

Article

High-Throughput ^1H -Nuclear Magnetic Resonance-based screening for the identification and quantification of heartwood diterpenic acids in four Black pine (*Pinus nigra* Arn.) marginal provenances in Greece.

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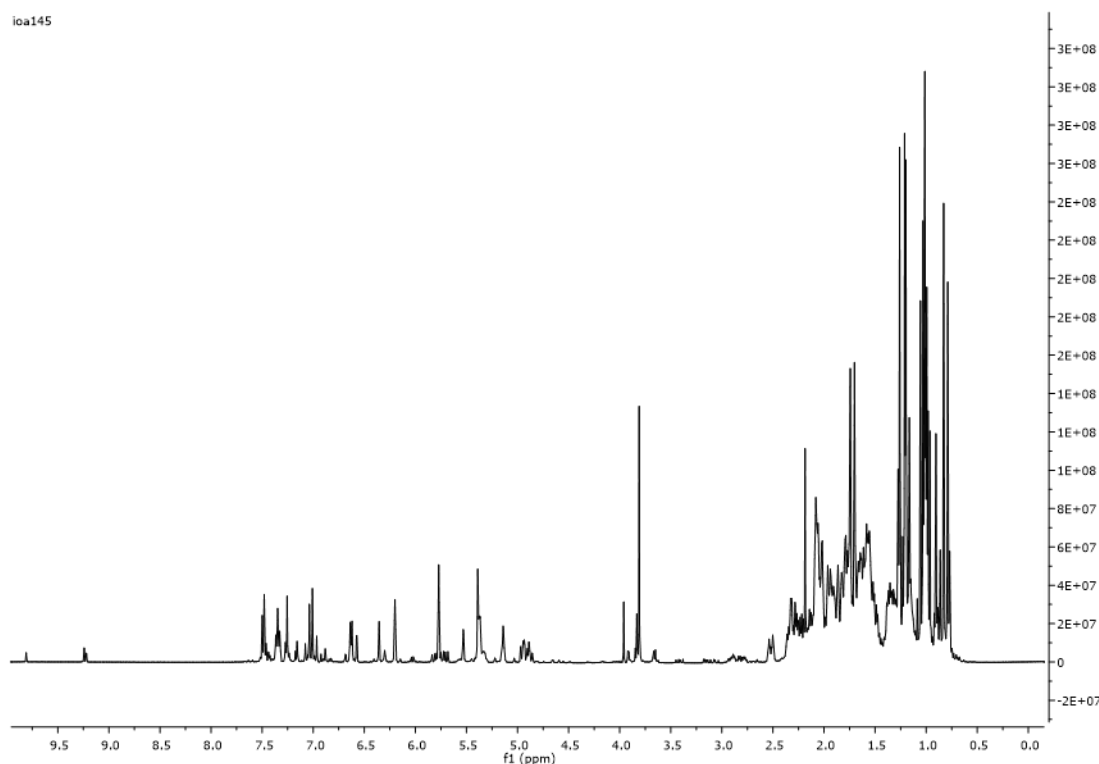


Figure S1. The ^1H -NMR spectrum (400MHz, CDCl_3 , δ -values in ppm) of Black pine (*P. nigra* Arn.) heartwood extract.

