Supplementary Materials for

Partial Purification, Identification, and Quantitation of Antioxidants from Wild Rice *Zizania latifolia*

Mei-Jun Chu, Xin-Min Liu, Ning Yan, Feng-Zhong Wang, Yong-Mei Du * and Zhong-Feng Zhang *

Contents

Table S1. Phenolic acids and flavonoids found in wild rice in previous reports.

Compound	Species	Collection Region of Sample	Identification Method	Reference
Phenolic acids and their derivatives				
Trans-ferulic acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
Cis-ferulic acid				
Ferulic acid	*Z. aquatica*	Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
		Canada, Greece, Hungary, Cambodia	HPLC	[3]
Chlorogenic acid	*Z. aquatica*	Canada, Greece, Hungary, Cambodia	HPLC	[3]
Caffeic acid				
Gallic acid				
Ellagic acid				
o-Coumaric acid				
Protocatechuic acid ethyl ester				
Cinnamic acid				
Trans-*p*-coumaric acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
Cis-*p*-coumaric acid				
p-Coumaric acid	*Z. aquatica*	Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
		Canada, Greece, Hungary, Cambodia	HPLC	[3]
Trans-sinapic acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
Sinapic acid	*Z. aquatica*	Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
		Canada, Greece, Hungary, Cambodia	HPLC	[3]
p-Hydroxybenzoic acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
		Canada, Greece, Hungary,	HPLC	[3]

		Cambodia		
Protocatechuic acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
		Canada, Greece, Hungary, Cambodia	HPLC	[3]
Vanillic acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
		Canada, Greece, Hungary, Cambodia	HPLC	[3]
Syringic acid	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
		Canada, Greece, Hungary, Cambodia	HPLC	[3]
p-Hydroxybenzaldehyde	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
Protocatechuic aldehyde	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
Vanillin	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	HPLC	[1]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
O-*β*-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(*trans*-feruloyl)-*α*-L-arabinofuranosyl-(1→3)]-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose	*Z. aquatica*	North-Ontario and Saskatchewan, Canada	MS, NMR	[1]
{[5-*O*-(*trans*feruloyl)][*O*-*β*-D-xylopyranosyl				

-(1→ 2)]-*O*-*α*-L-arabinofuranosyl-(1→ 3)}-*O*-*β*-D-xylopyranosyl-(1→4)-*D*-xylopyranose				
O-[5-*O*-(*trans*-feruloyl)-*α*-L-arabinofuranosyl]-(1→3)-*O*-*β*-D-xylopyranosyl-(1→ 4)-D-xylopyranose				
D-xylopyranosyl-(1 → 2)-[5-*O*-(*trans*-feruloyl)-L-arabinofuranose				
5-*O*-(*trans*-feruloyl)-L-arabinofuranose				
Cyclic form of 8–8-coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
Noncyclic form of 8–8-coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
Cyclic form of 8–8-coupled dehydrodisinapic acid (thomasidioic acid)	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
Noncyclic form of 8–5-coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
Noncyclic form of 8–8/7–*O*–7-coupled dehydrodiferulic acid				
Noncyclic form of 8–8-coupled	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]

dehydrodisinapic acid		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
8−*O*−4-coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
Cyclic form of 8−5-coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
5−5-Coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
		Dinorwic, Ontario, Canada and Houston, Texas, USA	HPLC-MS/MS	[2]
Decarboxylated noncyclic form of 8−5-coupled dehydrodiferulic acid	*Z. aquatica*	obtained from a local German supplier	GC-MS, GC-FID	[4]
Flavonoids				
6,8-Di-*C*-glucosyl apigenin	*Z. palustris*, *Z. aquatica*	Wabigoon Lake, northwestern Ontario, Canada	HPLC-MS/MS	[5]
6-*C*-Glucosyl-8-*C*-arabinosyl apigenin				
6,8-Di-*C*-arabinosyl apigenin				
Procyanidin dimer				
Procyanidin trimer				
Procyanidin tetramer				
Procyanidin pentamer				
Catechin	*Z. palustris*, *Z. aquatica*	Wabigoon Lake, northwestern Ontario, Canada	HPLC-MS/MS	[5]
	Z. aquatica	Canada, Greece, Hungary,	HPLC	[3]

		Cambodia		
Epicatechin	*Z. palustris*, *Z. aquatica*	Wabigoon Lake, northwestern Ontario, Canada	HPLC-MS/MS	[5]
	Z. aquatica	Canada, Greece, Hungary, Cambodia	HPLC	[3]
Epigallocatechin	*Z. aquatica*	Canada, Greece, Hungary, Cambodia	HPLC	[3]
Rutin				
Quercetin				
Kaempferol				

Table S2. Physical properties of six macroporous resins.

