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Electronic Supplementary Information

Sugaring-out strategy for counter-current chromatography isolation: podophyllotoxins and flavones from *Dysosma versipellis* as examples

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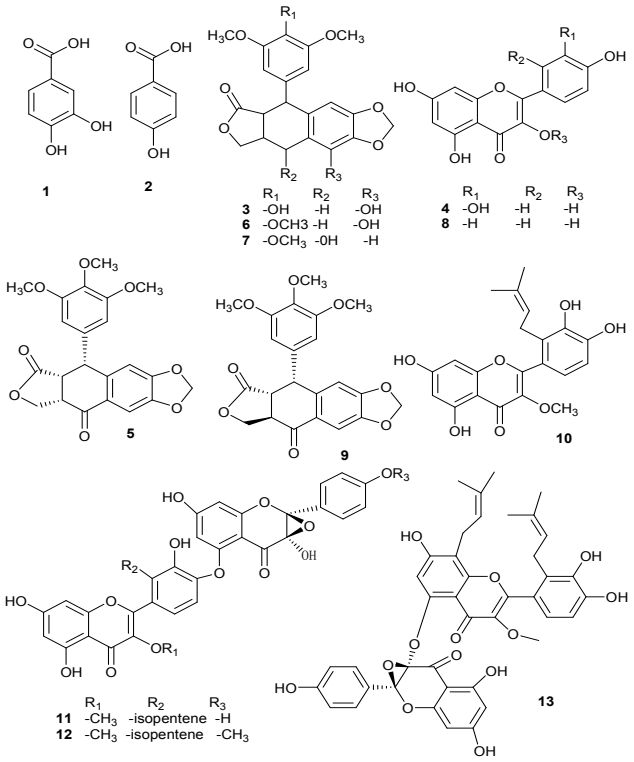

Fig. S1. The chemical structures of the 13 selected podophyllotoxins and flavones

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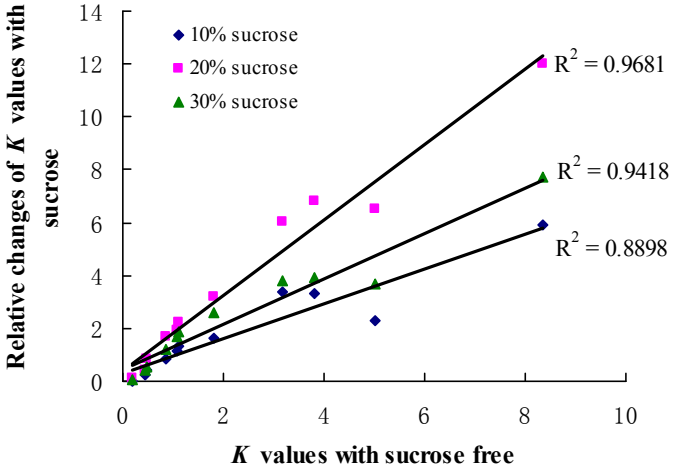

Fig. S2. Approximate linear responses of the relative changes of the partition coefficients for the selected components (**1**-**11**) in the HEMWat 4:6:4:6 (v/v) system. The relative changes of *K* values (*ΔK*) with sucrose were calculated as the equation: $\Delta K=K_s-K$, *K* is the partition coefficient of the target in the system without sucrose, while K_s is the partition coefficient in the system with sucrose.

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Table S1. *K* values of components in the HEMWat 4.5:5.5:4.5:5.5 (v/v) system adding sugars

Sugar concentration (w% in water)	K values											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
0	0.11	0.32	0.37	0.93	1.74	1.09	0.80	2.85	4.29	3.68	5.30	14.07
10% sucrose	0.14	0.34	0.47	1.06	2.09	1.33	0.85	3.02	4.45	4.18	6.30	15.46
20% sucrose	0.29	0.52	0.62	1.47	2.69	1.56	1.23	3.64	5.41	5.31	7.57	18.09
30% sucrose	0.18	0.38	0.54	1.14	2.36	1.39	0.96	3.48	5.08	5.06	6.67	16.58
10% glucose	0.19	0.45	0.55	1.10	2.21	1.52	1.06	3.17	4.48	4.29	6.85	15.83
20% glucose	0.30	0.65	0.67	1.51	2.88	1.79	1.32	4.24	7.07	5.88	11.61	23.95
30% glucose	0.26	0.46	0.65	1.27	2.39	1.62	1.18	3.71	6.06	5.17	9.85	18.83
10% fructose	0.23	0.55	0.61	1.42	2.73	1.70	1.18	4.26	6.36	6.05	13.42	38.32
20% fructose	0.29	0.58	0.62	1.69	2.89	1.84	1.21	5.28	7.38	6.38	17.23	50.17
30% fructose	0.05	0.14	0.17	0.38	0.64	0.44	0.32	0.88	1.46	0.94	2.63	8.84
10% maltose	0.10	0.39	0.40	1.10	2.24	1.20	0.92	3.10	4.81	4.61	8.59	28.29
20% maltose	0.18	0.54	0.62	1.55	2.97	1.81	1.31	4.55	7.48	6.31	12.48	45.05
30% maltose	0.38	1.16	1.34	2.02	3.88	2.97	1.93	5.99	8.87	7.76	14.35	52.34
10% D-galactose	0.20	0.51	0.55	1.43	2.67	1.63	1.07	4.23	6.15	5.73	12.22	32.13
20% D-galactose	0.28	0.85	1.02	1.79	3.57	2.54	1.30	5.75	8.12	7.48	15.32	36.04
30% D-galactose	without dissolution											
10% D-sorbose	0.14	0.44	0.48	1.16	2.38	1.38	0.81	3.36	4.89	4.80	9.57	25.23
20% D-sorbose	0.30	0.66	0.93	2.09	3.43	2.18	1.72	5.21	7.85	6.31	14.64	42.10
30% D-sorbose	0.17	0.49	0.54	1.28	2.39	1.55	1.10	3.72	5.45	4.89	9.84	26.26
10% mannose	0.13	0.41	0.44	1.13	2.25	1.26	0.93	3.14	4.77	4.68	8.64	30.67
20% mannose	0.18	0.52	0.53	1.26	2.45	1.51	1.09	3.83	5.48	4.90	9.51	41.22
30% mannose	0.06	0.22	0.26	0.42	0.79	0.54	0.39	1.16	1.86	1.74	3.90	8.48
10% rhamnose	0.09	0.26	0.31	0.71	1.57	0.90	0.63	2.11	3.13	2.92	5.24	11.79
20% rhamnose	0.10	0.30	0.35	0.80	1.69	1.04	0.76	2.32	3.60	3.40	5.28	13.92
30% rhamnose	0.17	0.37	0.40	1.00	1.86	1.23	0.89	2.99	4.57	4.25	8.05	20.30
10% xylopyranose	0.17	0.46	0.54	1.36	2.56	1.57	1.06	4.02	5.49	5.13	12.19	30.05
20% xylopyranose	0.19	0.48	0.55	1.37	2.59	1.61	1.07	4.04	6.03	5.32	12.59	50.33
30% xylopyranose	0.16	0.40	0.41	0.94	1.75	1.14	0.83	2.86	4.88	3.92	7.39	25.25

