



Identification of Constituents of Hydroethanolic Echinacea Extracts Active in Free Radical Quenching by *n*-Hexane and Ethyl Acetate Partitioning Aided by Chemometric Analysis



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Chandra Mohan Reddy Muthumula¹, O.C. Mcgehee¹, S.K. Nagumalli¹, H. A. Hussin¹, J.D. Carstens², C.V. Landingham³, K.A. ElSayed¹, and S.A. Meyer¹.

¹School of Basic Pharmaceutical and Toxicological Sciences, University of Louisiana at Monroe, Monroe, LA; ²North Central Regional Plant Introduction Station (NCRPIS), USDA-ARS, Ames IA; ³Ramboll Environ, Monroe, LA.

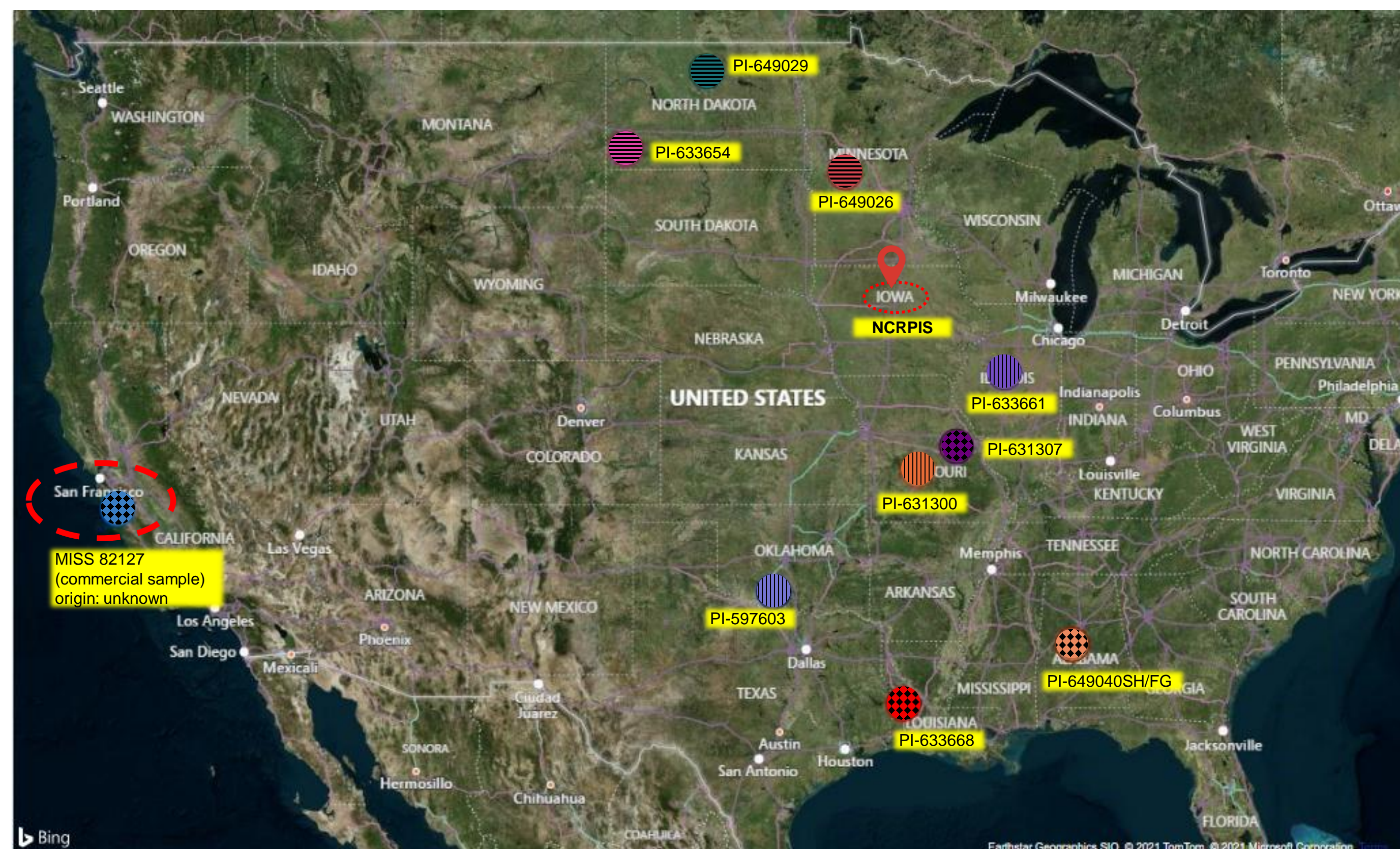
ABSTRACT

Echinacea spp. contains numerous chemical constituents known to quench free radicals, but relative potencies of these constituents *in situ* are unknown. To address this issue, we used partial least square (PLS) regression of spectral peaks from proton nuclear magnetic resonance (¹H-NMR, 400 MHz) spectroscopy against DPPH• quenching activity. Relative areas of proton spectral regions (0.04 ppm bins; 0.00 - 10.00 ppm) of *Echinacea* extract were integrated for aerial parts of the three medicinal species of *E. purpurea*, *E. pallida* and *E. angustifolia* obtained from USDA-ARS NCRPIS, Ames, IA. We used electron paramagnetic resonance (EPR) spectroscopy to evaluate the free radical quenching activity of 75% ethanolic extracts and fractions obtained by defatting with *n*-hexane and then partitioning with ethyl acetate. In addition, a commercial *E. purpurea* (Monterey Bay Spice Co.) extract was also evaluated. Quenching activity was measured as reduction of DPPH• signal by EPR and expressed relative to that of Trolox. Free radical quenching activity of *Echinacea* extract was modestly increased by defatting with *n*-hexane but was significantly increased by partitioning with ethyl acetate. Orthogonal PLS-regression analyses of the ¹H-NMR data identified several peaks well correlated with the free radical quenching activity in the 5.10-7.70 ppm region, mostly aromatic/olefinic constituents, that partitioned with ethyl acetate. In conclusion, these results show enrichment of constituents responsible for the free radical quenching in *Echinacea* by removal of *n* hexane-soluble constituents followed by partitioning with ethyl acetate. These observations are consistent with free radical quenching activity of unfractionated *Echinacea* being expressed by aromatic phenolic constituents *in situ*.

Objectives

- For a series of samples of the *Echinacea* species, *E. purpurea*, *E. angustifolia*, and *E. pallida*, determine the relative activities and to group plants with respect to quench free radical, DPPH•.
- Determine which chemical constituents 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.