Resin	Polarity	Particle Size (mm)	Surface Area (m^2/g)	Average Pore Diameter (Å)
HPD600	Polar	0.30 – 1.20	550 – 600	80
NKA-9	Polar	0.30 – 1.25	250 – 290	155 – 165
AB-8	Weak-polar	0.03 – 1.25	480 – 520	130 – 140
X-5	Non-polar	0.30 – 1.25	500 – 600	290 – 300
D101	Non-polar	0.30 – 1.25	550 – 600	90 – 100
HPD300	Non-polar	0.30 – 1.20	800 – 870	50 – 55

Table S3. The ion transitions, optimized MS parameters, and linear relationships of standards in UPLC-QqQ-MS/MS analysis.

Compound	Parent Ion (*m*/*z*)	Product Ion (*m*/*z*)	Collision Energy (V)	t_R [a] (min)	Calibration Curve[b]	r^2	LOD[c] (ng/mL)	LOQ[d] (ng/mL)
p-Hydroxybenzaldehyde	121.138	92.224	25	2.91	$y = 8.2754\times10^4x +5.1399\times10^4$	0.9991	0.992	3.298
		120.306	20					
p-Hydroxybenzoic acid	137.116	65.238	31	2.03	$y = 2.3214\times10^5x -3.1002\times10^5$	0.9982	4.691	14.539
		75.046	32					
		93.220	17					
Vanillin	151.120	51.600	50	4.05	$y = 6.5972\times10^5x -5.8837\times105$	0.9981	6.936	20.013
		92.239	23					
		107.962	25					
		136.138	16					
Protocatechuic acid	153.095	91.099	27	1.01	$y = 7.4267\times10^4x -9.8661\times10^3$	0.9995	0.770	2.566
		108.084	26					
		109.179	17					
p-Coumaric acid	163.080	93.221	37	3.18	$y = 4.4936\times10^5x -6.9351\times10^5$	0.9999	3.393	10.643
		117.187	37					
		119.199	18					
o-Coumaric acid	163.095	93.047	36	6.11	$y = 2.8759\times10^5x +3.9233\times10^5$	0.9988	5.075	16.251
		117.171	25					
		119.185	15					
Vanillic acid	167.042	91.078	22	2.84	$y = 2.0862\times10^5x -3.1034\times10^5$	0.9993	6.294	19.648
		108.118	19					
		123.106	13					
		152.027	16					
Gallic acid	169.081	79.288	24	0.61	$y = 7.3772\times10^4x -3.4494\times10^4$	0.9994	2.589	8.630
		97.217	20					
		124.132	27					
		125.176	15					
Protocatechuic acid ethyl ester	181.038	108.487	23	7.39	$y = 5.9352\times10^4x +2.5769\times10^6$	0.9992	0.147	0.491
		151.949	16					
		153.005	16					
Ferulic acid	193.081	133.097	30	5.16	$y = 5.8092\times10^4x -4.0636\times10^4$	0.9982	3.321	11.088
		134.166	18					
		149.184	13					
		178.117	14					
Syringic acid	197.084	95.222	35	3.36	$y = 2.6176\times10^5x -4.1087\times10^5$	0.9996	6.213	20.712
		123.166	25					

		167.117	21					
		182.117	16					
Sinapic acid	223.065	149.105	23	6.31	$y = 3.1595\times10^{5}x - 8.3289\times10^{4}$	0.9979	4.868	16.226
		164.141	18					
		193.081	25					
		208.100	17					
Catechin	289.041	203.256	21	2.97	$y = 1.3143\times10^{4}x - 9.6578\times10^{3}$	0.9995	8.395	27.984
		245.129	17					
Epicatechin	289.050	203.210	23	3.56	$y = 2.1011\times10^{4}x - 1.3420\times10^{4}$	0.9991	6.787	22.067
		245.137	18					
Quercetin	301.000	107.184	33	7.92	$y = 1.7855\times10^{5}x - 5.8558\times10^{4}$	0.9997	0.297	0.991
		121.176	30					
		151.074	24					
		179.060	20					
Epigallocatechin	305.033	125.138	27	2.35	$y = 3.9510\times10^{4}x - 1.5239\times10^{4}$	0.9990	2.744	9.148
		165.082	20					
		179.110	18					
		219.098	19					
Procyanidin B1	577.128	289.088	26	2.60	$y = 9.1478\times10^{4}x - 3.5465\times10^{4}$	0.9997	2.720	9.068
		407.009	23					
		425.148	18					
Procyanidin B2	577.137	289.010	26	2.85	$y = 1.1698\times10^{5}x - 8.5724\times10^{4}$	0.9989	1.894	6.332
		407.002	24					
		425.159	19					
Procyanidin B3	577.112	289.017	28	3.63	$y = 1.0529\times10^{5}x - 4.0134\times10^{4}$	0.9998	2.215	7.365
		407.021	24					
		425.098	17					
Rutin	609.122	151.051	52	6.79	$y = 2.5348\times10^{6}x + 2.4363\times10^{6}$	0.9989	0.012	0.039
		255.055	58					
		271.093	57					
		300.142	40					
Procyanidin C1	865.147	386.970	38	7.87	$y = 8.0104\times10^{4}x + 1.5888\times10^{4}$	0.9999	0.416	1.385
		524.985	29					
		695.291	26					
		713.302	29					

[a] t_R, retention time. [b] The calibration curve was constructed by plotting the peak area versus the concentration of each standard. [c] LOD, limits of detection. [d] LOQ, limits of quantification. Precision of the data obtained from repeated experiments (RSD%) < 7%.