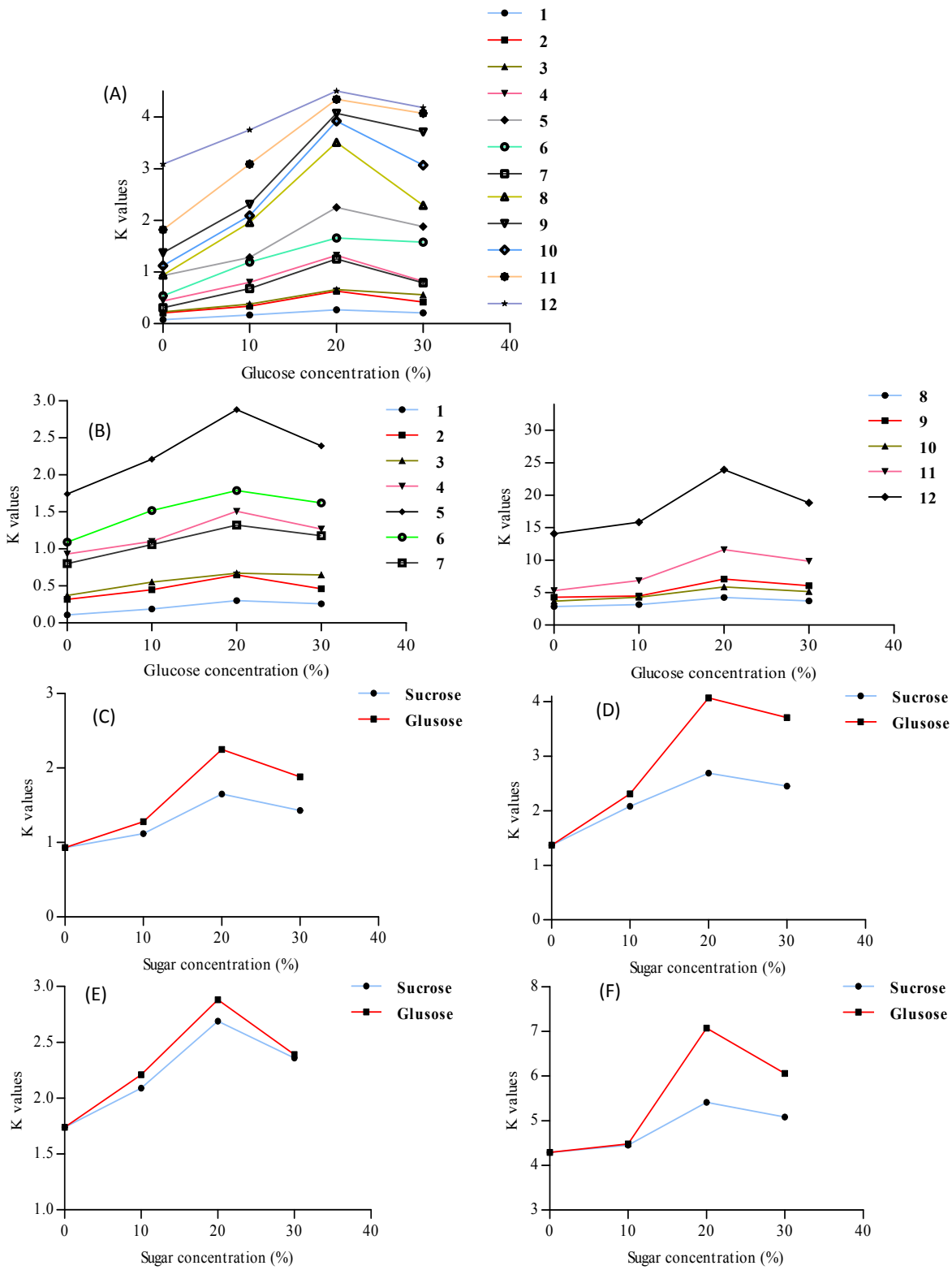

Fig. S3. Sugaring-out effects of glucose on the partition coefficients of the selected compounds in HEMWat (A) 5:5:5:5 and (B) 4.5:5.5:4.5:5.5 (v/v) systems. Sugaring-out effects of glucose and sucrose on the partition coefficients of compound **5** (C) and compound **9** (D) in HEMWat 5:5:5:5 system, and compound **5** (E) and compound **9** (F) in the 4.5:5.5:4.5:5.5 system.