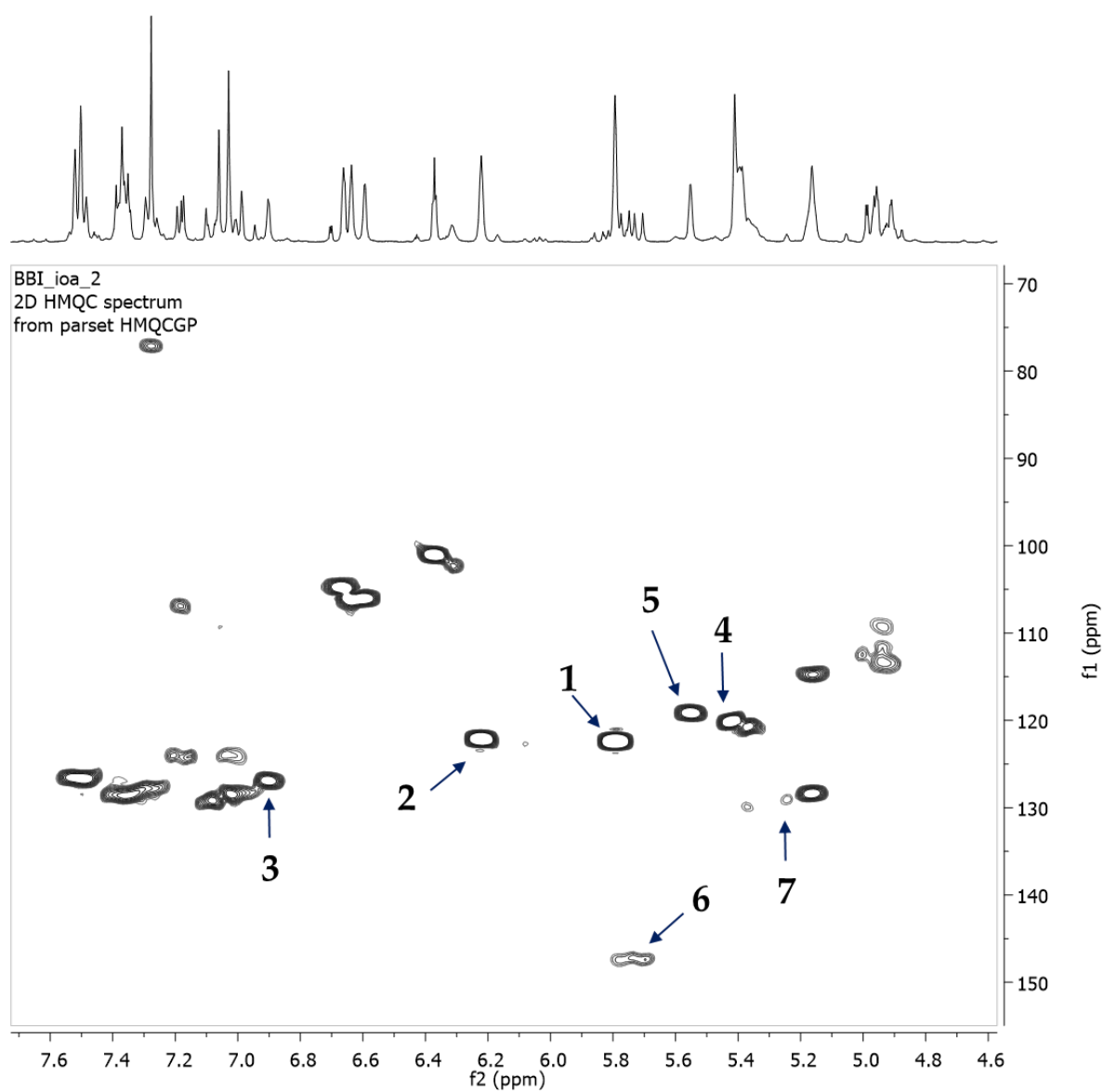


Figure S2. 2D HMQC spectrum (400MHz, CDCl₃, δ -values in ppm) of Black pine (*P. nigra* Arn.) heartwood extract with low concentration of isopimaric acid (8).