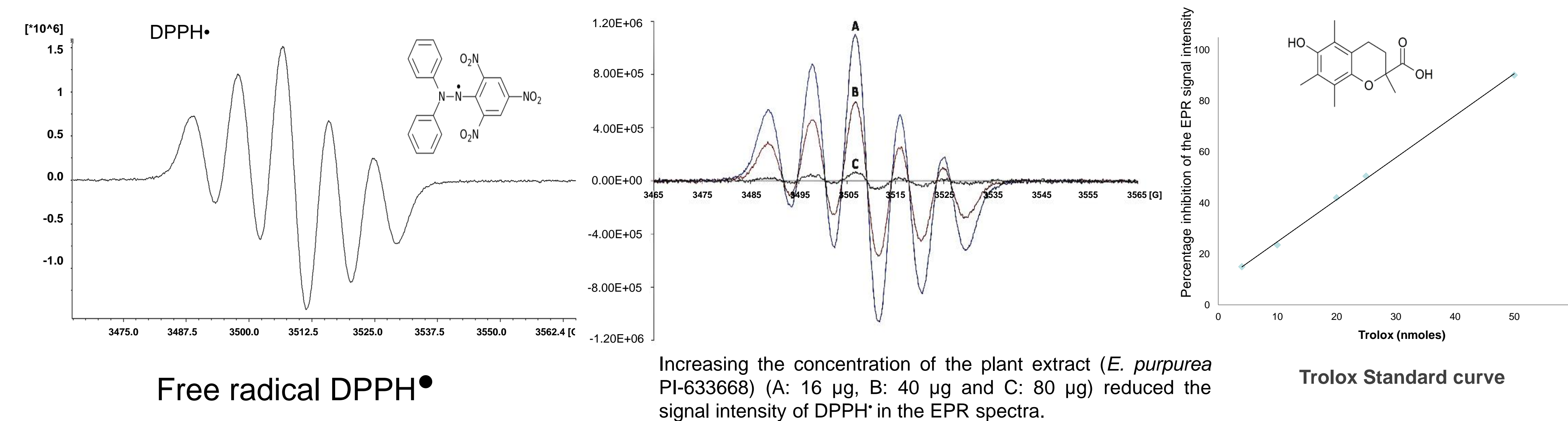
USDA PLANTS



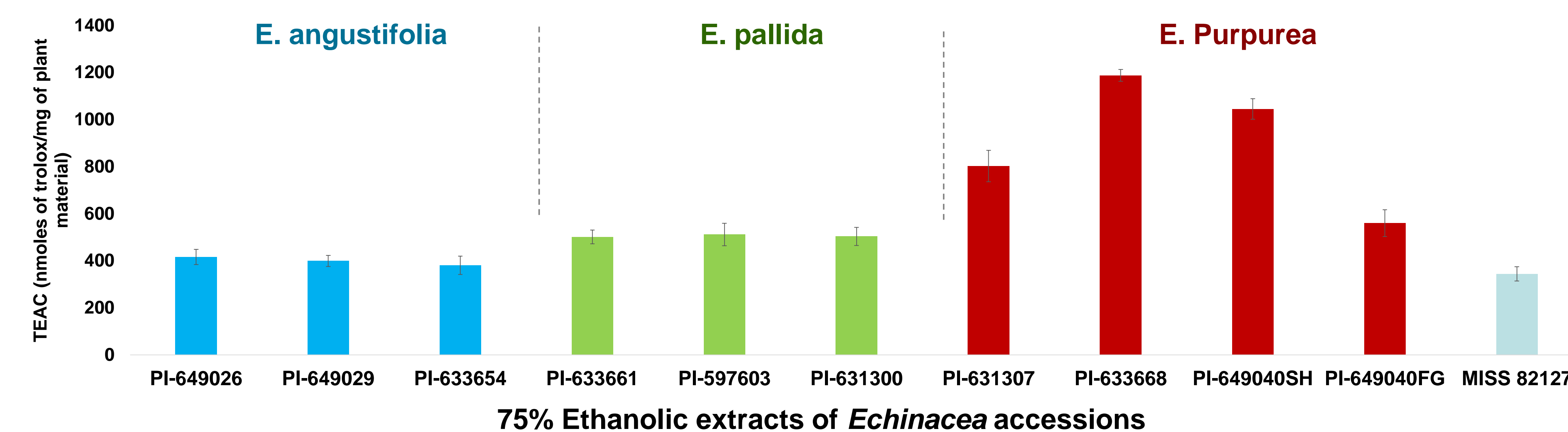
● PI-649026 ● PI-597603 ● PI-631307
● E. angustifolia ● PI-649029 ● E. pallida ● PI-631300 ● E. purpurea ● PI-633668
● PI-633654 ● PI-633661 ● PI-649040SH/FG ● MISS 82127

All these accessions of *Echinacea* were grown at NCRPIS, Ames, Iowa from the seeds originally collected from different geographical locations

