	UGT72E3	AT5G26310	Yang et al. 2018
Arabidopsis thaliana (At)	UGT84A1	AT4G15480	Milkowski et al. 2000b
Arabidopsis thaliana (At)	UGT84A2	AT3G21560	Milkowski et al. 2000b
Arabidopsis thaliana (At)	UGT84A3	AT4G15490	Milkowski et al. 2000b
Arabidopsis thaliana (At)	UGT84A4	AT4G15500	Milkowski et al. 2000b
Brassica napus (Bn)	UGT84A9	AAF98390	Milkowski et al. 2000a
Brassica napus (Bn)	UGT84A10	CAJ77650	Mittasch et al. 2007
Camellia sinensis (Cs)	UGT72AM1	ASA40331	Zhao et al. 2017
Camellia sinensis (Cs)	UGT84A22	ALO19890	Cui et al. 2016
Citrus unshiu (Cu)	CitLGT (UGT84A5)	BAA93039	Kita et al. 2000
Eucalyptus camaldulensis (Ec)	UGT84A25	BBB21213	Tahara et al. 2018
Eucalyptus camaldulensis (Ec)	UGT84A26	BBB21215	Tahara et al. 2018
Fragaria × *ananassa* (Fa)	FaGT2 (UGT84A6)	AAU09443	Schulenburg et al. 2016
Gentiana trifloral (Gt)	GtUF6CGT1	BAQ19550	Sasaki et al. 2015
Glycine max (Gm)	UGT72X4	Glyma.08G338100	Yin et al. 2017b
Glycine max (Gm)	UGT72Z3	Glyma.08G338200	Yin et al. 2017b
Hieracium pilosella (Hp)	UGT72B11	ACB56923	Witte et al. 2009
Lotus japonicus (Lj)	UGT72V3	chr3.CM0282.950.r2.m	Yin et al. 2017a
Lotus japonicus (Lj)	UGT72Z2	LjSGA_016571	Yin et al. 2017a
Lotus japonicus (Lj)	UGT72AD1	chr1.LjT10H09.40.r2.m	Yin et al. 2017a
Lotus japonicus (Lj)	UGT72AF1	chr6.CM0118.1240.r2.d	Yin et al. 2017a
Lotus japonicus (Lj)	UGT72AH1	chr3.CM0846.120.r2.d	Yin et al. 2017a
Medicago truncatula (Mt)	UGT72L1	ACC38470	Pang et al. 2008
Nicotiana benthamiana (Nb)	UGT72B34	UHH90499	Sun et al. 2019
Nicotiana benthamiana (Nb)	UGT72B35	UHH90500	Sun et al. 2019
Nicotiana benthamiana (Nb)	UGT72AX1	UHH90503	Sun et al. 2019
Nicotiana benthamiana (Nb)	UGT72AY1	UHH90560	Sun et al. 2019

Nicotiana benthamiana (Nb)		Niben101Scf00817g06013	This study
Nicotiana benthamiana (Nb)		Niben101Scf01341g00002	This study
Nicotiana benthamiana (Nb)		Niben101Scf02565g03002	This study
Nicotiana benthamiana (Nb)		Niben101Scf03434g01013	This study
Nicotiana benthamiana (Nb)		Niben101Scf04240g00006	This study
Nicotiana benthamiana (Nb)		Niben101Scf07353g02006	This study
Nicotiana benthamiana (Nb)		Niben101Scf13710g02002	This study
Populus fremontii × *P. angustifolia* (Pfa)	UGT84A17	AII32448	Babst et al. 2014
Populus tremula × *P. alba* (Pta)	UGT72B37	QLI54353	Speeckaert et al. 2020
Populus tremula × *P. alba* (Pta)	UGT72B39	QLI54355	Speeckaert et al. 2020
Populus tremula × *P. alba* (Pta)	UGT72AZ2	QLI54351	Speeckaert et al. 2020
Punica granatum (Pg)	UGT72BD1	QHB92369	Chang et al. 2019
Punica granatum (Pg)	UGT84A23	ANN02875	Ono et al. 2016
Punica granatum (Pg)	UGT84A24	ANN02877	Ono et al. 2016
Quercus robur (Qr)	UGT84A13	AHA54051	Mittasch et al. 2014
Solanum lycopersicum (Sl)	SlUGT5	ADI33725	Louveau et al. 2011
Vitis labrusca (Vl)	VlRSgt	ABH03018	Hall and De Luca 2007
Vitis vinifera (Vv)	UGT72B27	QZP12103	Härtl et al. 2017
Vitis vinifera (Vv)	VvgGT1	AEW31187	Khater et al. 2012