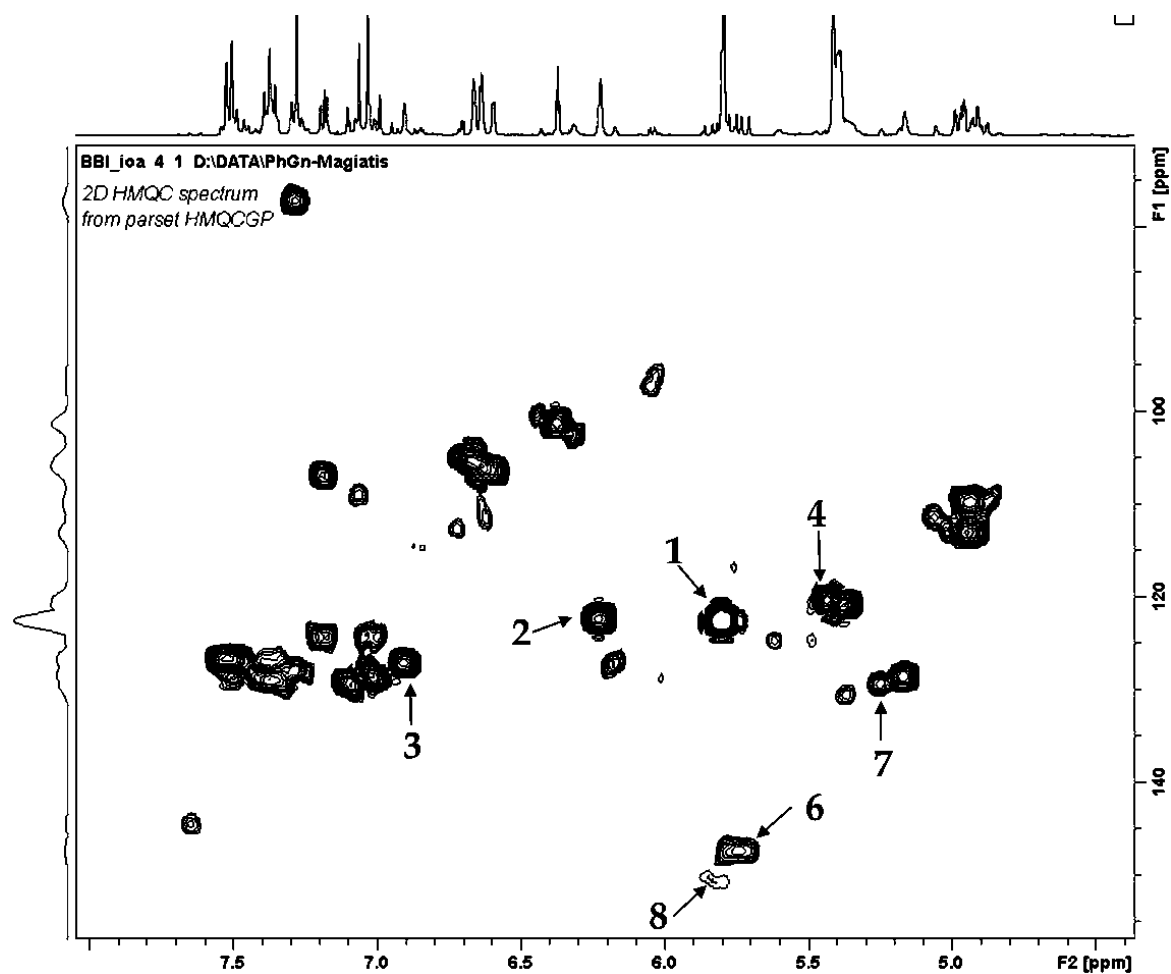


Figure S3. The 2D HMQC ^1H -NMR spectrum (400MHz, CDCl_3 , δ -values in ppm) of Black pine (*P. nigra* Arn.) heartwood extracts with low concentration of levopimaric acid (5).