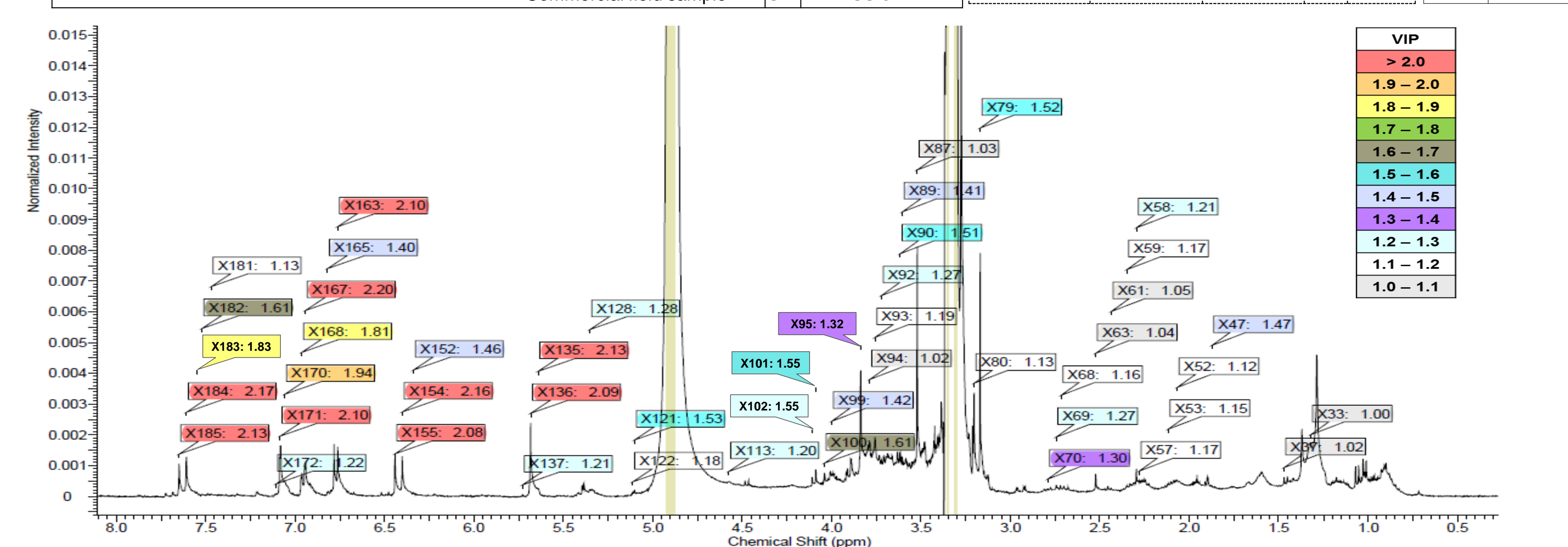
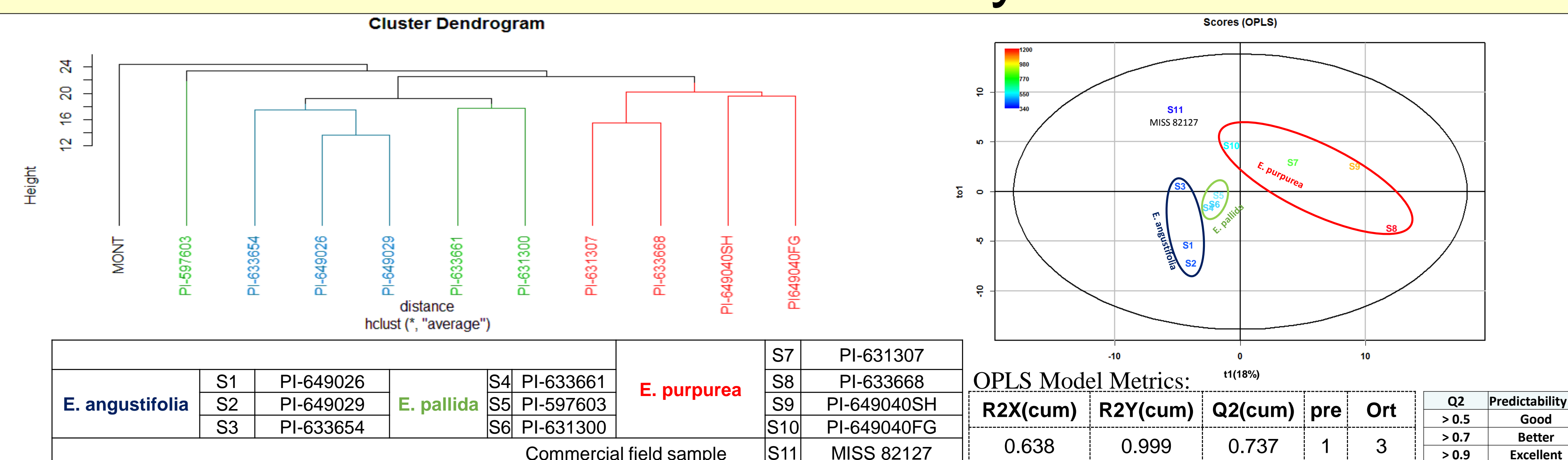
EPR spectra