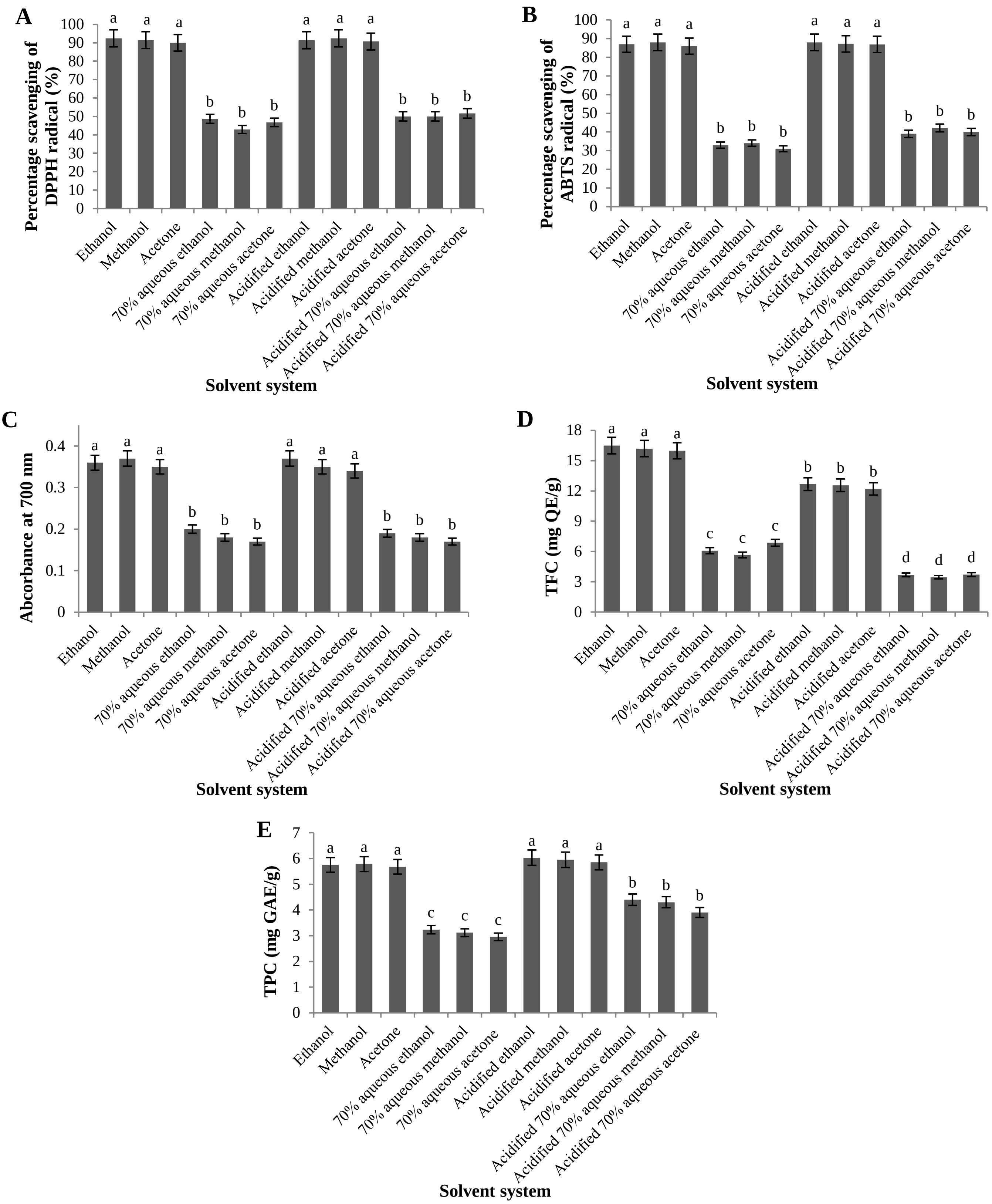


Figure S1. The radical scavenging activities of DPPH (**A**) and ABTS (**B**), reducing power (**C**), total flavonoid content (TFC) (**D**), and total phenolic content (TPC) (**E**) of different solvent extracts of wild rice collected from Jingzhou. Different letters within the same figure mean statistical difference ($p < 0.05$).

