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Supplementary information

High-performance thin-layer chromatography/bioautography and liquid chromatography-mass spectrometry hyphenated with chemometrics for the quality assessment of *Morus alba* samples

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Content

Table S1. List of *Morus alba* samples with voucher code, plant organ, geographical origin (GPS data), collection season/purchase date and image of the sample.

No.	Voucher number	Plant organ	Geographical origin	Collection season/ purchased	Image of the sample
1	RP-20180214A1	Sang bai pi (Root bark)	Latitude (N): 44 º23'38.242" Longitude (E): 19º34' 0.947"	February, 2018	
2	RP-20180122A2	Sang bai pi (Root bark)	Latitude (N): 42º36'41.396" Longitude (E): 22 º2'13.97"	November, 2017	
3	RP-20180122A3	Sang bai pi (Root bark)	Latitude (N): 42º33' 31.751" Longitude (E): 21º58'35.205"	November, 2017	
4	RP-20180122A4	Sang bai pi (Root bark)	Latitude (N): 42º24'27.123" Longitude (E): 11º3'55.878 "	November, 2017	

5	RP-20180122A5	Sang bai pi (Root bark)	Latitude (N): 42º25'8.177" Longitude (E): 22º 4'33.487"	November, 2017	
6	RP-20180122A6	Sang bai pi (Root bark)	Latitude (N): 42º 34' 13.529" Longitude (E): 22º 0'53.787"	November, 2017	
7	RP-20180122A7	Sang bai pi (Root bark)	Latitude (N): 42º 32'26.361" Longitude (E): 21º54' 37.086"	November, 2017	
8	RP-201801222A8	Sang bai pi (Root bark)	Latitude (N): 42º36' 0.445" Longitude (E): 22º2' 6.977"	November, 2017	
9	RP-201801223A9	Sang bai pi (root bark)	Latitude (N): 44º24' 14.978" Longitude (E): 19º38'6.903"	November, August, 2017	

10	RP-20180214A10	Sang bai pi (root bark)	Latitude (N): 44º21'16.957" Longitude (E): 20º9'31.031"	February, 2018	
11	RP-20180214A11	Sang bai pi (root bark)	Latitude (N): 44º23'35.891" Longitude (E): 19º45'27.838"	February, 2018	
12	RP-20180123A12	Sangzhi (branches)	Jiangsu, Tongrentang Beijing	September 2017	
13	RP-20180123A13	Sang bai pi (root bark)	Pharmacy in Beijing	September 2017	
14	RP-20180122A16	Sang bai pi (root bark)	Guangxi, Yiren Drug Store, Guilin	September 2017	

15	RP-20180123A17	Sang bai pi (root bark)	Huimintang Drug Store, Yangshou	September 2017	
16	RP-20180123A18	Sang bai pi (root bark)	LBX Pharmacy, Yulin	September 2017	
17	RP-20180123A19	Sang bai pi (root bark)	TCM Pharmacy in the first affiliation of Guangxy TCM college (Nanning)	September 2017	
18	RP-20180123A20	Sang zhi (branches)	TCM Pharmacy in the first affiliation of Guangxy TCM college (Nanning)	September 2017	

Table S2. Statistical performances of the principal component analysis (PCA) models.

	254 nm	**366 nm**	**Vanillin-sulphuric acid reagent**			**DPPH assay**	***B. subtilis* assay**	***E.coli* assay**	**LTQ-MS/MS**
			Red channel	**Green channel**	**Blue channel**				
Preprocessing techniques	Median filtering, baseline correction warping, mean centering	COW, auto scaling	COW, mean centering	COW, mean centering	COW, mean centering	Median filtering, smoothing, baseline correction, standard normal variate, COW, mean centering	SNV, mean centering	Median filtering, warping, SNV, mean centering	COW, mean centering
Number of PCs	2	5	3	3	3	3	3	3	4
% Variance captured - Total	73.02%	72.62%	78.80%	75.32%	71.71%	80.03%	81.14	83.79%	68.80 %
% Variance captured – PC1/PC2	51.59%/ 21.43%	26.59%/ 21.16%	47.01%/ 25.27%,	35.47%/ 26.10%	38.05% /20.23%	45.91%/ 22.01%	60.59%/ 13.08%	4.98%/ 16.93%	37.18/ 14.02 %