Table S3 Orders, families, and genera in which hydrolyzable tannins were reportedly identified

Order	Family	Genus
Nymphaeales	Nymphaeaceae	*Nuphar*
Gunnerales	Myrothamnaceae	*Myrothamnus*[1]
Fabales	Fabaceae	*Caesalpinia, Ceratonia, Haematoxylum*
Rosales	Elaeagnaceae	*Elaeagnus, Hippophae, Shepherdia*
	Rosaceae	*Agrimonia, Filipendula, Fragaria, Geum, Rosa, Rubus, Potentilla, Sanguisorba, Sieversia*
Fagales	Betulaceae	*Alnus, Carpinus, Corylus*
	Casuarinaceae	*Casuarina*
	Fagaceae	*Castanea, Castanopsis, Quercus*
	Juglandaceae	*Juglans, Rhoiptelea*
Cucurbitales	Coriariaceae	*Coriaria*
Oxalidales	Elaeocarpaceae	*Elaeocarpus*
Malpighiales	Euphorbiaceae	*Alchornea, Aleurites, Euphorbia, Excoecaria, Macaranga, Mallotus*
	Phyllanthaceae	*Antidesma, Phyllanthus*
Geraniales	Geraniaceae	*Geranium*
Myrtales	Combretaceae	*Anogeissus, Combretum, Terminalia*
	Lythraceae	*Cuphea, Lagerstroemia, Punica, Trapa, Woodfordia*
	Melastomataceae	*Bredia, Heterocentron, Medinilla, Melastoma, Tibouchina*
	Myrtaceae	*Eucalyptus, Eugenia, Feijoa, Melaleuca, Psidium, Syzygium*
	Onagraceae	*Epilobium, Fuchsia, Oenothera*
Crossosomatales	Stachyuraceae	*Stachyurus*
Malvales	Cytinaceae	*Cytinus*
Sapindales	Aceraceae	*Acer*
	Anacardiaceae	*Cotinus, Mangifera, Rhus*
Vitales	Vitaceae	*Vitis*
Saxifragales	Altingiaceae	*Liquidambar*
	Cercidiphyllaceae	*Cercidiphyllum*
	Hamamelidaceae	*Hamamelis, Loropetalum*
	Paeoniaceae	*Paeonia*
	Saxifragaceae	*Bergenia, Tellima*
Caryophyllales	Polygonaceae	*Rheum*
	Tamaricaceae	*Reaumuria, Tamarix*
Cornales	Cornaceae	*Cornus*
	Nyssaceae	*Camptotheca*
Ericales	Ericaceae	*Arctostaphylos, Pyrola*
	Lecythidaceae	*Barringtonia*
	Theaceae	*Camellia, Gordonia, Schima*

Hydrolyzable tannin-containing genera reviewed by Okuda et al. (2000) were revised according to APG IV unless otherwise noted. [1]Engelhardt et al. (2016) reported the presence of hydrolyzable tannins in this genus.

Table S4 PCR primers used for subcloning and the quantitative real-time PCR analysis

