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Supplementary materials: Metabolite profiling of the microalga diatom, *Chaetoceros calcitrans* and correlation with antioxidant and nitric oxide inhibitory activities via ^{1}H NMR-based metabolomics

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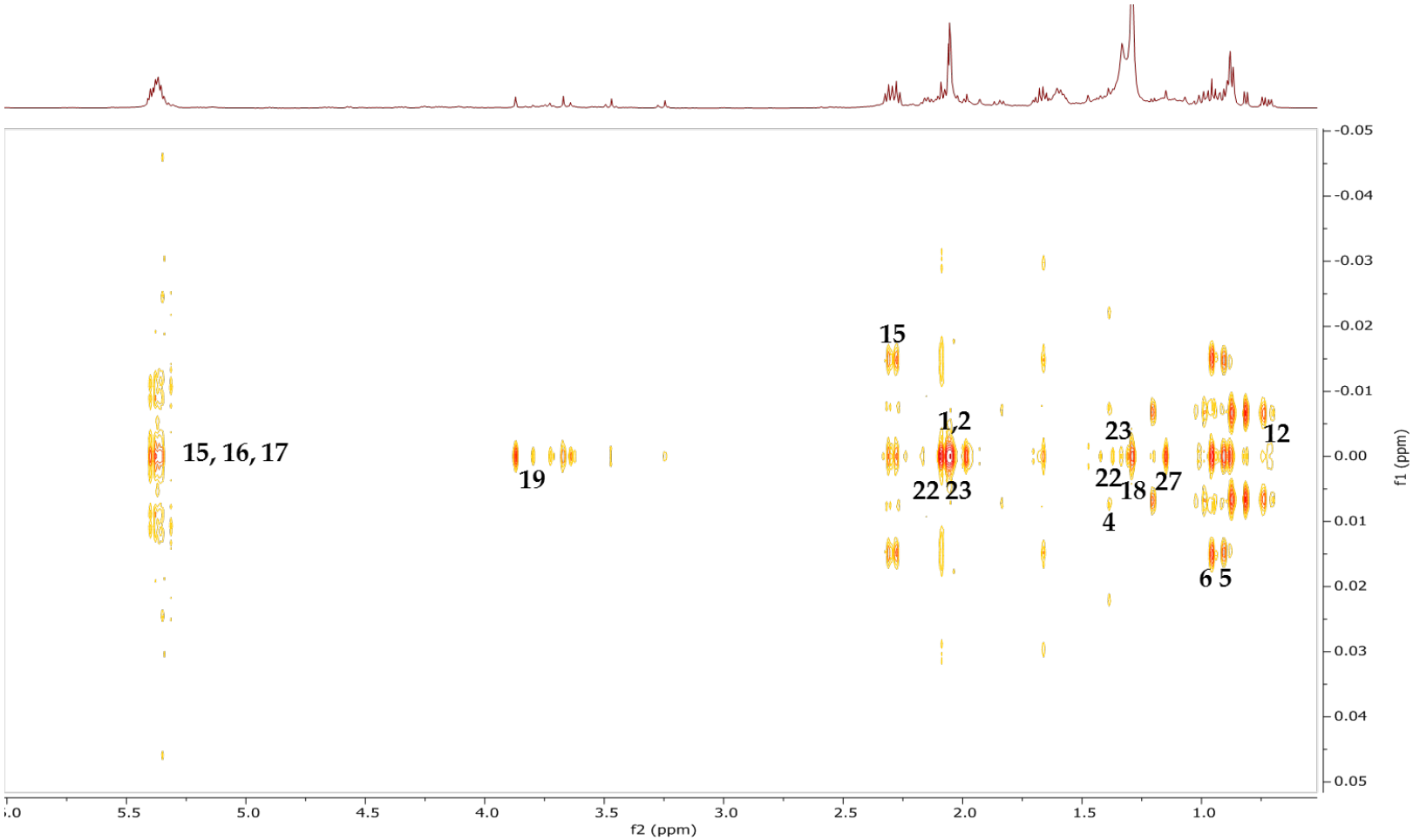

Figure S1. 2D NMR ^{1}H (*J*-resolved) spectrum of chloroform extract of *C. calcitrans*.