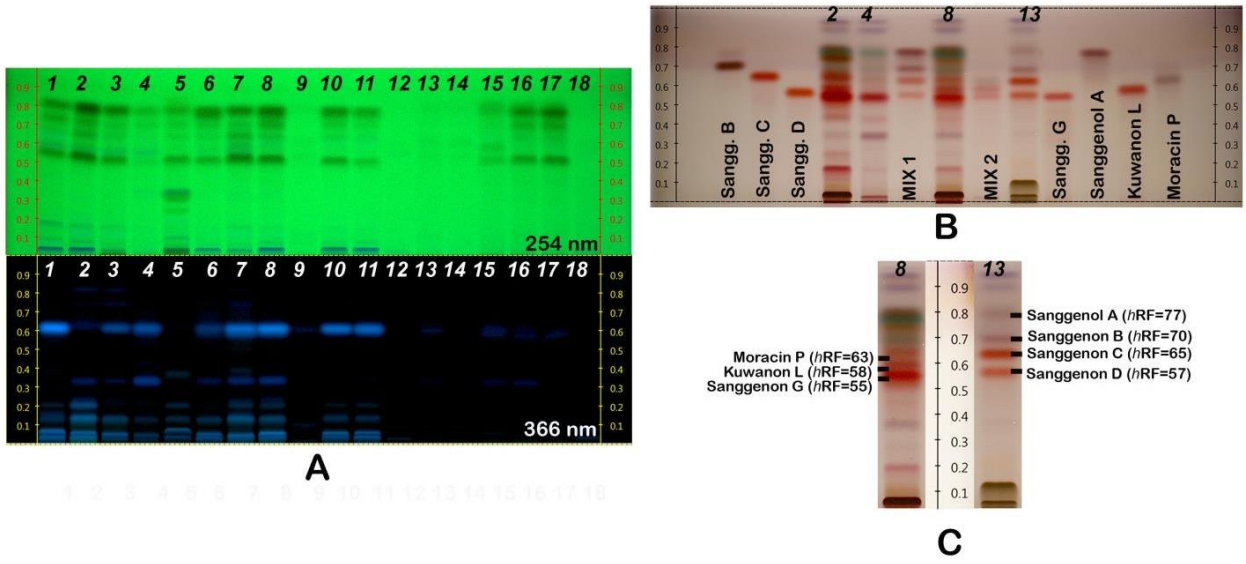

Fig. S1. HPTLC profile of *M. alba* root bark samples: A) under 254 nm and 366 nm, B) reference compounds, reference mix 1 (sanggenons B, C, D, sangenol A), reference mix 2 (sanggenon G, kuwanon L, moracin), C) Serbian (***8***) and Chinese (***13***) samples with reference compounds.

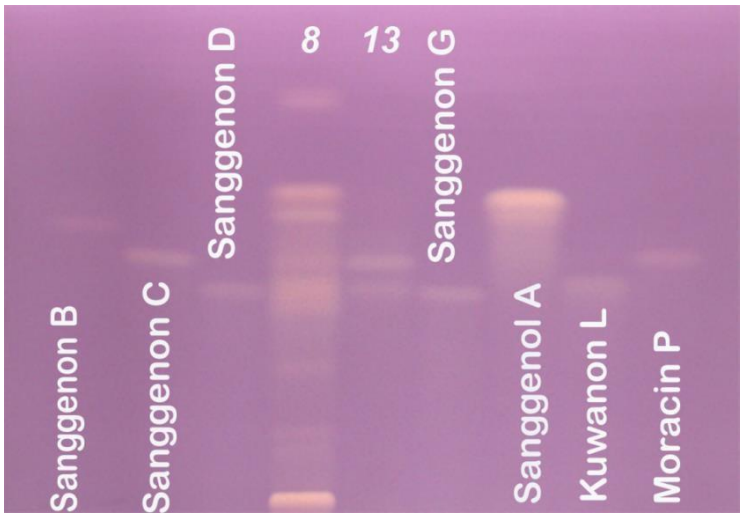

Fig. S2. HPTLC-DPPH profile of *M. alba* root bark samples ***8***, ***13*** and reference compounds.

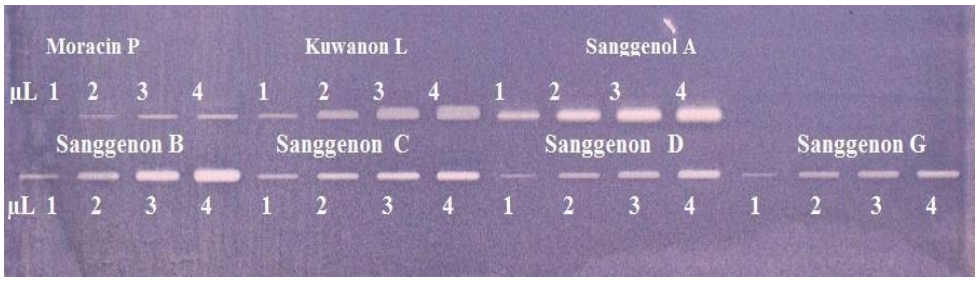

Fig. S3. HPTLC-antimicrobial assay of reference compounds against *B. subtilis*.

Fig. S4. HPTLC-antimicrobial assay of reference compounds against *E. coli*.

