Identification of Constituents of Hydroethanolic Echinacea Extracts Active in Free Radical Quenching by n-Hexane Partitioning and Chemometric Analyses

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Objectives:

- For a series of samples (75% ethanolic extracts and hexane washed ethanolic extracts) of the *Echinacea species*, E. purpurea, E. angustifolia, and E. pallida, determine the relative activity to quench free radical, DPPH•.

- Determine which constituent chemical class(es) of the *Echinacea spp*. preparations mediates free radical quenching by correlating activity with previously determined integrated areas of regions of 1H-NMR spectra by (O)PLS regression.

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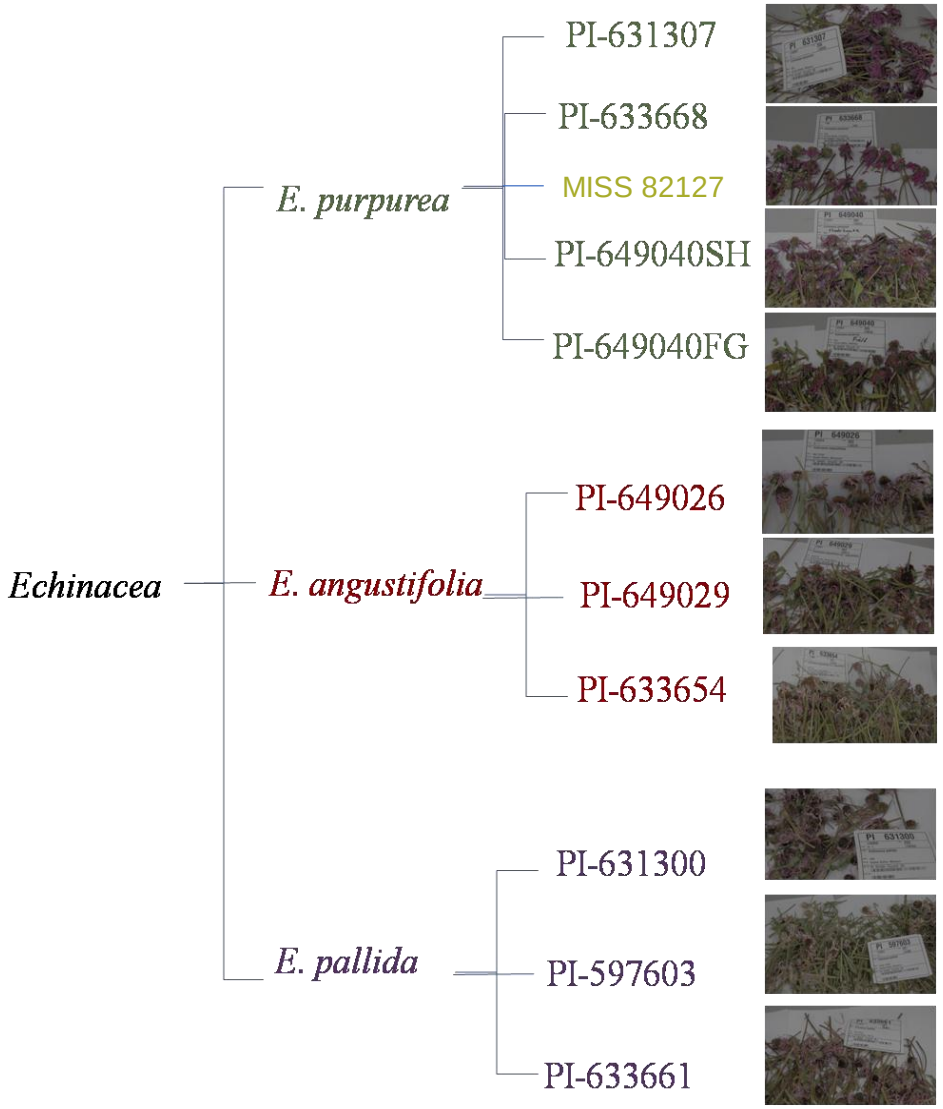
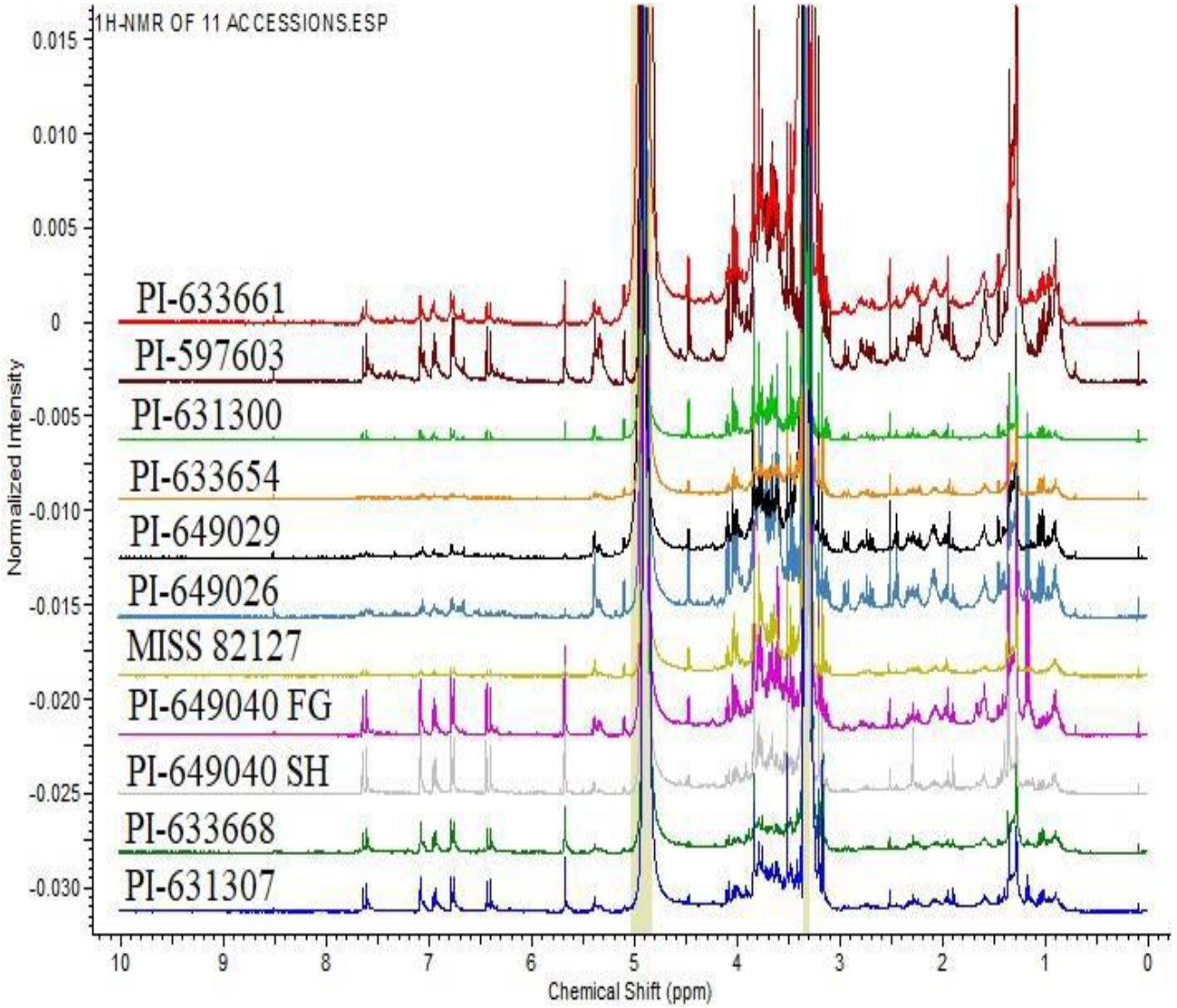