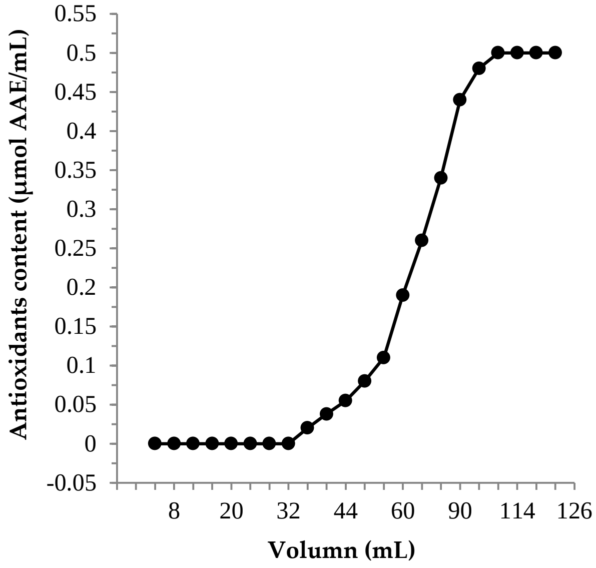
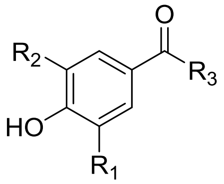
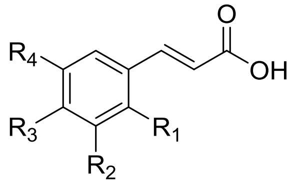


Figure S2. Dynamic breakthrough curve of antioxidants on D101 resin column.

Phenolic acids and their derivatives

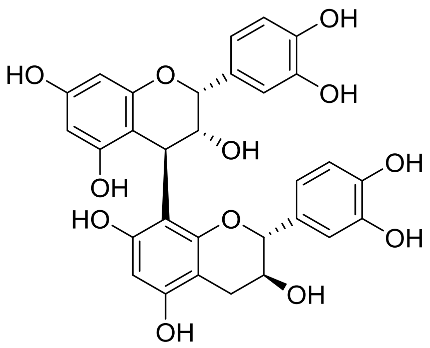




A1: R_1=OH, R_2=OH, R_3=OH
A2: R_1=H, R_2=OH, R_3=OH
A3: R_1=H, R_2=H, R_3=OH
A4: R_1=H, R_2=OMe, R_3=OH
A5: R_1=H, R_2=H, R_3=H
A6: R_1=OMe, R_2=OMe, R_3=OH
A7: R_1=H, R_2=OMe, R_3=H
A8: R_1=H, R_2=OH, R_3=OEt

A9: R_1=H, R_2=H, R_3=OH, R_4=H
A10: R_1=OH, R_2=H, R_3=H, R_4=H
A11: R_1=H, R_2=OMe, R_3=OH, R_4=H
A12: R_1=H, R_2=OMe, R_3=OH, R_4=OMe
B21: R_1=H, R_2=OMe, R_3=OMe, R_4=OMe

Procyanidins



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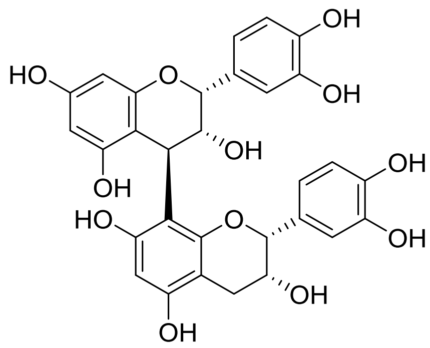

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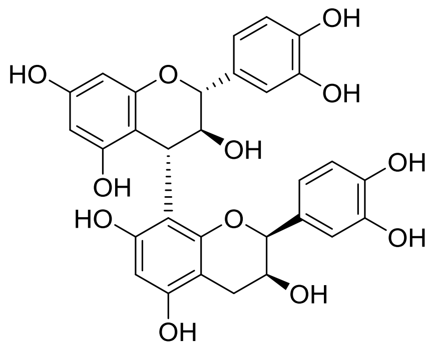

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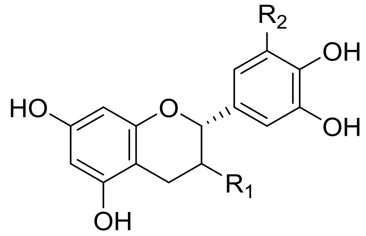
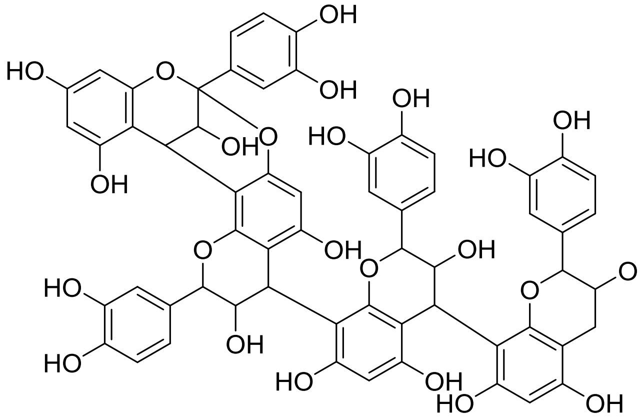