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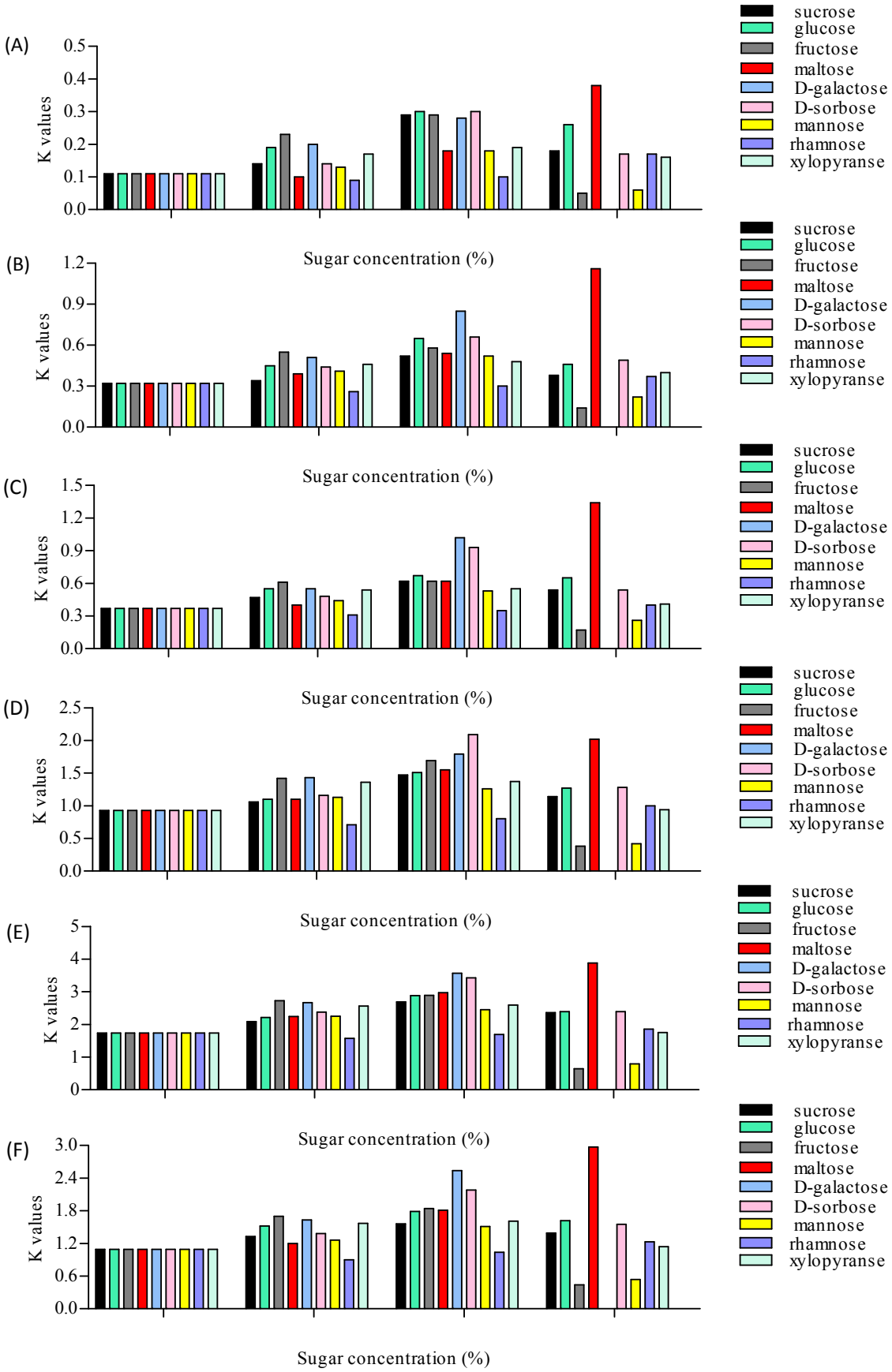