A

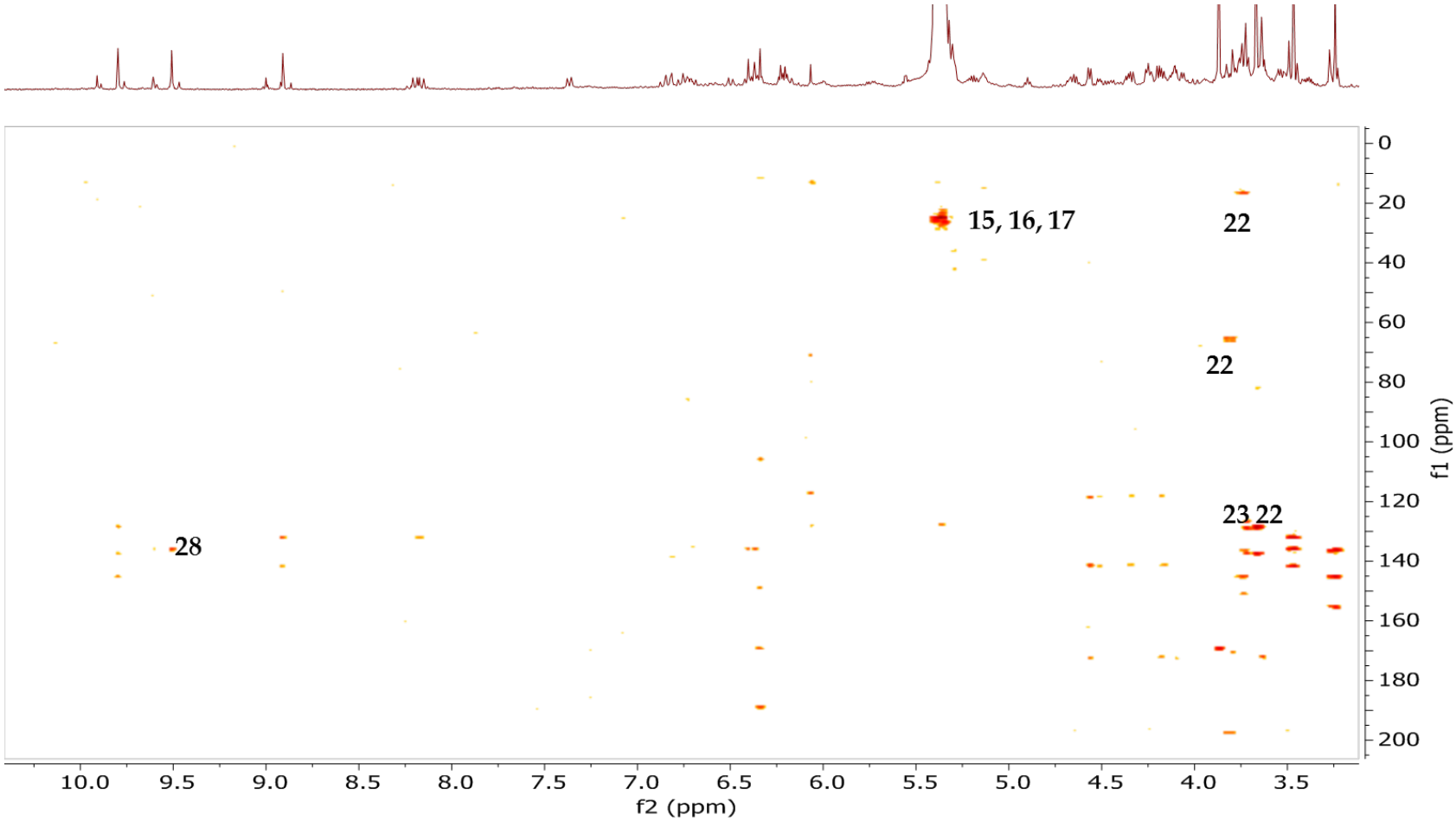

Figure S2 2D NMR ^{1}H-^{13}C (HMBC) spectrum of the chloroform extract of *C. calcitrans* (**A**) at 3.5 – 10 ppm region.