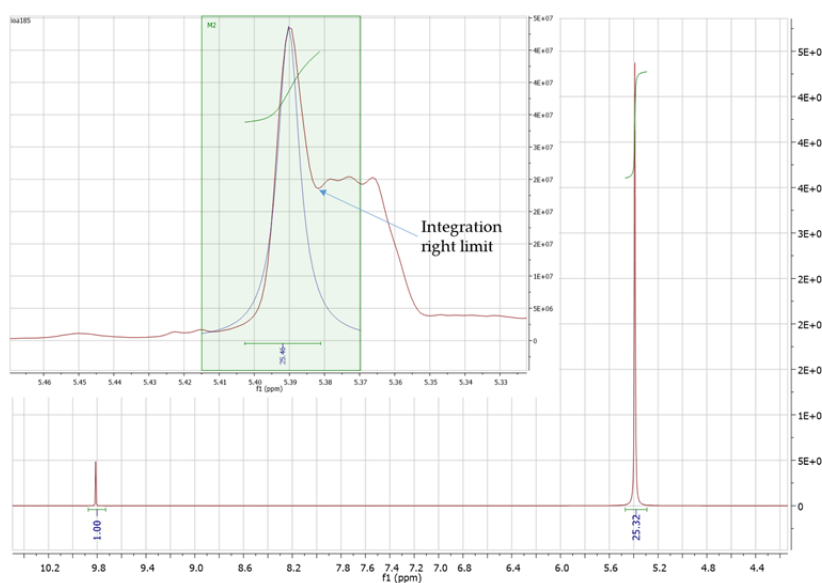


Figure S4. Deconvolution of a spectrum (400MHz, CDCl_3 , δ -values in ppm) in order to quantify the partial overlapping of peak 4.

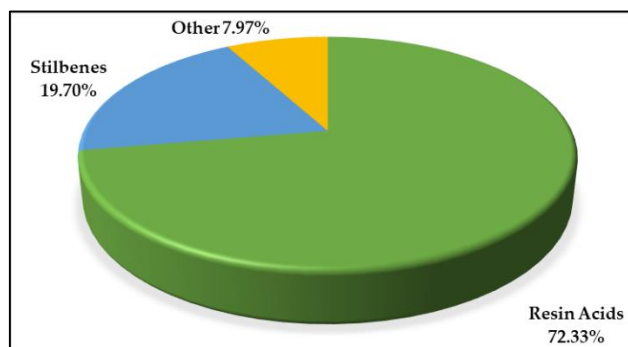


Figure S5. The major components of acetone extractives.

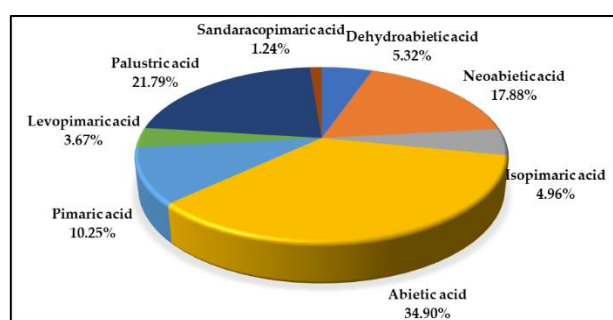


Figure S6. The mean amounts of each individual resin acid.

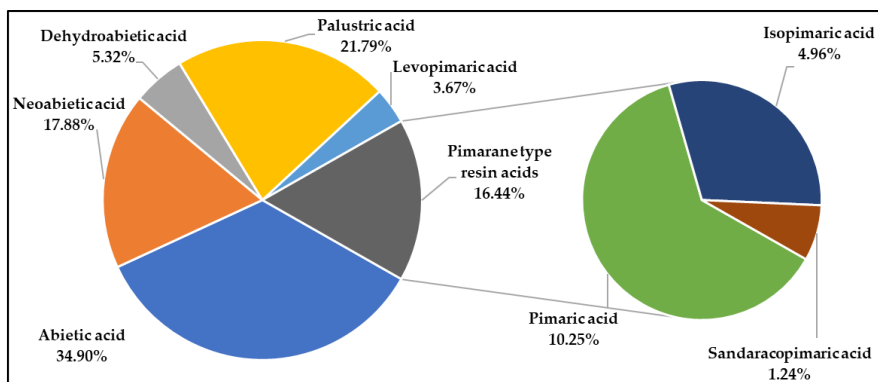


Figure S7. The mean percentage of abietane and pimarane types of resin acids.