^{1}H NMR spectra



Free Radical Quenching Activity of Echinacea

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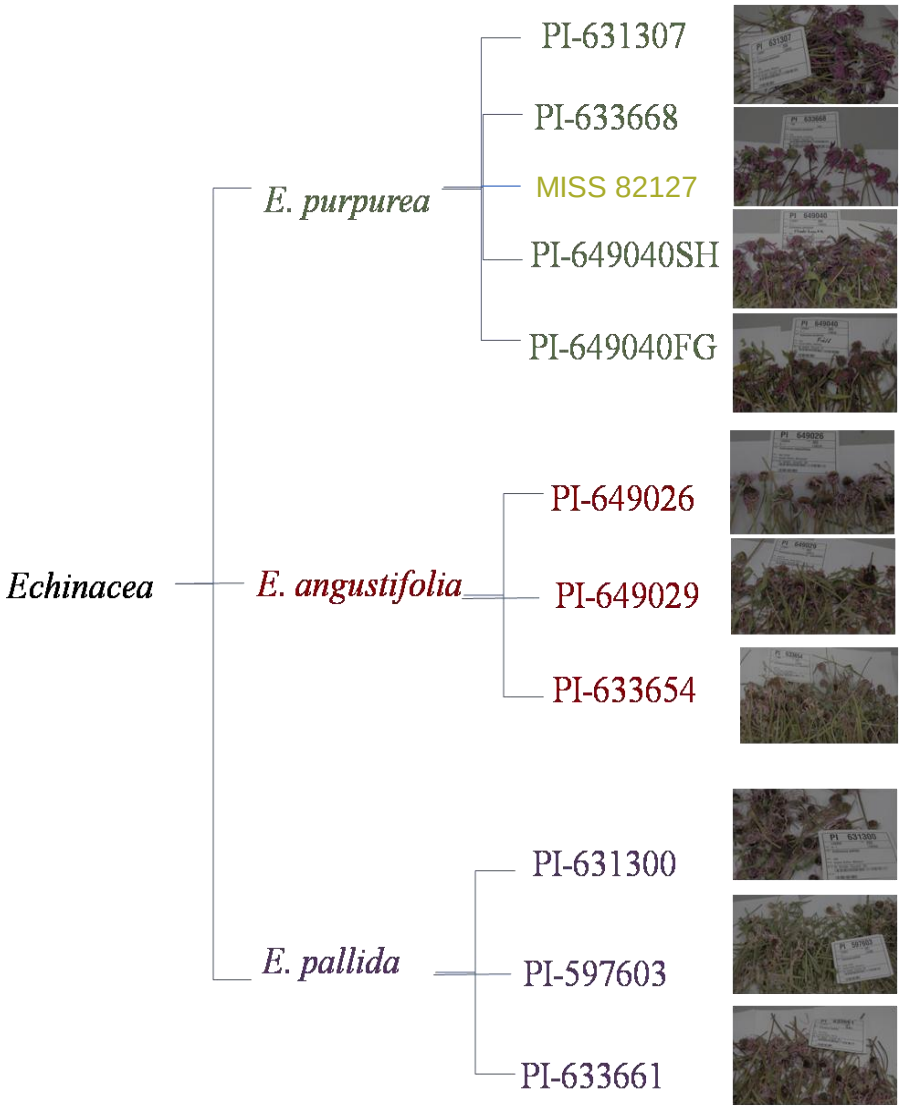
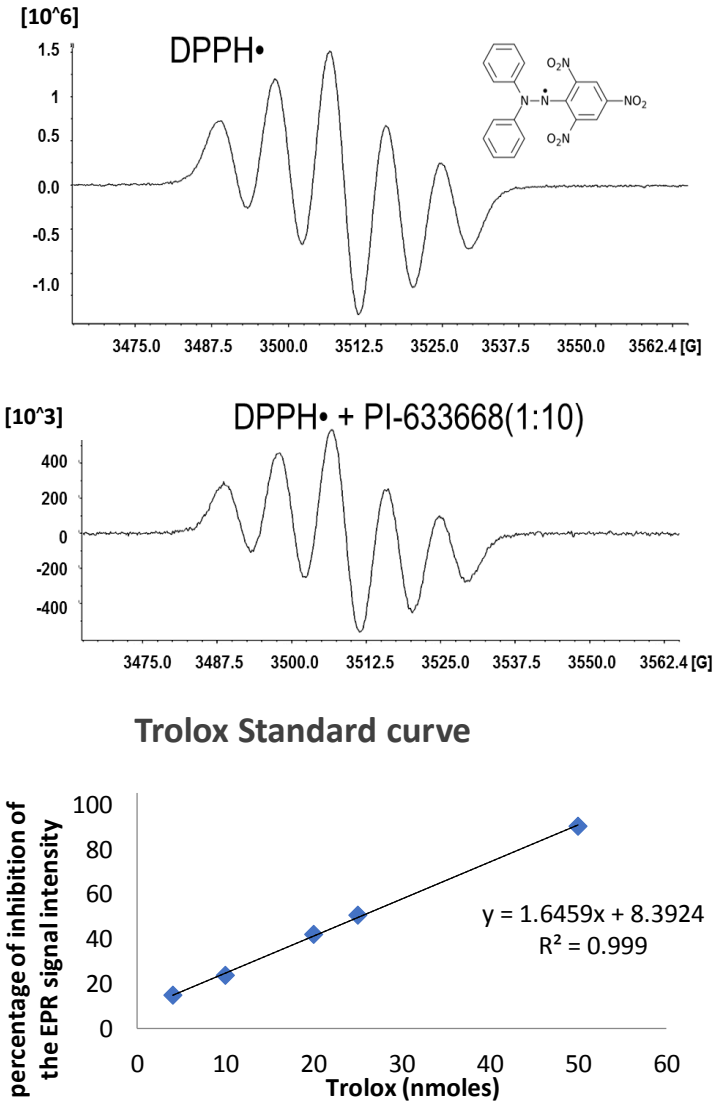