B

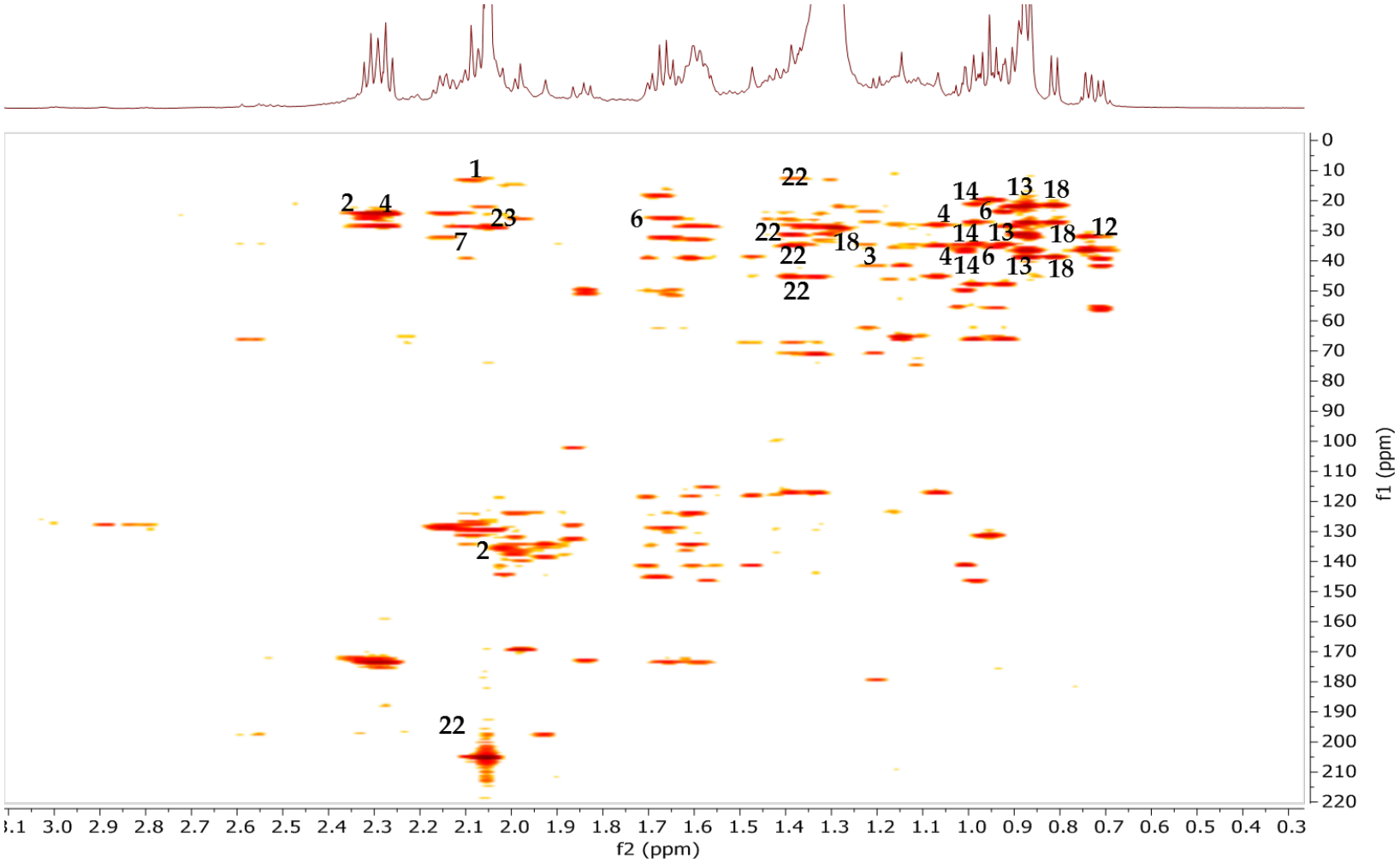

Figure S2. 2D NMR ^{1}H-^{13}C (HMBC) spectrum of the chloroform extract of *C. calcitrans* (**B**) at 0.30- 3.00 ppm region.