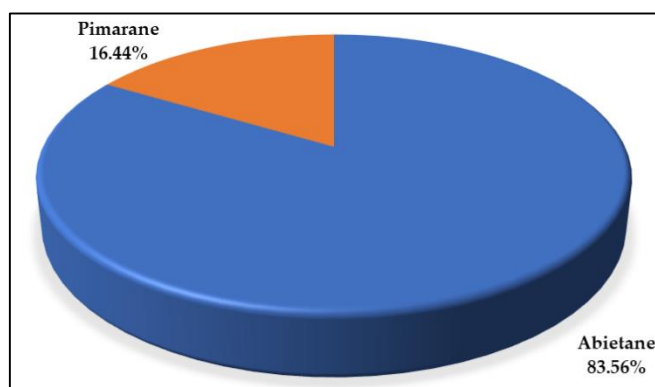
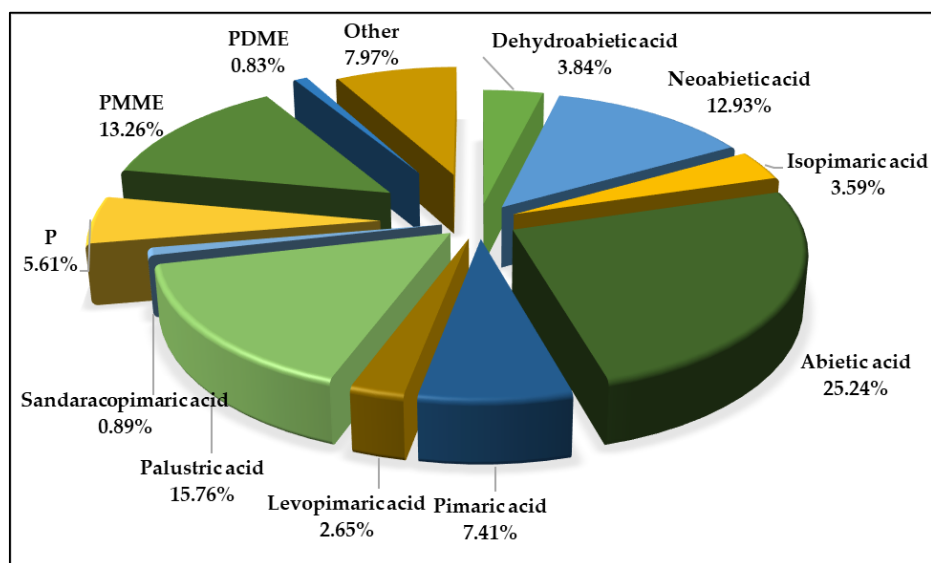
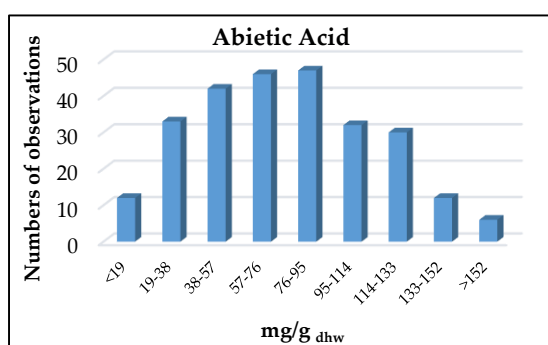
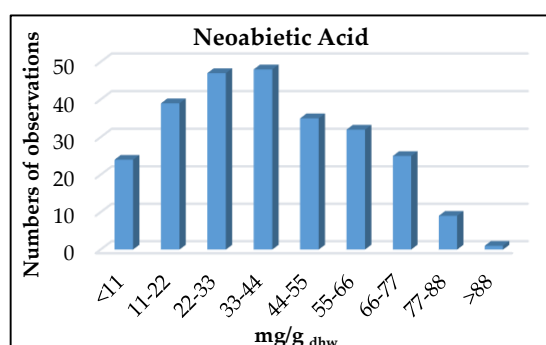
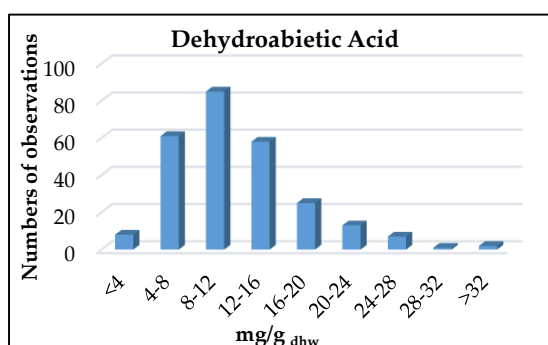


Figure S8. The mean percentage composition of the two resin acids types, i.e. abietane and pimarane.**Figure S9.** The mean percentage content of the constituents in the Black pine's heartwood acetone extraction from the Peloponnese (P= pinosylvin, PMME and PDME = monomethylether and dimethylether of pinosylvin respectively).