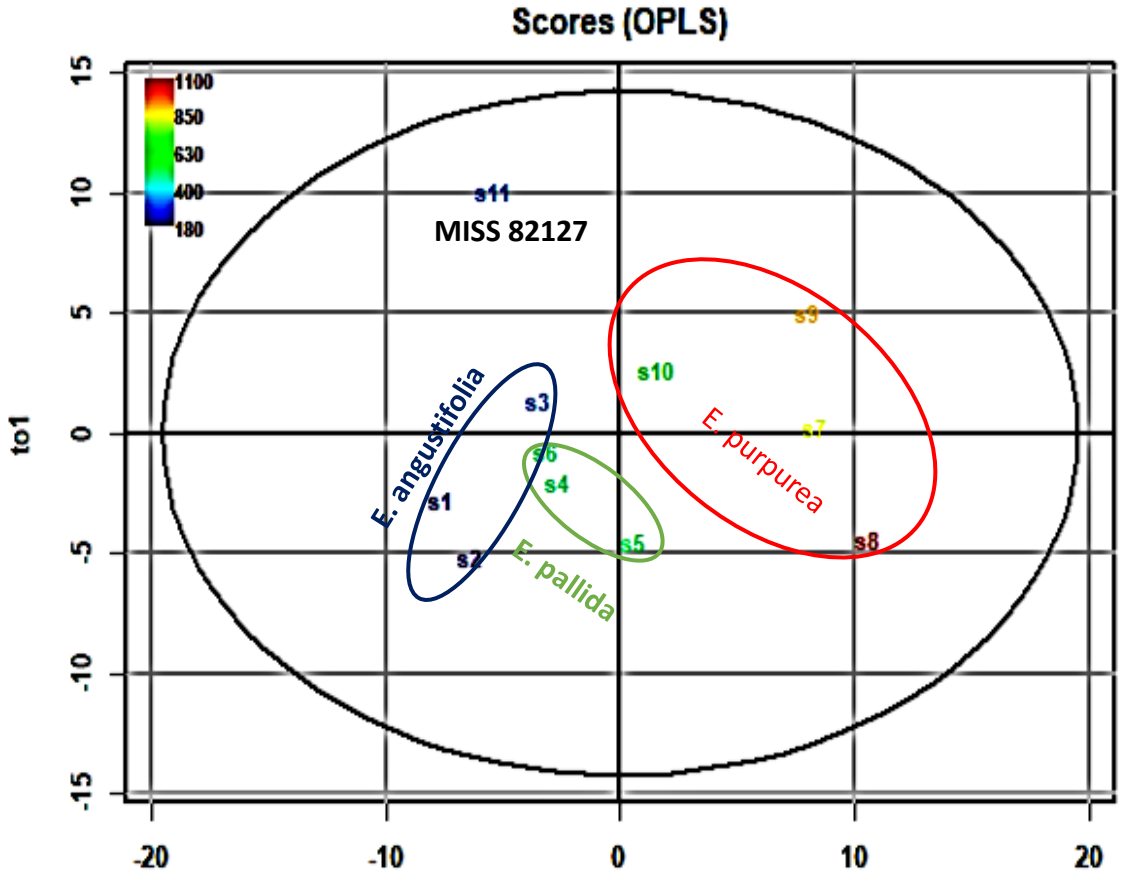
EPR spectra



Trolox equivalents

Echinacea Species	75% ETOH Extracts
PI-633661	485.1
PI-597603	519.1
PI-631300	492.9
PI-633654	311.2
PI-649029	176.4
PI-649026	197.6
MISS 82127	306.7
PI649040FG	582.1
PI-649040SH	896.9
PI-633668	**1080.1**
PI-631307	828.4

Multivariate Data Analysis (Chemometrics)



E. angustifolia	S1	PI-649026
	S2	PI-649029
	S3	PI-633654
E. pallida	S4	PI-633661
	S5	PI-597603
	S6	PI-631300
E. purpurea	S7	PI-631307
	S8	PI-633668
	S9	PI-649040SH
	S10	PI-649040FG
Commercial field sample	S11	MONT