Purpose	Gene	Forward or reverse	Sequence (5' –3')
Subcloning	*EcDQD/SDH2*	Fw	tacaattacagatcccATGACTCTCAGCAGCATCC
		Rev	gtcggtacccccgggACTGTTCTTCACCAATGTAT
	EcDQD/SDH3	Fw	gctgtcgaccccgggATGGGCAGCGTTCCGTTCAC
		Rev	gacttatctagttacccTGCATGTTTCTCCATGAG
	UGT84A25	Fw	ttacaattacagatcccATGGGGTCGGAGGCGCTC
		Rev	gtcggtacccccgggCGACACTACCTTCAGCTCCC
	UGT84A26	Fw	gctgtcgaccccgggATGGGGTCGGAGGCACTTGT
		Rev	ctcgtcgacggtacccccCGACACCACCTTTAACT
Real-time PCR	*EcDQD/SDH2*	Fw	TGCCATCTACACGCCAAAATTG
		Rev	CAGTTGCTTTGGTGCAGGATAC
	EcDQD/SDH3	Fw	ATGCCATTTACACACCAAAGGAC
		Rev	GTCCTGAACAATTCCTCCGGG
	UGT84A25	Fw	AGAACGCGATGAAGTGGAGC
		Rev	GCCATTGGCCGTTGACTTGC
	UGT84A26	Fw	TCCTCAGACCGGAACATCCAG
		Rev	CCATTGCCCGCCACTTTGTTG
	NbEF-1α	Fw	AGCTTTACCTCCCAAGTCATC
		Rev	CAGAACGCCTGTCAATCTTGG

Table S5 β-Glucogallin and related compounds identified and quantified by UPLC-Q-TOF-MS

Compound	Retention time (min)	Elemental composition	Measured ion	Calculated exact mass	Measured accurate mass	Error (ppm)
Quinic acid	0.66	$C_7H_{12}O_6$	$[M-H]^-$	192.0634	192.0653	9.8
Shikimic acid	0.83	$C_7H_{10}O_5$	$[M-H]^-$	174.0528	174.0531	1.6
3-Dehydroshikimic acid	1.02	$C_7H_8O_5$	$[M-H]^-$	172.0371	172.0381	5.3
β-Glucogallin	2.26	$C_{13}H_{16}O_{10}$	$[M-H]^-$	332.0744	332.0761	5.2
Gallic acid	2.29	$C_7H_6O_5$	$[M-H]^-$	170.0215	170.0240	14.7
3-Glucogallic acid	2.64	$C_{13}H_{16}O_{10}$	$[M-H]^-$	332.0744	332.0755	3.5
4-Glucogallic acid	3.75	$C_{13}H_{16}O_{10}$	$[M-H]^-$	332.0744	332.0768	7.5