B4: R_1= OH, R_2=OH
B5: R_1= OH, R_2=H
B6: R_1= OH, R_2=H



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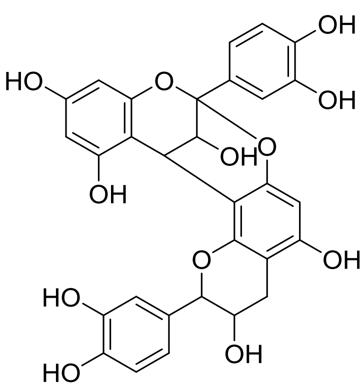

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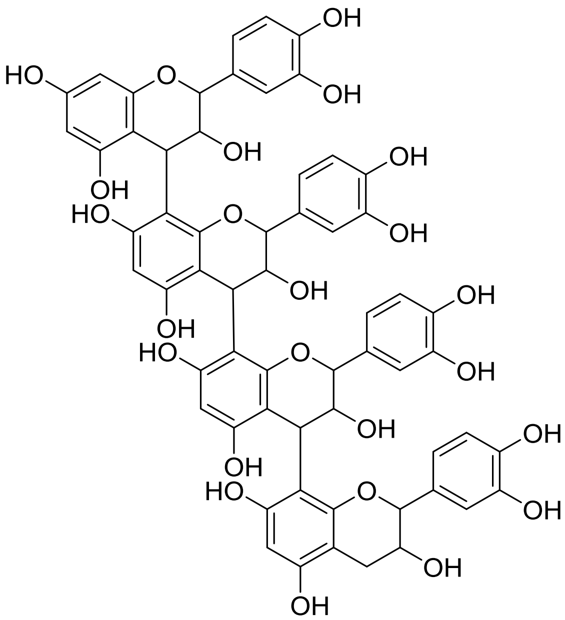

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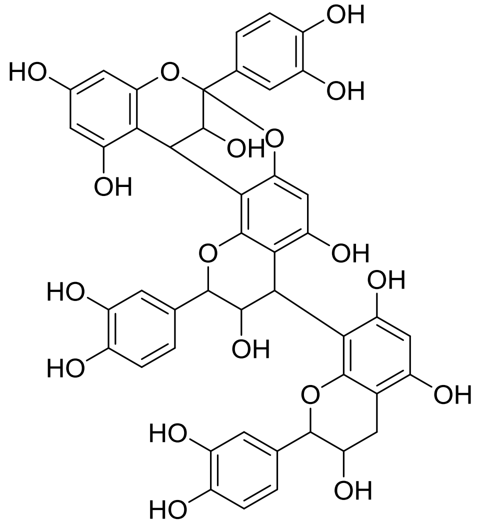

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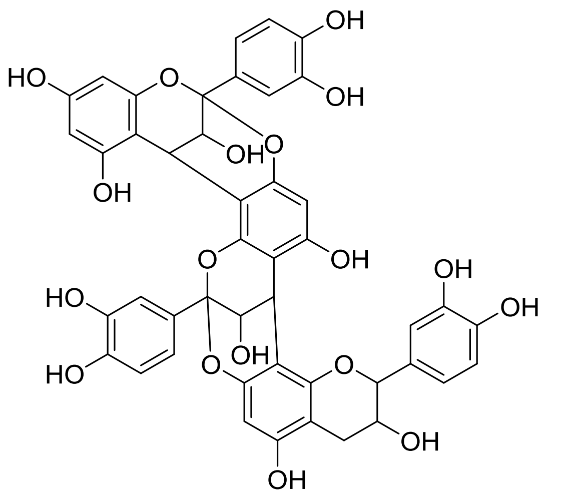

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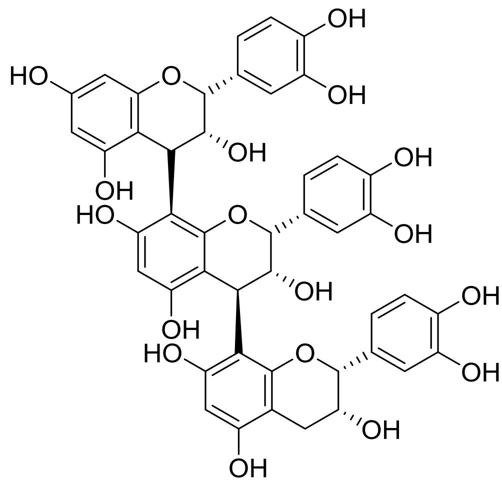
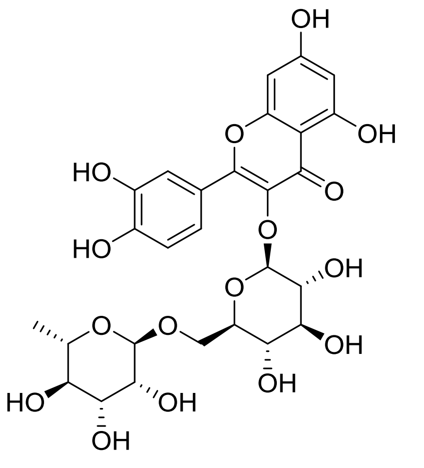