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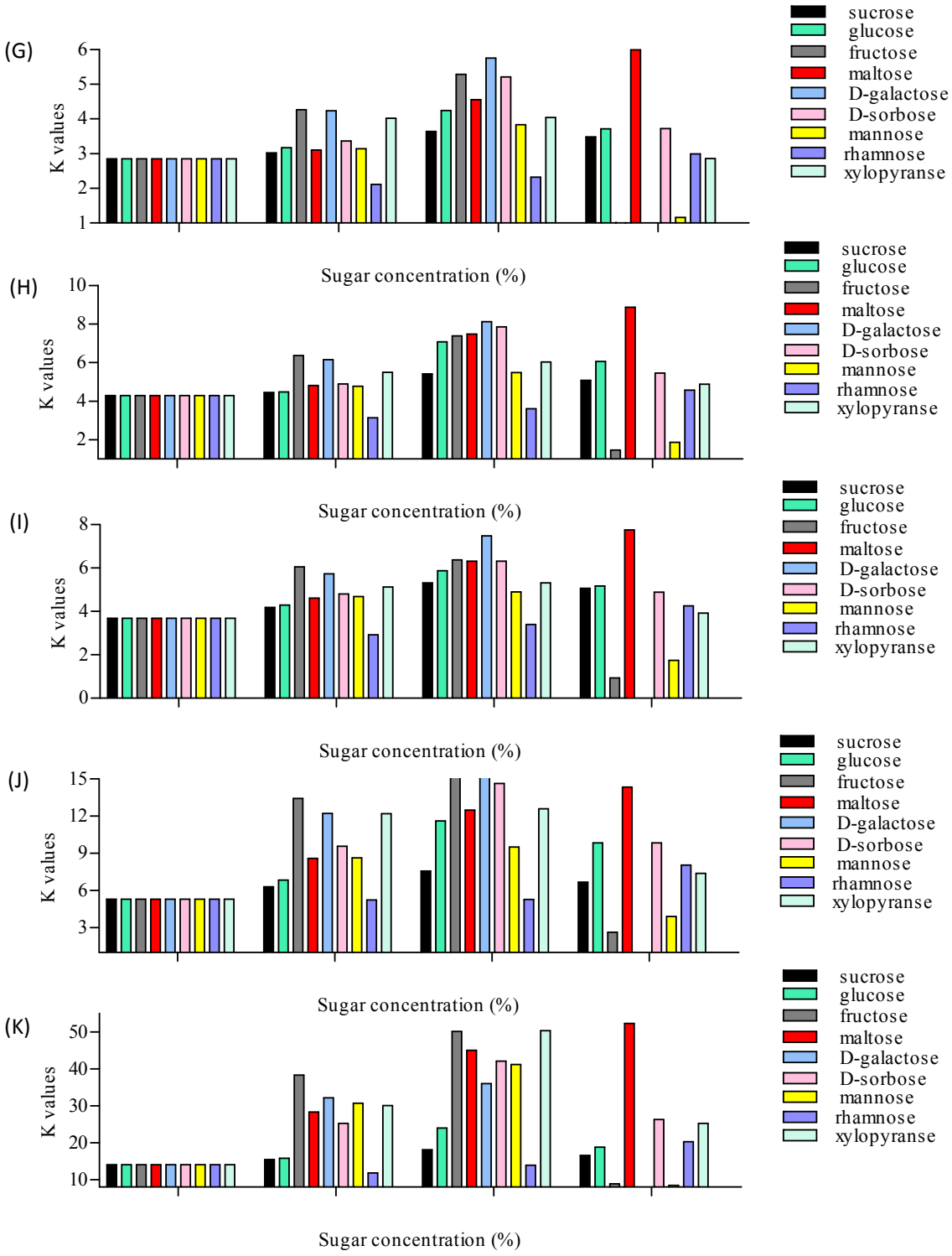

Fig. S4. Sugaring-out effects of different types of sugars on the partition coefficients of the selected compounds in HEMWat 4.5:5.5:4.5:5.5 (v/v) system, (A) compound **1**, (B) compound **2**, (C) compound **3**, (D) compound **4**, (E) compound **5**, (F) compound **6**, (G) compound **8**, (H) compound **9**, (I) compound **10**, (J) compound **11**, (K) compound **12**.

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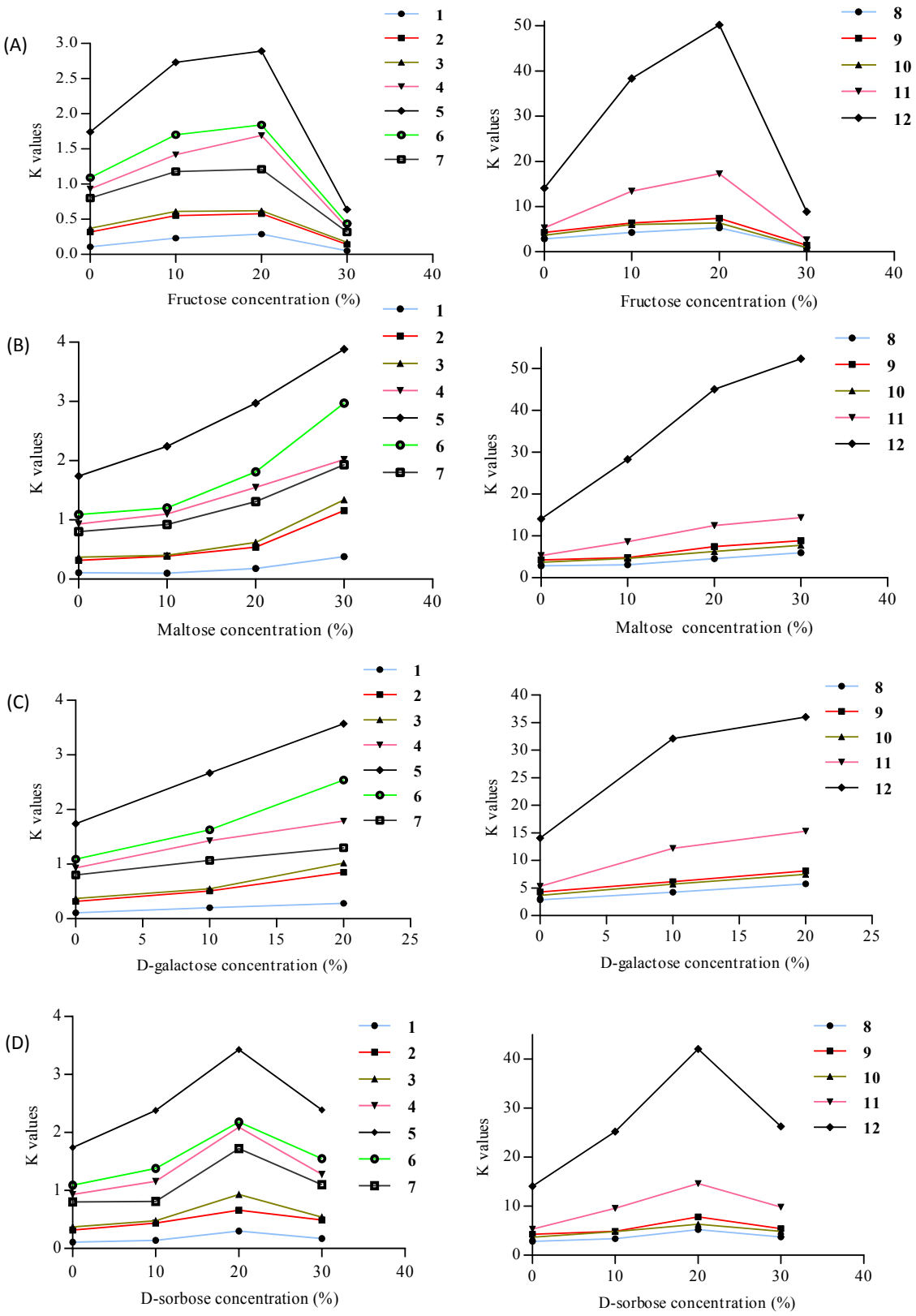