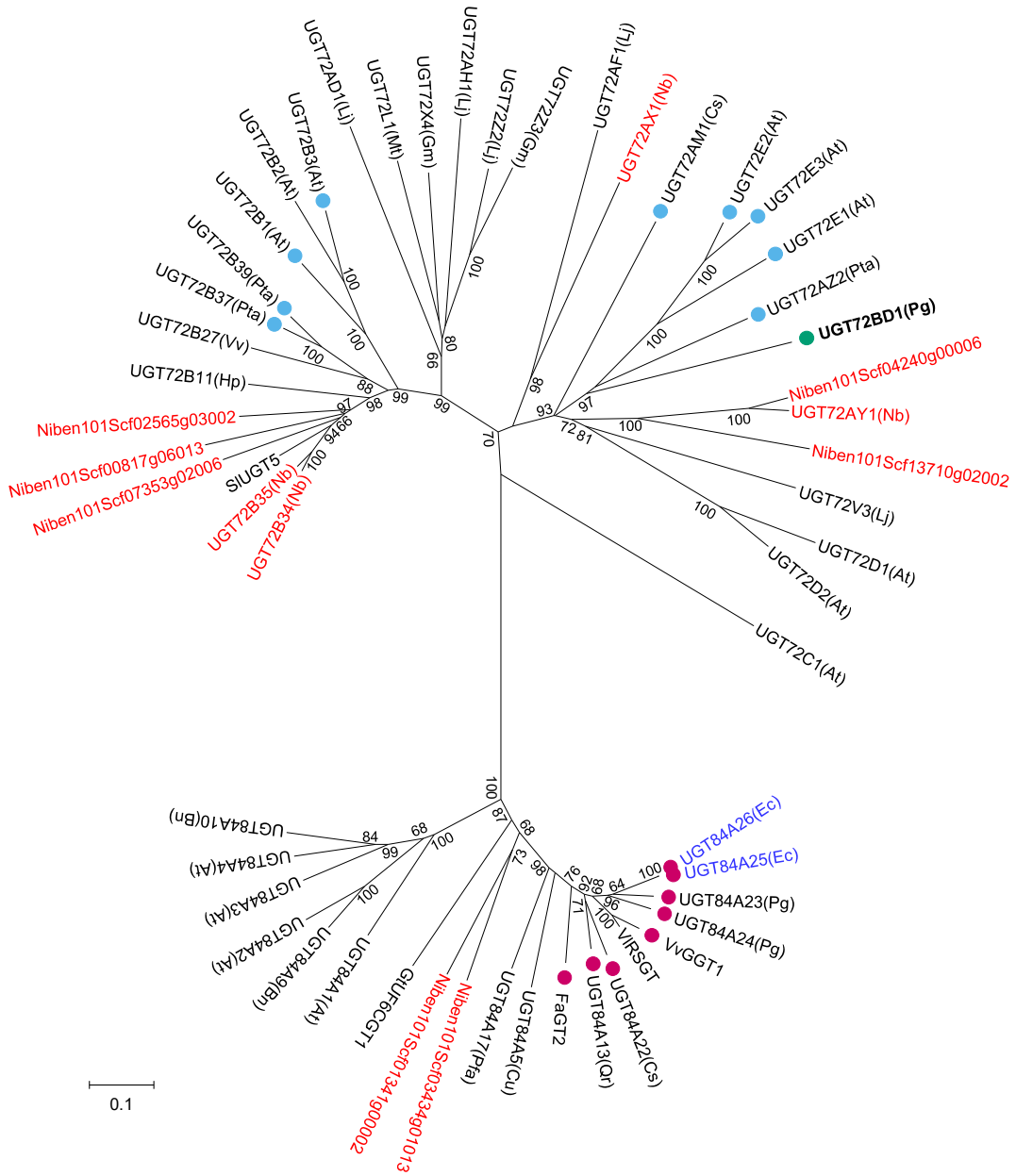

Fig. S1 Phylogenetic analysis of UGT84As and UGT72s from *Nicotiana benthamiana* and *Arabidopsis thaliana*, and their family members functionally characterized in seed plants. The phylogenetic tree was constructed based on an alignment of multiple full-length protein sequences according to the neighbor-joining method. The scale bar represents 0.1 fixed mutations per site. Bootstrap values (1,000 replicates) greater than 60% are indicated. The accession numbers of the UGTs are listed in Table S2. The UGTs from *N. benthamiana* and *Eucalyptus camaldulensis* are highlighted in red and blue letters, respectively. Magenta, green and blue dots indicate enzymes with activities of the formation of β-glucogallin, 4-glucogallic acid, and phenylpropanoid 4-*O*-glucoside, respectively.