B19

Flavonoid glycosides



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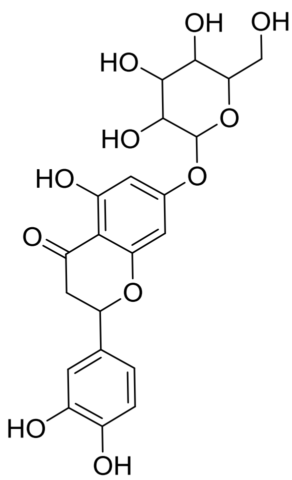

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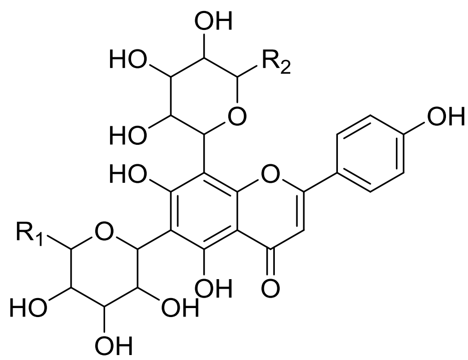
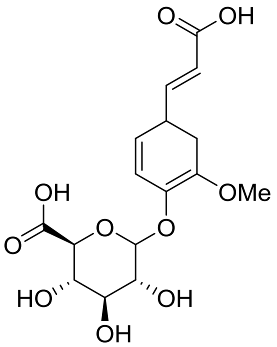


B12: R_1=CH_2OH, R_2=CH_2OH
B15: R_1=CH_2OH, R_2=H
B16: R_1=H, R_2=CH_2OH
B17: R_1=H, R_2=H

Others



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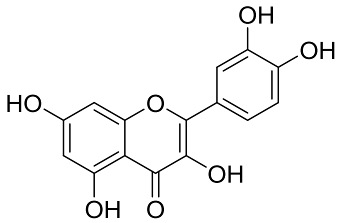
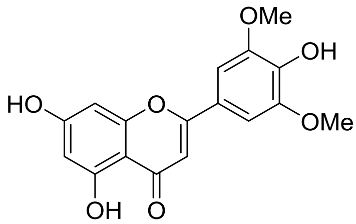
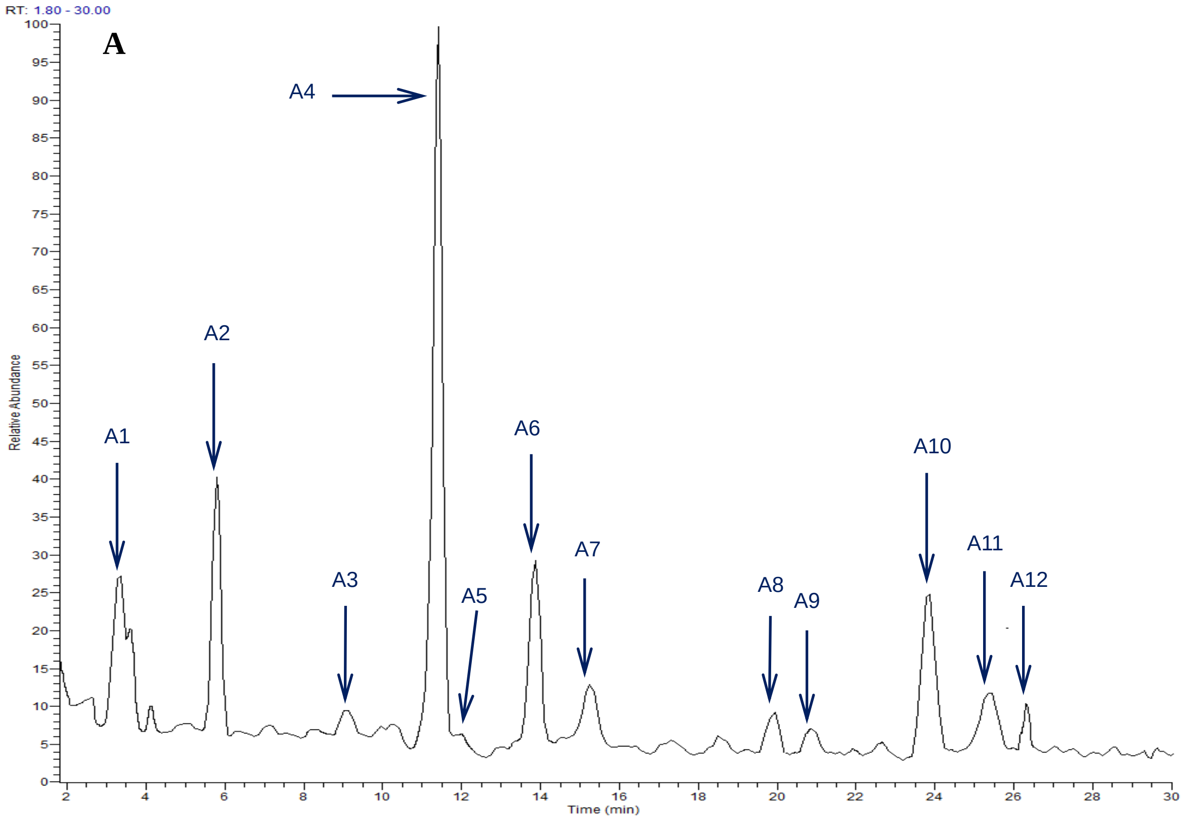