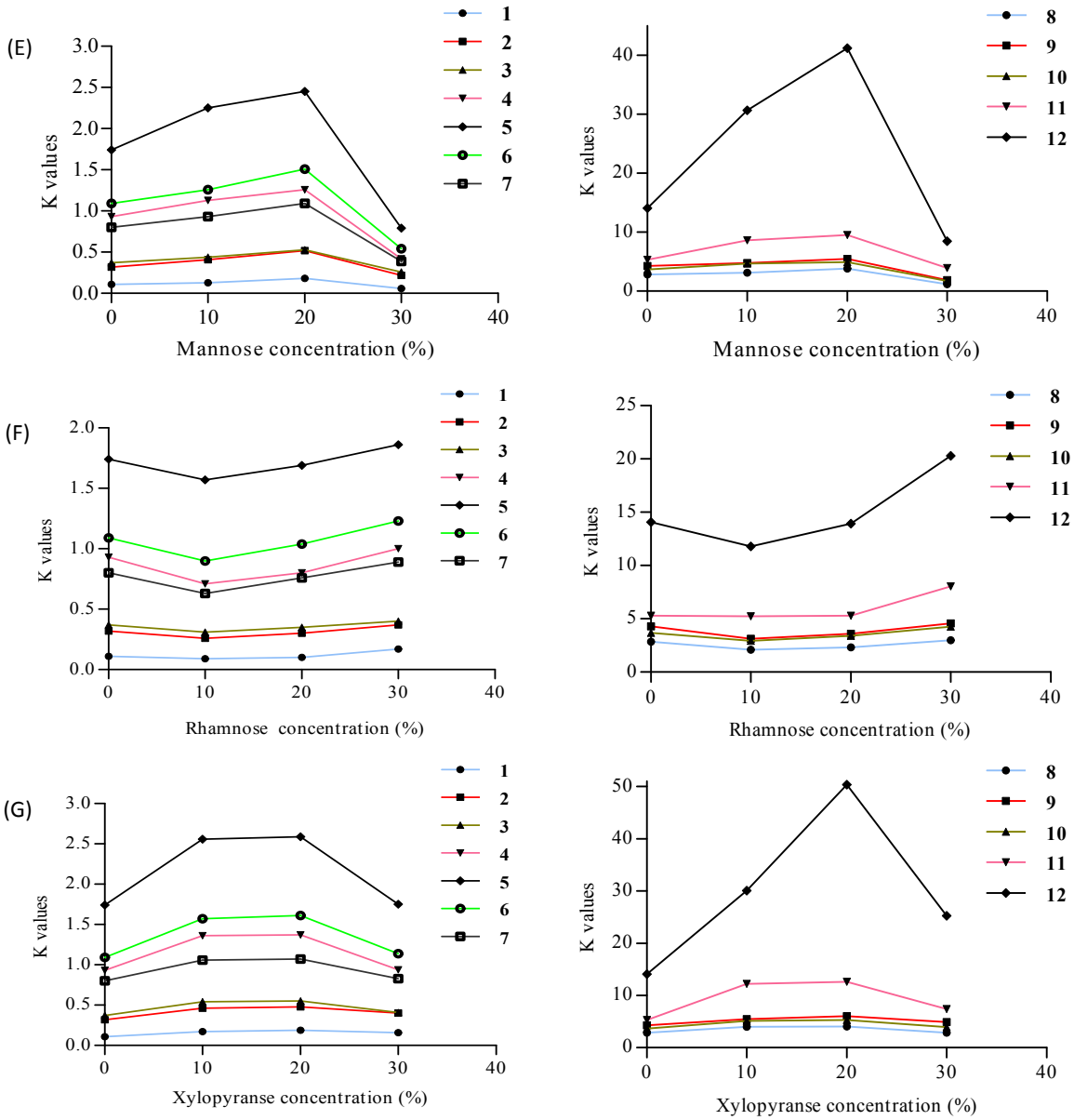

Fig. S5. Sugaring-out effects of sugars on the partition coefficients of the selected compounds in HEMWat 4.5:5.5:4.5:5.5 (v/v) system, (A) fructose, (B) maltose, (C) *D*-galactose, (D) *D*-sorbose, (E) mannose, (F) rhamnose, (G) xylopyranose.