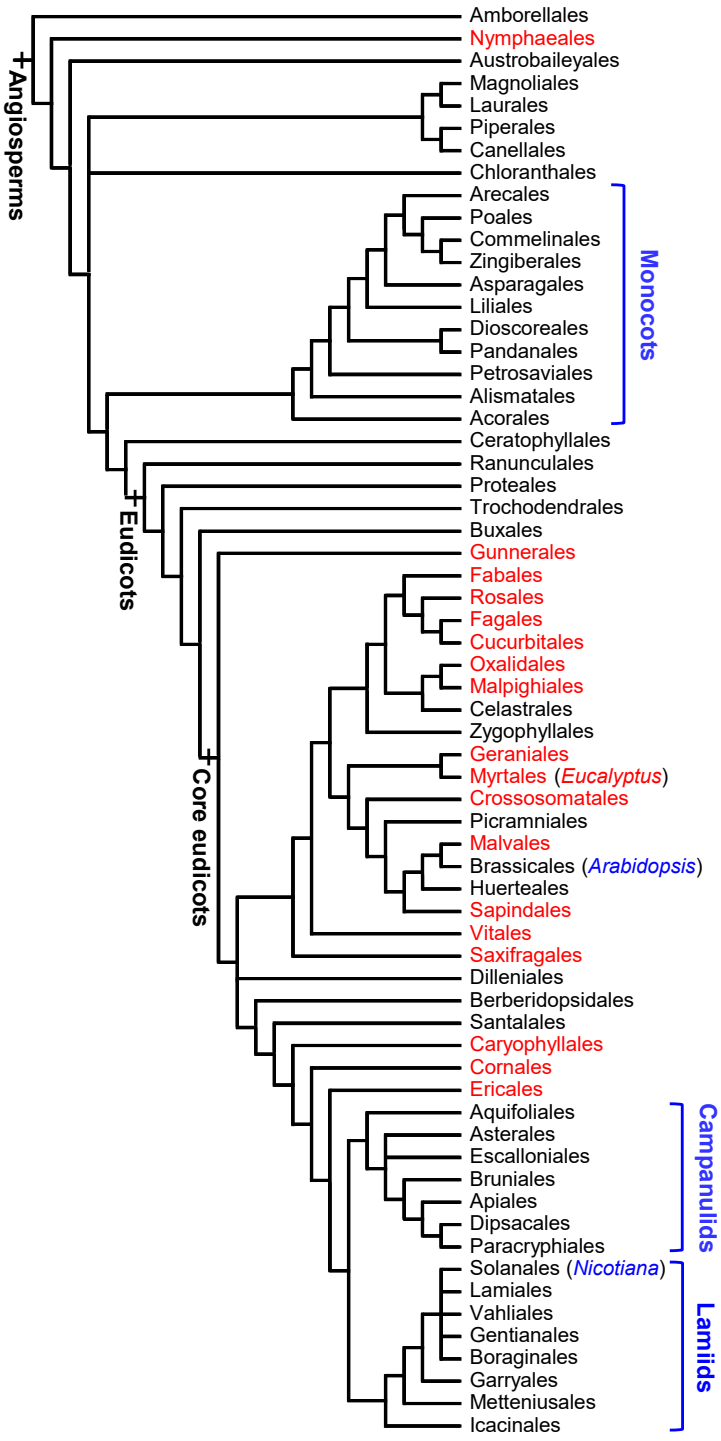

Fig. S2 Distribution of hydrolyzable tannins (HTs) in flowering plants. Orders in which HTs were reportedly identified are indicated in red in the phylogenetic tree constructed based on the Angiosperm Phylogeny Group classification (APG IV). Families and genera in which HTs were reportedly identified are listed in Table S3.

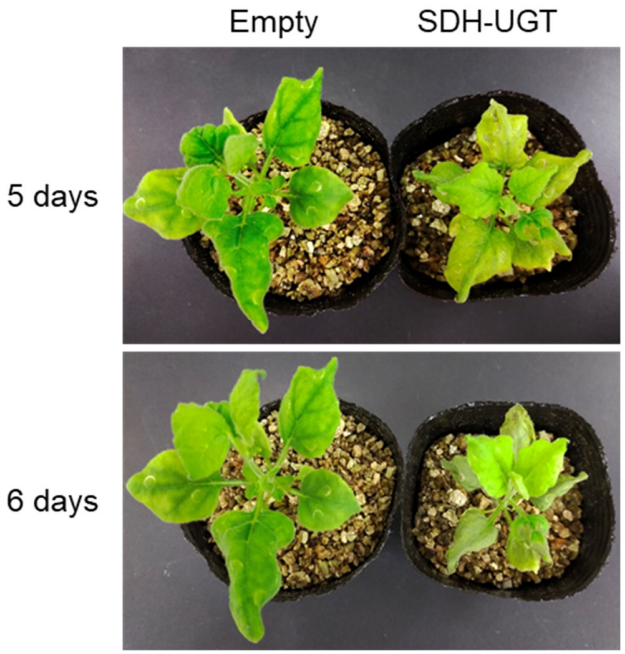

Fig. S3 Harmful effects of the long-term incubation of *N. benthamiana*. The *N. benthamiana* plants infiltrated with *A. tumefaciens* harboring the empty vector or the SDH-UGT vector are presented.