Table S1. Relative quantification of compounds in the extracts of *Chaetoceros calcitrans*.

	***P* value[b]**									
Compound	**A vs C**	**A vs H**	**A vs M**	**A vs 7E**	**C vs H**	**C vs M**	**C vs 7E**	**H vs M**	**H vs 7E**	**M vs 7E**
Chlorophyll-*c1*	1.000	1.000	0.566	1.000	1.000	0.564	1.000	0.563	1.000	0.564
Chlorophyll-*a*	1.000	0.010	0.004	1.000	0.010	0.005	1.000	0.997	0.010	0.004
Arachidic acid	0.010	0.000	0.005	0.006	0.000	0.000	0.000	0.000	0.000	1.000
α-Linolenic acid	0.022	0.000	0.011	0.012	0.000	0.000	0.000	0.000	0.000	1.000
Astaxanthin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Canthaxanthin	0.000	0.162	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044
Lutein	0.001	0.000	0.000	0.000	0.910	0.000	0.000	0.000	0.000	1.000
Sucrose	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.631	0.496	0.999
Palmitic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.009	1.000
Fucoxanthin	0.676	0.009	0.005	0.006	0.156	0.000	0.000	0.000	0.000	1.000
Stearic acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.062	0.103	0.999
Isoleucine	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Violaxanthin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.018	1.000
Zeaxanthin	0.079	0.706	0.000	0.000	0.609	0.000	0.000	0.000	0.000	0.999
Cholestrol	0.000	1.000	0.000	0.000	0.000	0.000	0.000	0.510	0.543	0.564
Leucine	0.344	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000
Glucose	0.037	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.998	0.993
Proline	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.988	1.000	0.984
Myo-inositol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.754	0.634	0.102
Glycine	0.425	0.000	0.000	0.000	0.000	0.000	0.000	0.999	0.926	0.983

[†] *P* values were results of Tukey-HSD pairwise multiple-comparison tests using SPSS 16.0. Significant level: $P > 0.050$, not significant; $0.050 \geq P > 0.010$, significant*; $0.010 \geq P > 0.001$, very significant**; and $0.001 \geq P$, highly significant***. Letters indicate the three extraction solvents of *Chaetoceros calcitrans*: (A) Acetone, (C) Chloroform, (H) Hexane, (M) Methanol and (7E) 70% Ethanol