Trolox equivalents



Chemometric Analysis

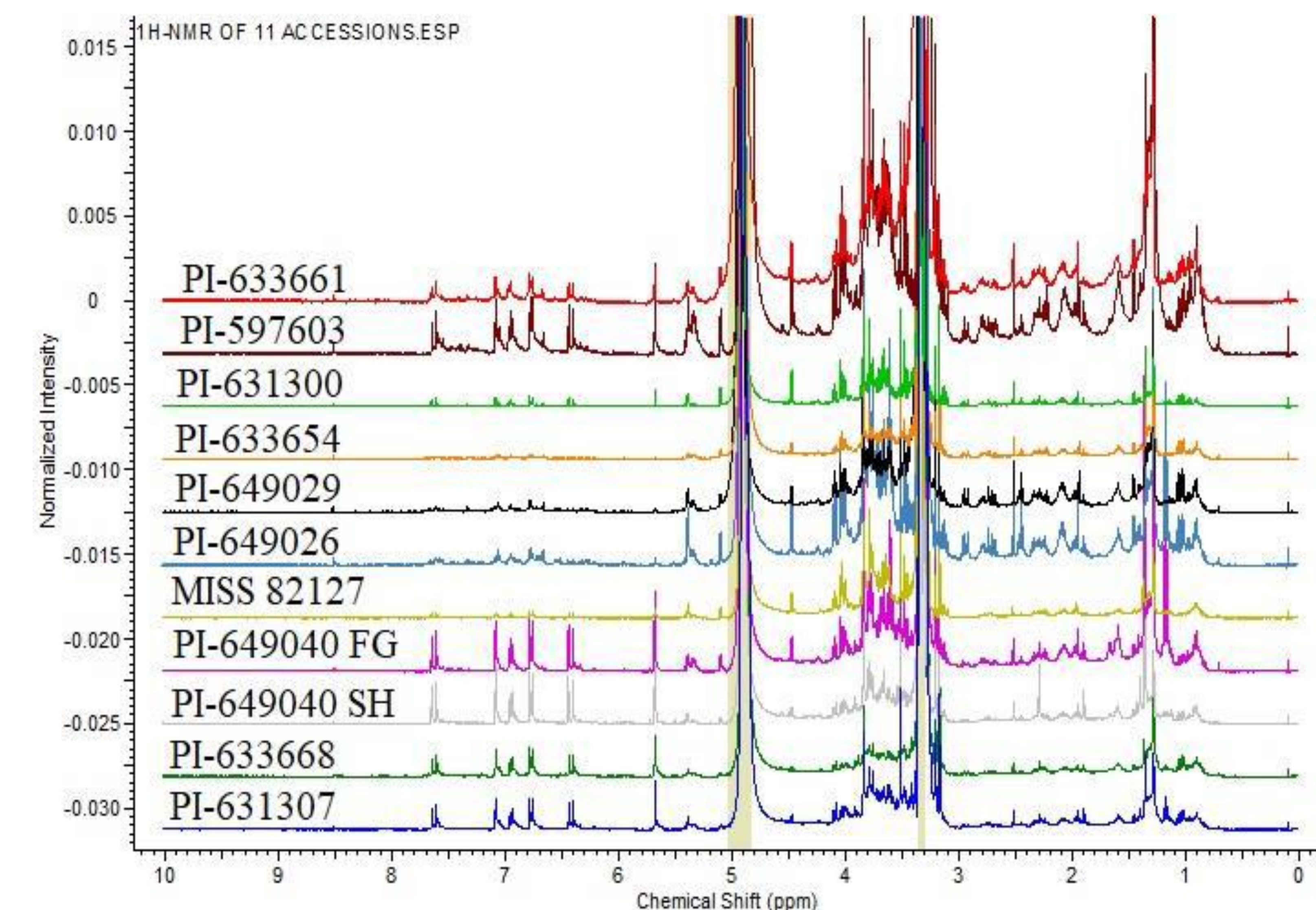


Variables with VIP>1.0 contributing to free radical quenching identified on PI-633668. It can be inferred that the olefinic/aromatic regions (chemical shift range of 5.10-7.70 ppm) best correlate with the free radical quenching activity.