B20



B22

Figure S3. Structures of phenolic compounds identified in wild rice.



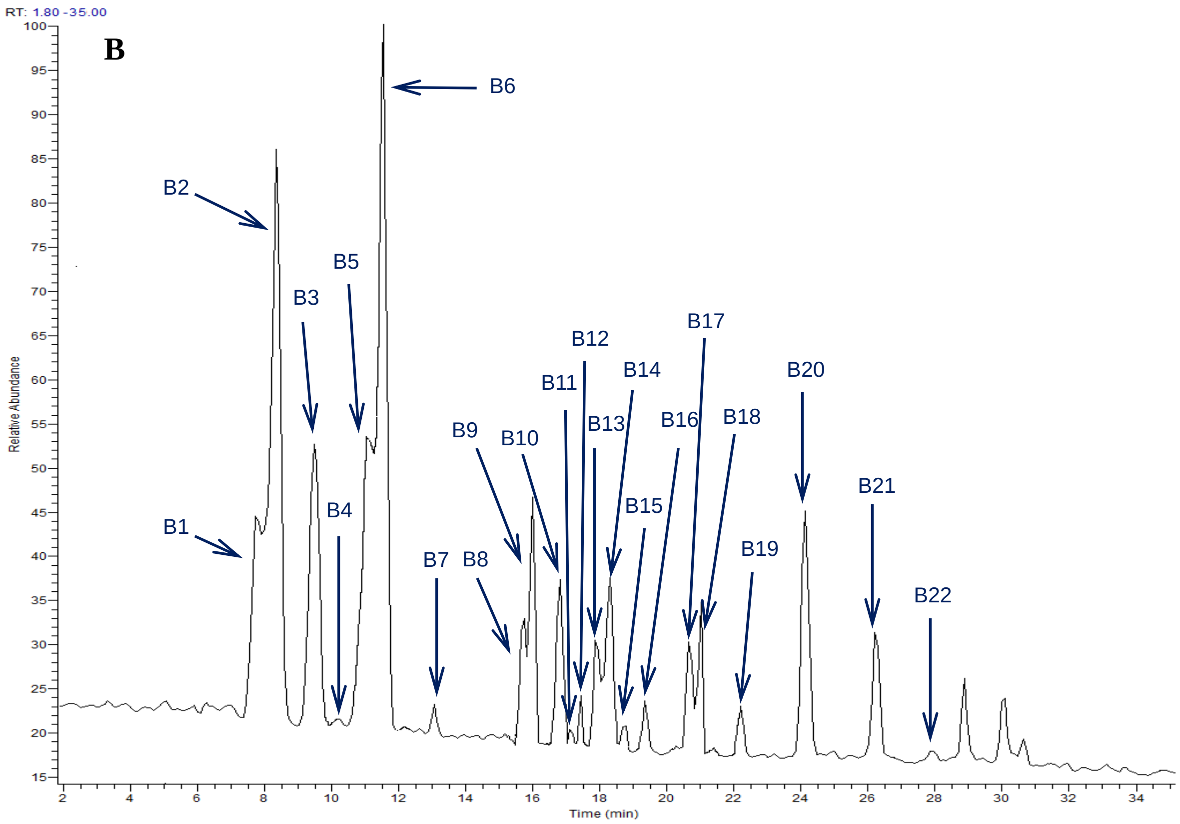


Figure S4. Base peak chromatograms of Fr. 1 (**A**) and Fr. 2 (**B**) in HPLC-LTQ-Orbitrap-MS^n analysis.