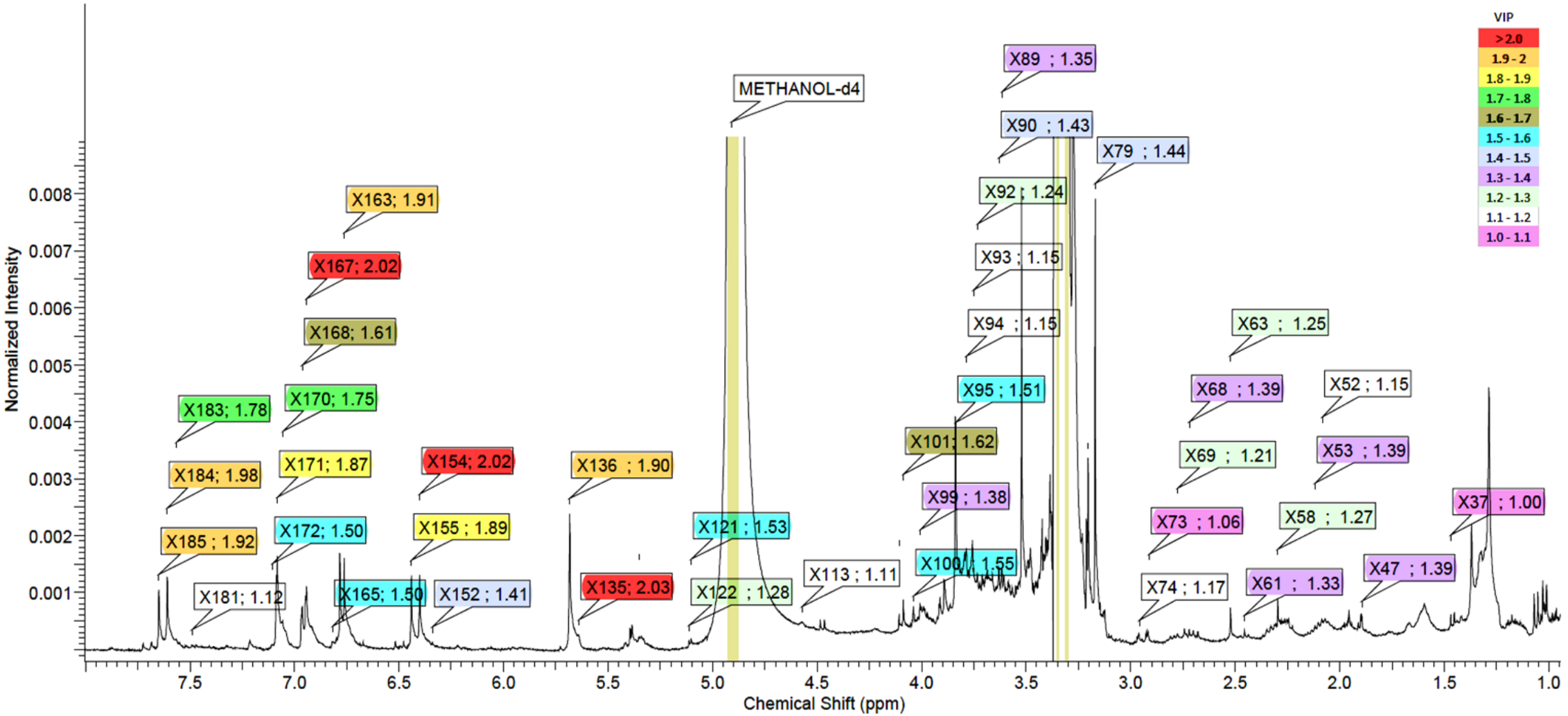
OPLS Model Metrics:

R2X(cum)	R2Y(cum)	Q2(cum)*	pre	Ort	pR2Y	pQ2
0.392	0.956	0.714	1	1	0.15	0.05

Q2 represents predictive performance of the model

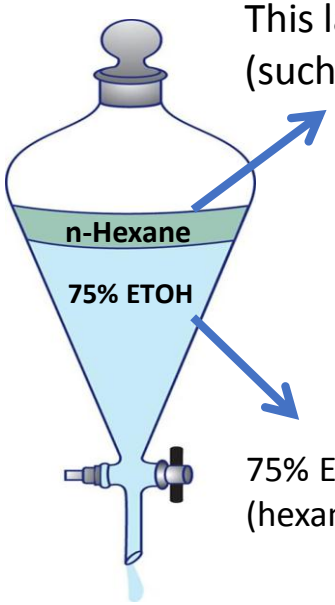
Q2	Predictability
> 0.5	Good
> 0.7	Better
> 0.9	Excellent