Conclusions

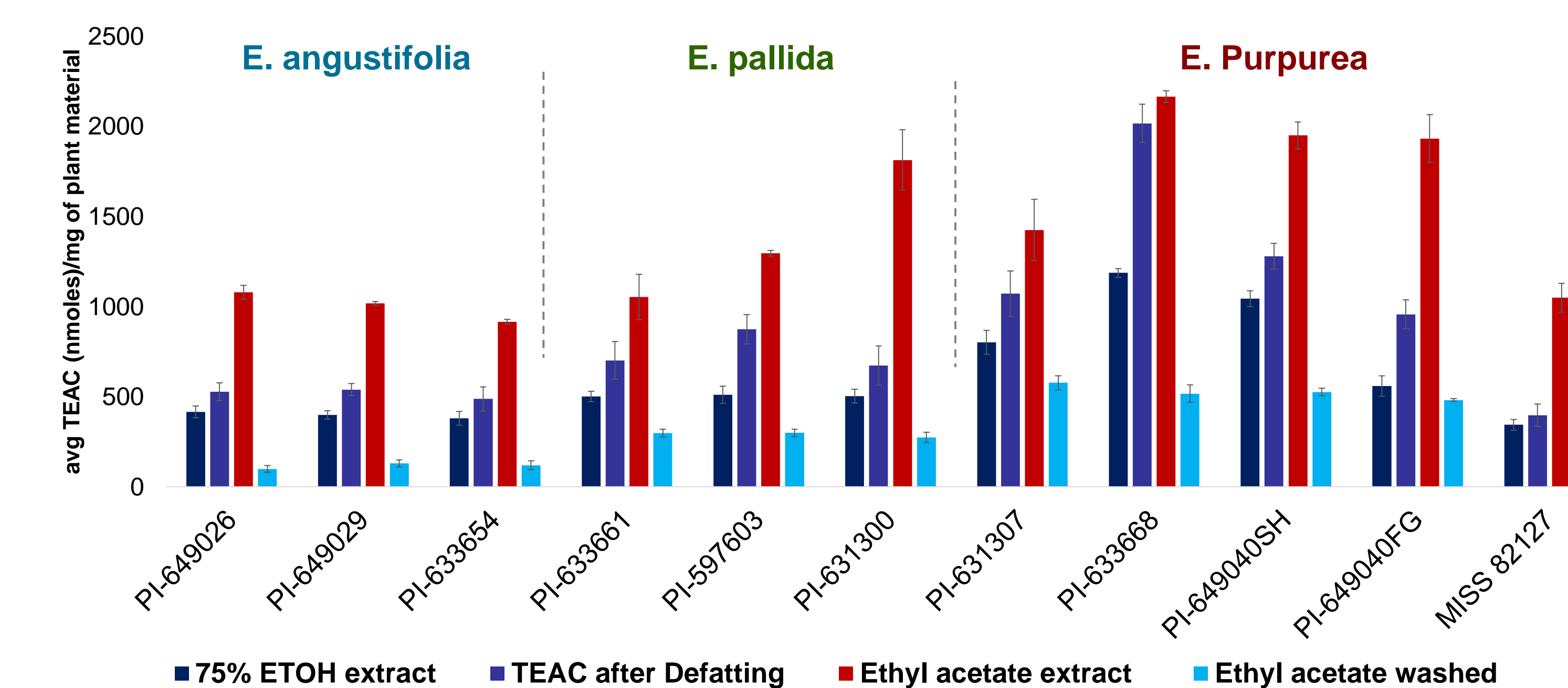
- E. purpurea* showed relatively high quenching activity than *E. pallida* and *E. angustifolia*.
- With (O)PLS regression analysis we were able to identify the (aromatic) regions of ¹H-NMR likely contributing to the free radical quenching activity.
- The *n*-hexane-soluble constituents are not significantly responsible for the free radical quenching activity of *Echinacea* *in vitro*, while ethyl acetate extracts (from defatted samples) showed improved quenching activity in all accessions.
- Application of chemometrics is supported here as a tool to identify the constituent chemicals that are active within a complex mixture such as *Echinacea*.