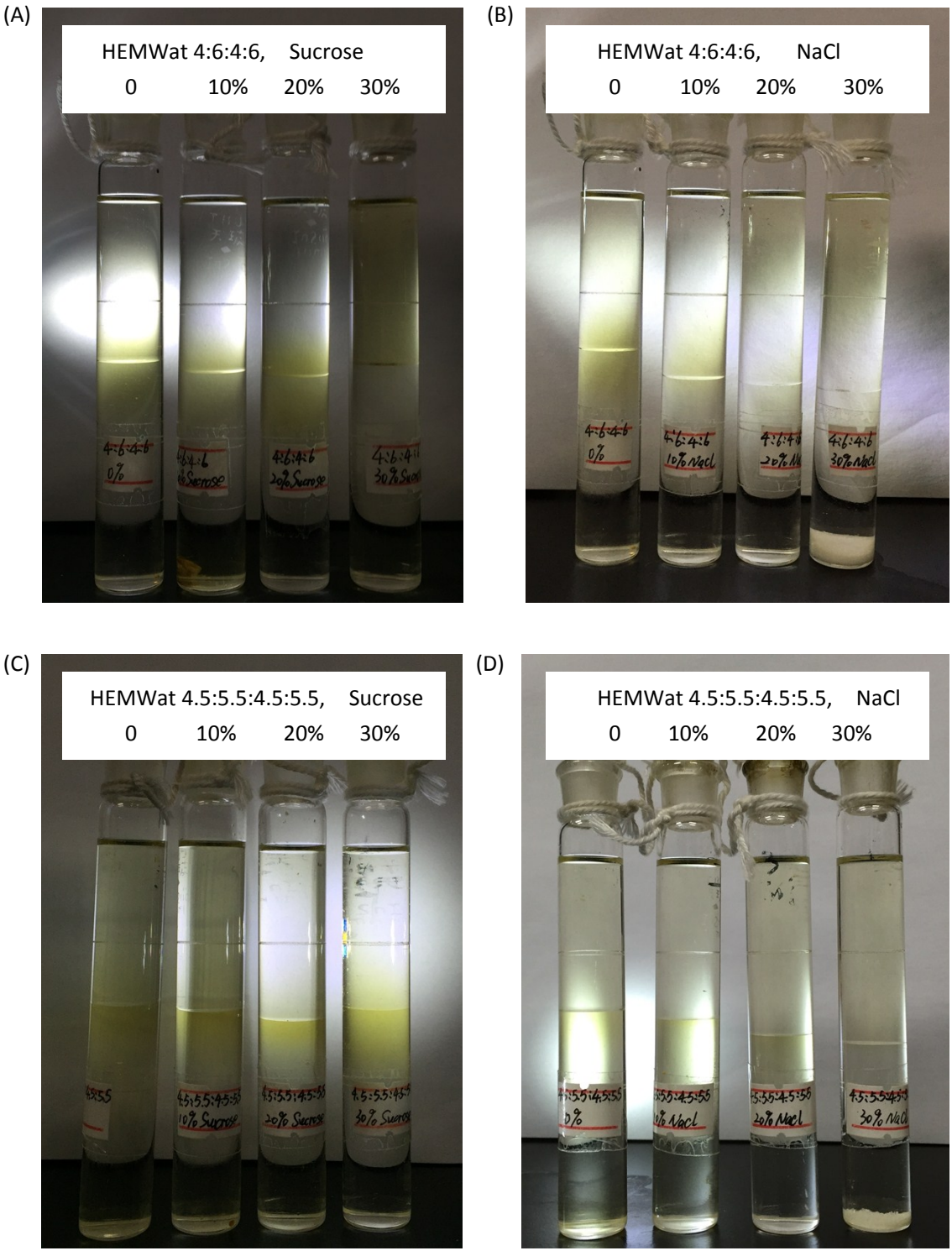

Fig. S6. The photographs of the effects of sucrose and NaCl on the two-phase partition of HEMWat 4:6:4:6 and 4.5:5.5:4.5:5.5 systems (the total volume of two phases was 14 mL in each test tube). Minor sample (yellow powder) of *D. versipellis* was added into the two-phase interface in order to clearly show the interface of two phases of the selected solvent system. In an extremely high salt concentration (30%), some of NaCl was precipitated from the HEMWat system.

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Table S2. The volume changes of two phases after adding sucrose and NaCl in the total 40 mL HEMWat systems

HEMWat system	The volume of two phase solvents (upper phase/lower phase, v/v)						
	0%	Sucrose			NaCl		
		10%	20%	30%	10%	20%	30%
4:6:4:6	18/22	19/21	20/20	19.5/21.5	21/19	22/18	22.5/17.5
4.5:5.5:4.5:5.5	16/24	16.5/23.5	17/23	16.5/23.5	19/21	20/20	22/18