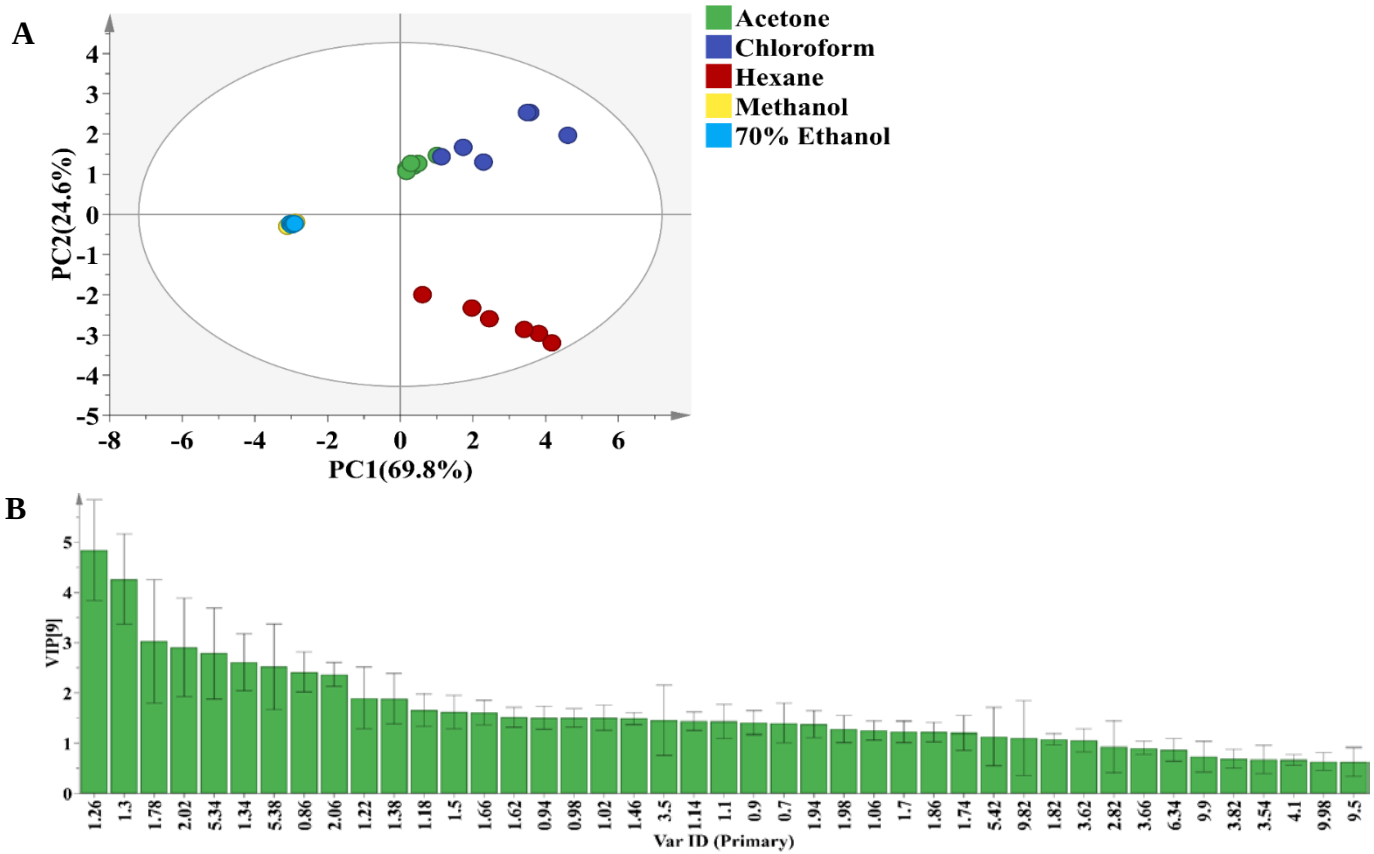

Figure S3. Partial least squares discriminant analysis (PLSDA) (**A)** score and (**B**) variable importance in projection (VIP) plots of *C. calcitrans* extracts. Chemical shift (ppm) as listed in VIP plot: arachidic acid (δ 1.26; 1.62), α-linolenic acid (δ 1.3; 2.06; 2.82; 5.34), astaxanthin (δ 1.34; 1.82; 1.94), stearic acid (δ 1.46; 1.78), canthaxanthin (δ 1.18; 1.86 2.02), sucrose (δ 3.66; 5.38), lutein (δ 0.86; 1.02; 1.06; 1.74; 5.42), fucoxanthin (δ 1.22; 1.38; 1.5), palmitic acid (δ 0.9; 1.66), isoleucine (δ 0.94), violaxanthin (δ 0.98; 1.14), zeaxanthin (δ 1.1; 1.98; 6.34), glucose (δ 3.5; 3.82), cholesterol (δ 0.7), leucine (δ 1.7), chlorophyll c_1 (δ 9.82; 9.99), myo-inositol (δ 3.62), glycine (δ 3.54), proline (δ 4.1), chlorophyll *a* (δ 9.5).