¹H NMR spectra

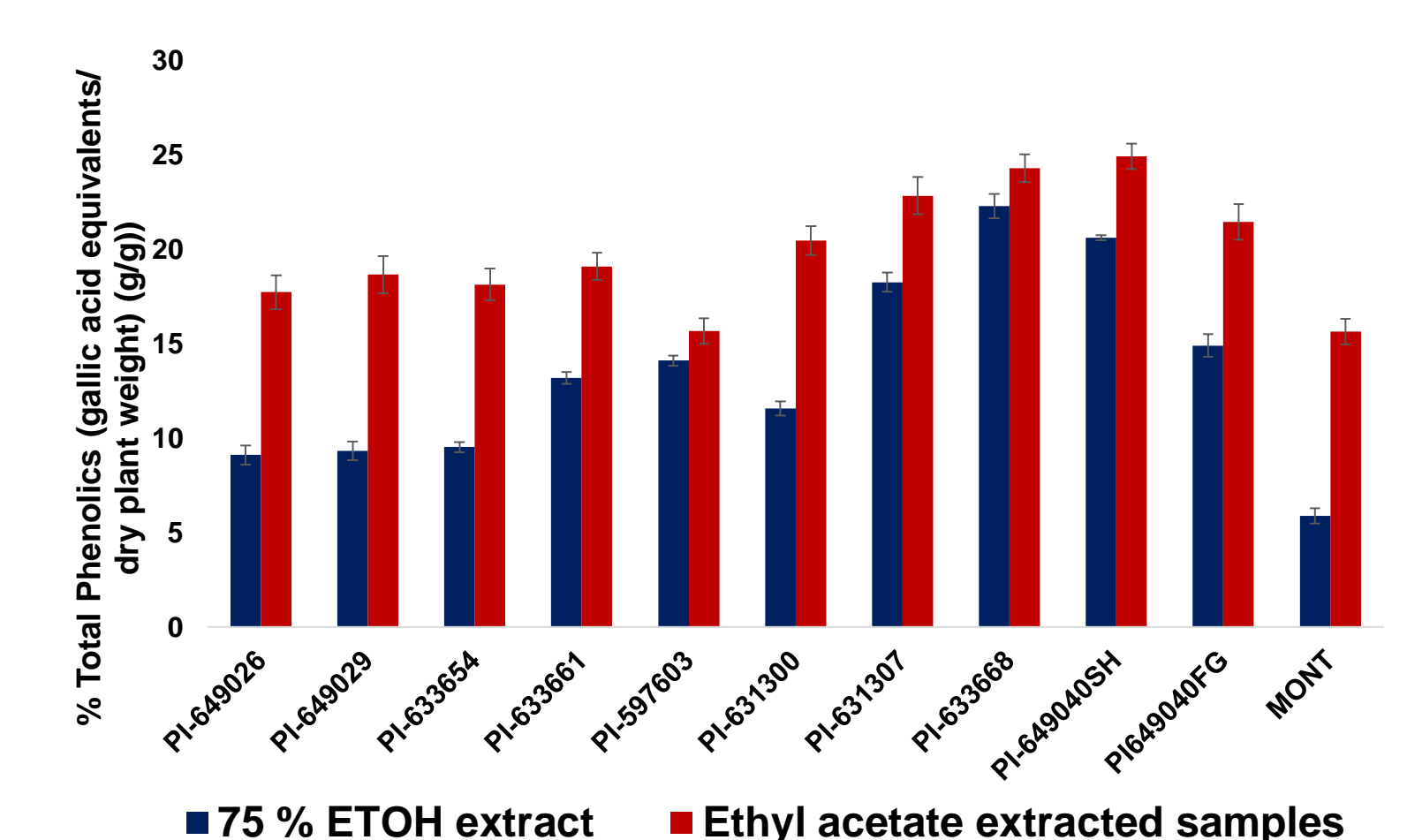


¹H NMR spectra of all accessions (Ethanolic extracts) of *E. purpurea*, *E. angustifolia*, and *E. pallida*.

Trolox equivalents using different Echinacea extracts



Total phenolic content of different Echinacea extracts



Trolox equivalents of Echinacea (PI-633668; polyphenol enriched and removed) extracts.