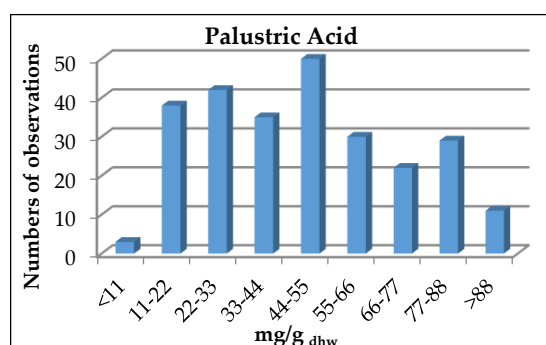
(a)



(b)



(c)



(d)

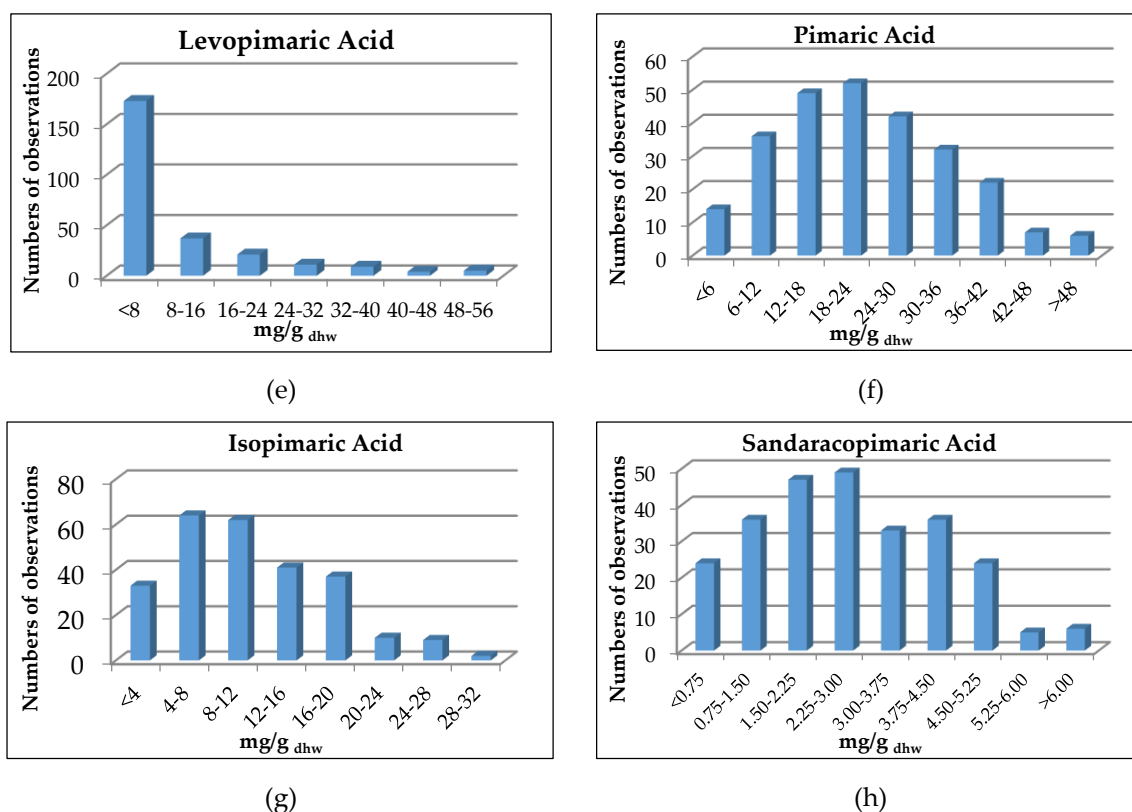


Figure S10. Resin acids frequency distribution of Black pine (*P. nigra* Arn.): (a) abietic acid; (b) neoabietic acid; (c) dehydroabietic acid, (d) palustric acid, (e) levopimaric acid, (f) pimaric acid, (g) isopimaric acid and (h) sandaracopimaric acid.