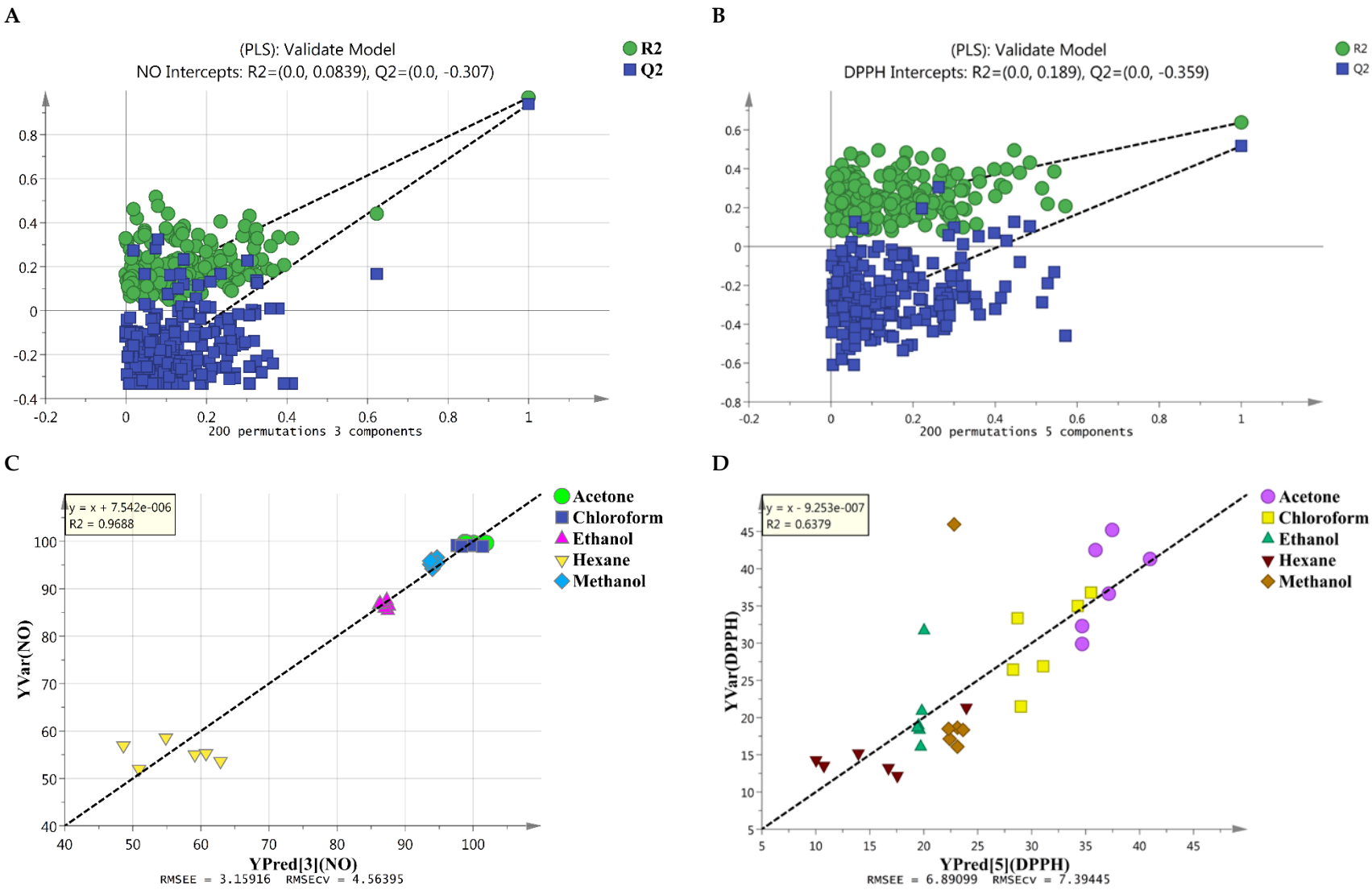

Figure S4. Validation of PLS model using permutation test (200 permutations) of NO (**A**) and DPPH (**B**) inhibitory activity. PLS derived relationship between observed vs predicted of NO (**C**) and DPPH (**D**) activity