Multivariate Data Analysis (Chemometrics)



Variables with VIP>1.0 contributing to free radical quenching identified on PI-633668. It can be inferred that the olefinic/aromatic regions (chemical shift range of 5.10-7.70 ppm) possibly best correlate with the free radical quenching activity.

n-Hexane Partitioning

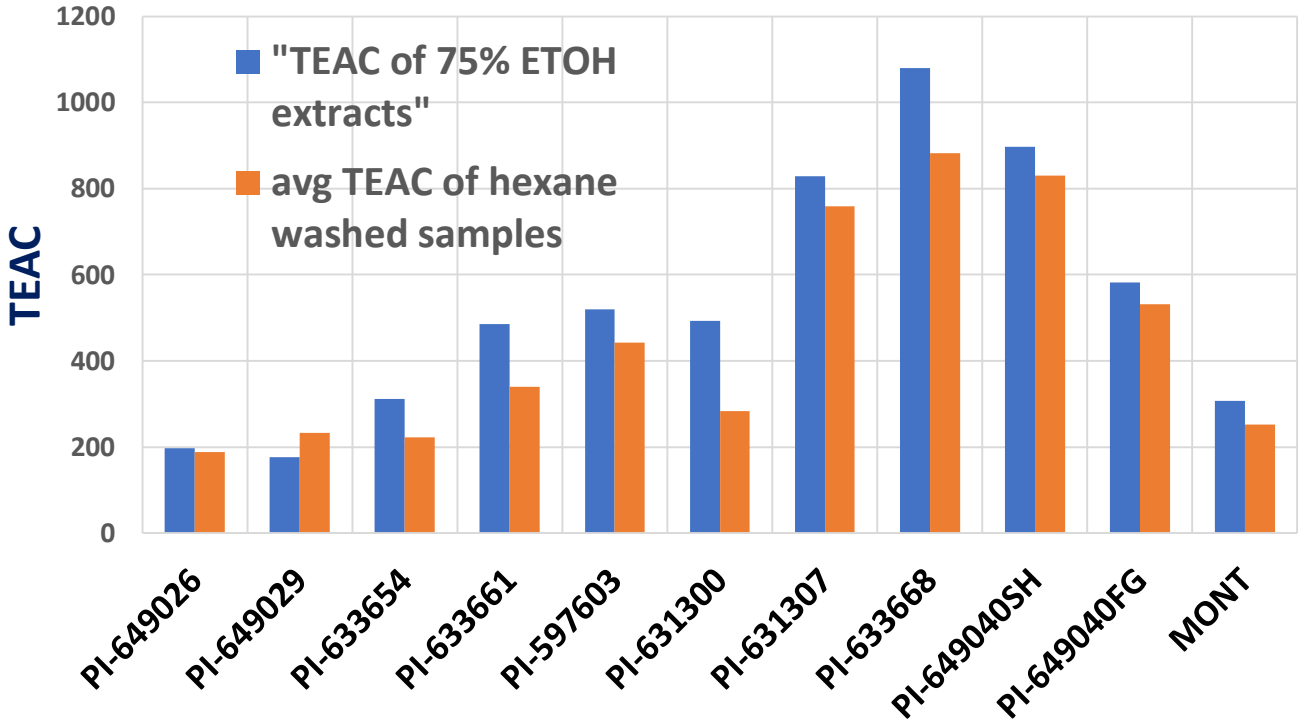


This layer contains lipophilics (such as alkylamides).

Dried and tested for quenching activity in EPR spectrometer.

75% Ethanol (hexane washed) layer

TEAC before and after n-Hexane washing



Summary and Conclusions:

- With (O)PLS regression analysis we were able to identify the regions of ^{1}H-NMR likely contributing to the free radical quenching activity.

- Removal of hexane-soluble constituents (such as alkylamides and phytosterols) in most preparations has a little loss of quenching effect, but two preparations (PI-633668 and PI-631300) shows a sizable loss of quenching activity. Further studies will try to potentially differentiate constituent classes for activity.

- In conclusion, these data support the use of chemometrics to suggest constituent classes bioactive within a complex mixture and individual active constituents in some cases.