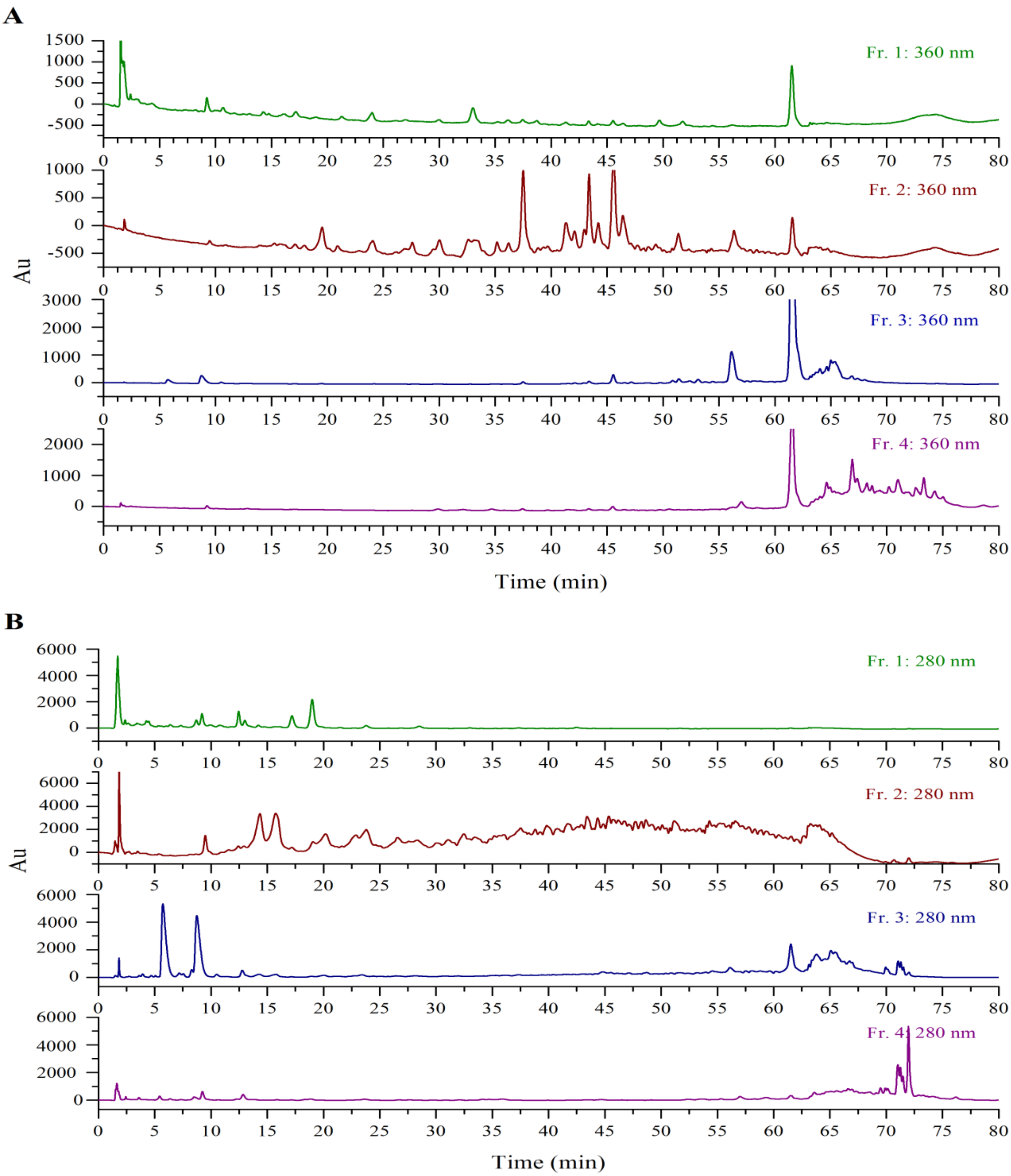


Figure S5. HPLC fingerprint chromatograms at 360 nm (**A**) and 280 nm (**B**) of the four fractions (Frs. 1–4) eluted from D101 resin column. The gradient solvent system consisting of A (methanol) and B (water containing 0.1% acetic acid, *v*/*v*) was as follows: 0–10 min, 5–10% A; 10–30 min, 10–15% A; 30–40 min, 15–20% A; 40–50 min, 20–25% A; 50–60 min, 25–35% A; 60–70 min, 35–90% A; 70–80 min, 90–60% A.

References

1. Bunzel, M.; Allerdings E.; Sinwell, V.; Ralph J.; Steinhart H. Cell wall hydroxycinnamates in wild rice (*Zizania aquatica* L.) insoluble dietary fibre. *Eur. Food. Res. Technol.* **2002**, *214*, 482–488.
2. Qiu, Y.; Liu Q.; Beta, T. Antioxidant properties of commercial wild rice and analysis of soluble and insoluble phenolic acids. *Food Chem.* **2010**, *121*, 140–147.
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4. Bunzel, M.; Ralph, J.; Kim, H.; Lu, F.; Ralph, S. A.; Marita, J. M.; Hatfield, R. D.; Steinhart, H. Sinapate dehydrodimers and sinapate–ferulate heterodimers in cereal dietary fiber. *J. Agric. Food Chem.* **2003**, *51*, 1427–1434.
5. Qiu, Y.; Liu Q.; Beta, T. Antioxidant activity of commercial wild rice and identification of flavonoid compounds in active fractions. *J. Agric. Food Chem*. **2009**, *57*, 7543–7551.