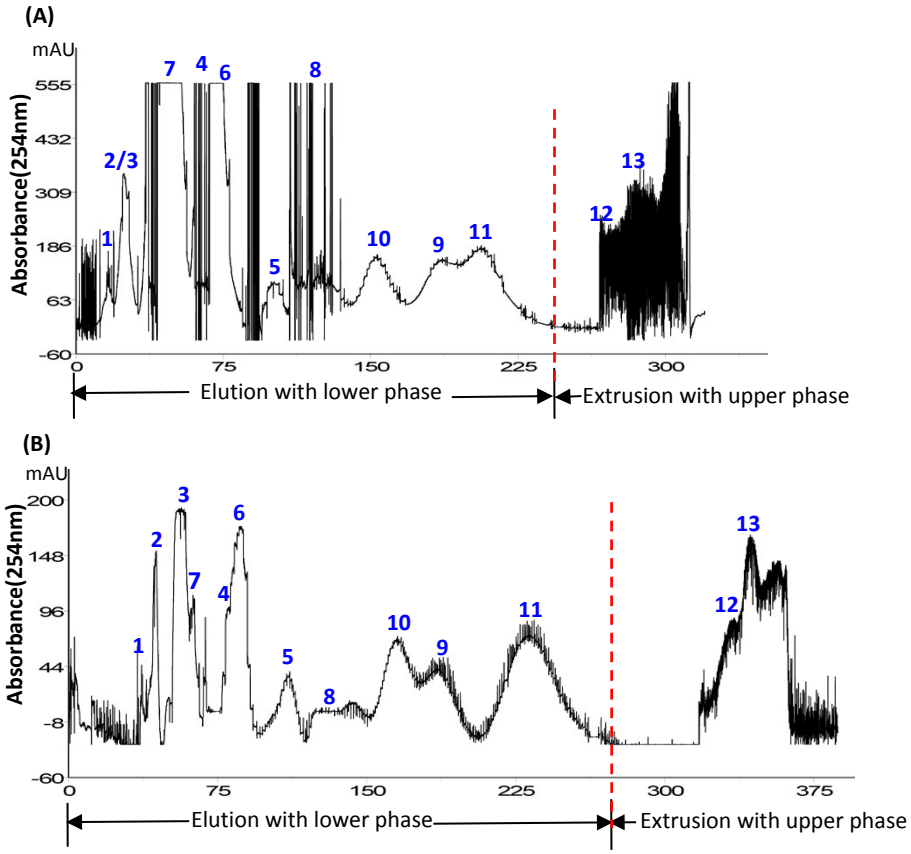

Fig. S7. The CCC Profiles of *Dysosma versipellis* (A) without sucrose and (B) with 30% sucrose CCC conditions: the solvent system, HEMWat (4.5:5.5:4.5:5.5, v/v); Elution mode: elution-extrusion, the upper phase as stationary phase and the lower phase as mobile phase in elution stage while in extrusion stage upper phase was directly pumped in the same flow rate; flow rate, 3 mL/min; rotation speed, 900 rpm; detection wavelength, 254 nm; sample, 250 mg was dissolved in a mixed solution composed of 3 mL upper phase and 3 mL lower phase. The retention of the stationary phase was both 55.6% for (A) and (B). The switch time was set at 232 min for (A), at 276 min for (B).