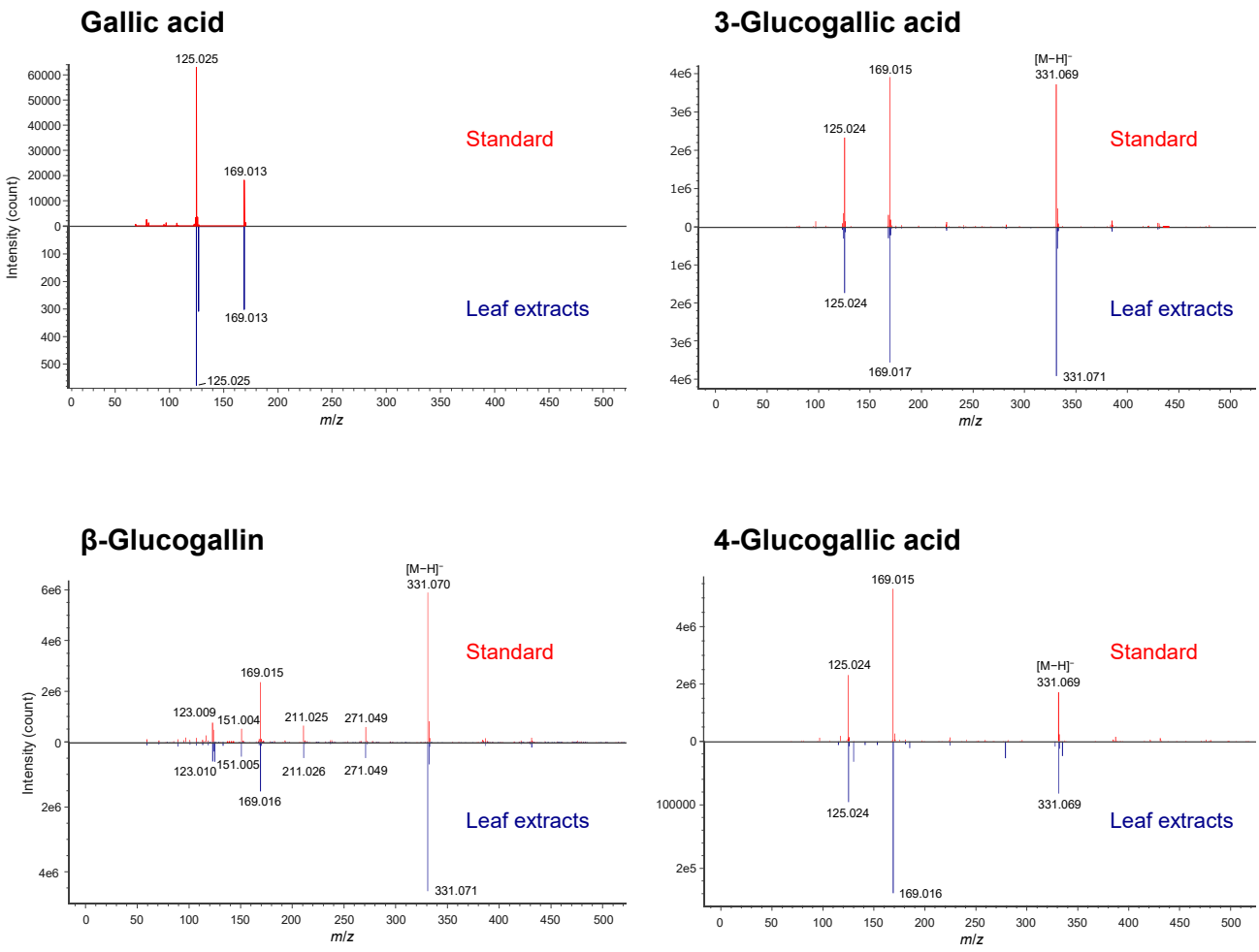

Fig. S4 Product ion spectra of gallic acid, β-glucogallin, 3-glucogallic acid, and 4-glucogallic acid. Product ion spectra were acquired at high collision energies (10–45 eV) using the UPLC-Q-TOF-MS system and then compared between authentic standards and leaf extracts.

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