Table S1. Precision data.

Precision													
ID	NMR Spectrum	Sampling Site	Provenance	Clone	Replication	Abietic acid	Neoabietic acid	Dehydroabietic acid	Palustric acid	Levopimaric acid	Pimaric acid	Sandaracopimaric acid	Isopimaric acid
799-21B	loa330	3	Feneos	21	1	8.05	0.99	2.63	3.88	0.18	0.86	0.24	0.25
799-21B	loa331	3	Feneos	21	2	7.09	0.90	2.17	3.80	0.20	0.76	0.19	0.23
799-21B	loa332	3	Feneos	21	3	7.93	1.05	2.64	3.68	0.16	0.80	0.23	0.20
					Average	7.69	0.98	2.48	3.79	0.18	0.81	0.22	0.23
					Standard Deviation (s)	0.4271	0.0616	0.2192	0.0822	0.0163	0.0411	0.0216	0.0205
					Coefficient of Variance (CV)	5.55	6.29	8.84	2.17	9.07	5.09	9.82	9.07
578-36A	loa333	2	Parnonas	36	1	12.48	2.26	5.02	6.94	0.42	1.68	0.42	1.47
578-36A	loa334	2	Parnonas	36	2	14.28	2.65	5.46	7.87	0.45	2.02	0.45	1.49
578-36A	loa335	2	Parnonas	36	3	12.47	2.29	5.32	7.55	0.40	1.83	0.43	1.38
					Average	13.08	2.40	5.27	7.45	0.42	1.84	0.43	1.45
					Standard Deviation (s)	0.8509	0.1772	0.1835	0.3858	0.0205	0.1391	0.0125	0.0478
					Coefficient of Variance (CV)	6.51	7.38	3.49	5.18	4.85	7.55	2.88	3.31
821-47B	loa336	3	Taigetos	47	1	14.26	8.70	2.24	8.44	0.54	1.08	0.62	0.54
821-47B	loa337	3	Taigetos	47	2	13.73	8.44	2.38	10.61	0.67	1.29	0.66	0.61
821-47B	loa338	3	Taigetos	47	3	13.03	7.65	2.32	10.38	0.60	1.26	0.62	0.51
					Average	13.67	8.26	2.31	9.81	0.60	1.21	0.63	0.55
					Standard Deviation (s)	0.5037	0.4465	0.0573	0.9733	0.0531	0.0927	0.0189	0.0419
					Coefficient of Variance (CV)	3.68	5.40	2.48	9.92	8.81	7.66	2.98	7.57

Table S2. Recovery data.

Recovery												
ID	NMR Spectrum	Sampling Site	Provenance	Clone	Abietic acid	Neoabietic acid	Dehydroabietic acid	Palustric acid	Levopimaric acid	Pimaric acid	Sandaracopimaric acid	Isopimaric acid
799-21B	loa339	3	Feneos	21	91.29	95.92	88.71	93.40	94.44	93.80	90.91	86.76
578-36A	loa340	2	Parnonas	36	88.99	92.50	89.75	88.19	97.64	90.24	88.46	86.18
821-47B	loa341	3	Taigetos	47	90.49	92.13	87.90	89.91	80.11	94.21	96.84	89.16
				Average	90.26	93.52	88.78	90.50	90.73	92.75	92.07	87.37
				Standard Deviation (s)	0.9535	1.7043	0.7573	2.1656	7.6221	1.7867	3.5186	1.2892
				Coefficient of Variance (CV)	1.06	1.82	0.85	2.39	8.40	1.93	3.82	1.48