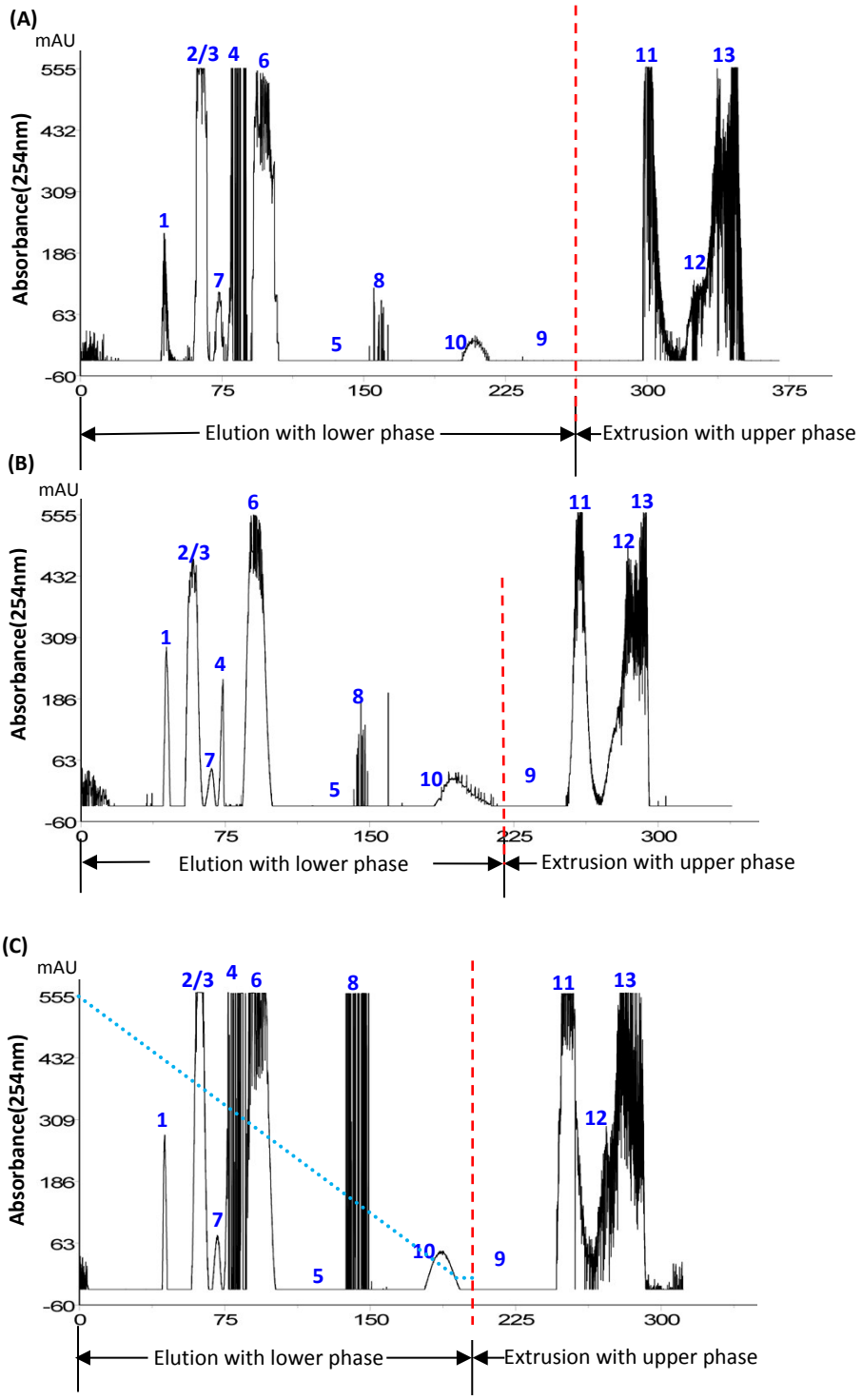

Fig. S8. The sugaring-out effects of glucose on the CCC separation of extract of *Dysosma versipellis*. The HEMWat 4.5:5.5:4.5:5.5 (v/v) solvent system with 20% glucose was for (A), with 30% glucose was for (B) and with 30% glucose was for (C) in a linear gradient mode (M, the lower phase without glucose, N, the lower phase with 30% glucose): 0–200 min, M from 0% to 100% and N from 100% to 0%; after 200 min, M was maintained at 100% until extrusion. The retention of the stationary phase was 60% for (A), 55.6% for (B) and 60% for (C). Elution mode: elution-extrusion. The switch time was set at 260 min for (A), at 220 min for (B) and at 206 min for (C).

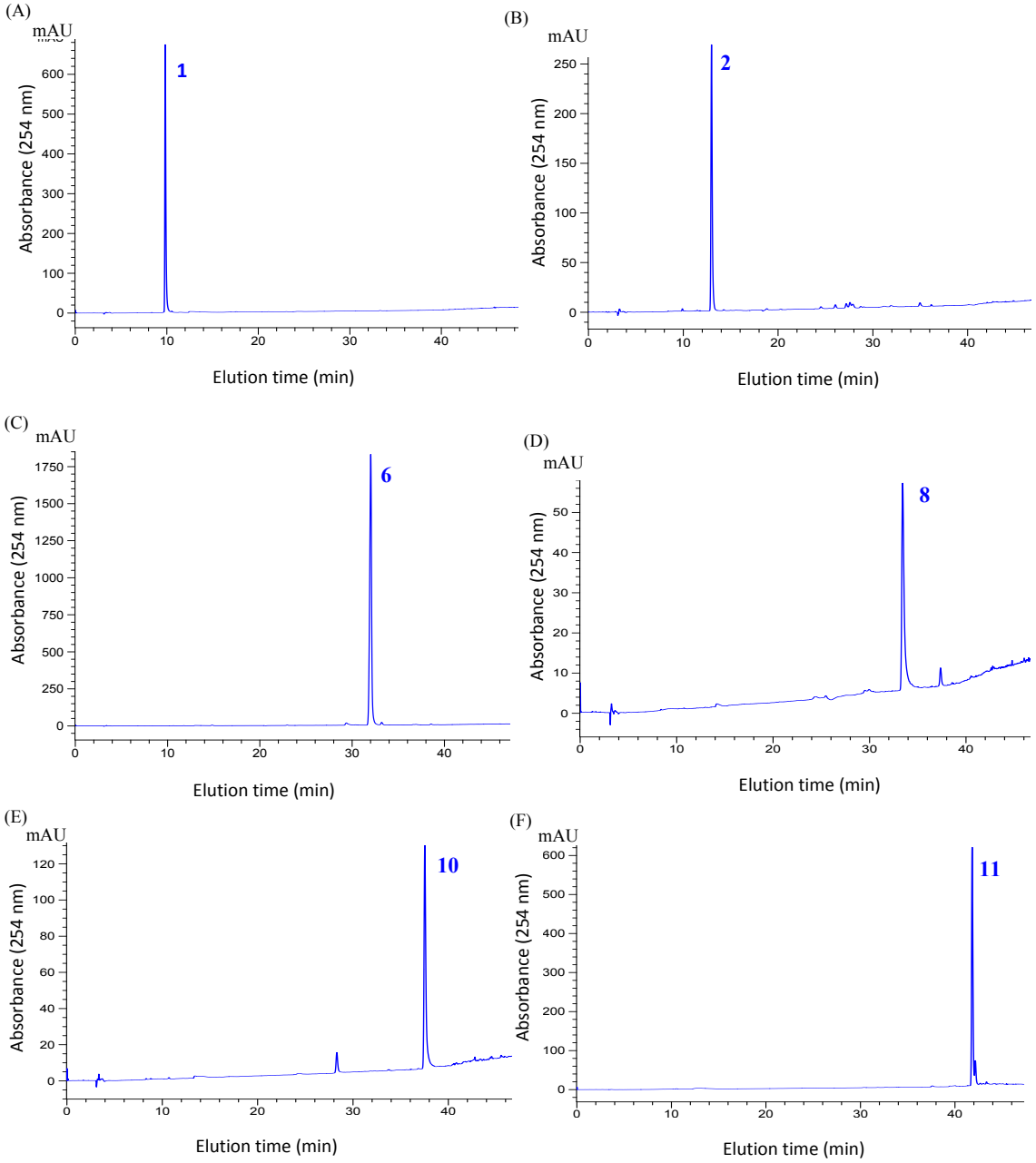

Fig. S9. The representative HPLC profiles of purified components of *Dysosma versipellis* using the solvent system of HEMWat (4.5:5.5:4.5:5.5, v/v) with 20% glucose. HPLC conditions were same to Fig.1.