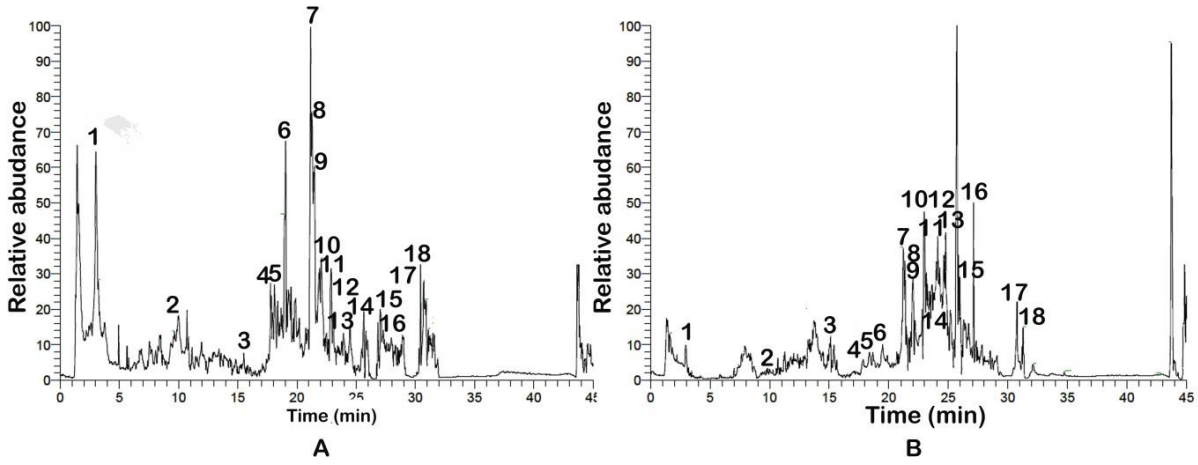

Fig. S5. UPLC-MS/MS chromatograms (total ion chromatograms (TIC): relative abundance of the base peaks vs. retention time) of *Morus alba* root bark samples collected from Serbia (***1***) and China (***17***).

S 1.1 UPLC-MS/MS analysis

Using the negative ionization mode, compound **I** produced ions at *m/z* values of 405, 243, 225, and 199, corresponding to the successive neutral losses of one glucosyl group, another glucosyl group, and a H_2O moiety or a CO_2 molecule (Table 1). Compound **II** recognized as moracin M has a main fragment at *m/z* 197 (Table 1) [22]. Compound **III** with a molecular ion at *m/z* 325 gave ion species *m/z* 283 and 253; ion at *m/z* 253 was formed by elimination of a C_4H_8O group [22]. Compounds **IV** and **V** (presumably sanggenon T and its isomer) with a molecular ion at *m/z* 711 was identified in Chinese samples. In the case of compounds **IV** and **V**, three ions, *m/z* 601 and 549, 491, were obtained [22]. Compound **VI** recognized as kuwanon L produced fragment ions at *m/z* 499, 471, 389, whereas an ion at *m/z* 499 was formed by elimination of $C_6H_6O_2$ [20, 22] (Table 1). Compound **VII** with a molecular ion at *m/z* 707, and characteristic fragments at *m/z* 489, 369, 325, 300, 259, 227 was compared with the reference compound and was accordingly identified as sanggenon D (Table 1) [21, 22]. Compound **IX** (kuwanon G) and its

isomer (compound **XVIII**) with a molecular ion at *m/z* 691 produced two characteristic fragment ions at *m/z* 581 and 471, corresponding to the loss of one and two resorcinol ($C_6H_6O_2$) groups [21, 22]. Compounds **VIII**, **X** and **XI** with molecular ions at *m/z* 569, 707, 693 were recognized as sanggenons B, C, G, respectively. The mass spectra of the above mentioned compounds were confirmed with reference compounds and literature data [20, 22]. In case of sanggenon C, neutral losses of two resorcinol ($C_6H_6O_2$, 110 Da) groups were also observed for the deprotonated ion (*m/z* 707) of sanggenon C under negative ionization [22]. Sanggenon G with a molecular ion at *m/z* 693, produced an ion at *m/z* 583, which corresponds to $C_{34}H_{31}O_9^-$ and a fragment at *m/z* 531 obtained after a loss of $C_9H_5O_3$ corresponding to $C_{31}H_{31}O_8^-$ (Table 1). Compound **XII** with a molecular ion at *m/z* 423, recognized as sanggenol A, produced ions at *m/z* 298, 245, 151, 126: the fragmentation pattern was confirmed by comparison with the reference compound and literature data [22]. Compound **XIII** (kuwanon C) produced fragment ions of *m/z* 352, 309, 231, confirmed by literature data [22]. Compound **XIV** with a quasi-molecular ion of *m/z* 419, produced ions at *m/z* 375, 350, 309, and 297 based on the mass spectral profiles. The quasi-molecular ions of compound **XV** (*m/z* 437) corresponding to morunigrol was tentatively identified based on the mass spectral profiles (Table 1) [22]. Further, the diagnostic product ions of the deprotonated ion (*m/z* of 243 for $[M-H]^-$) of compound **XVI** (sanggenol B) were generated at *m/z* values of 445, 364, 351, 309 and 257, as described in literature [22]. The molecular ion of compound **XVII** at *m/z* 487 was tentatively assigned as andalasin A: there are two characteristic ions at *m/z* 349 and 231 [22].