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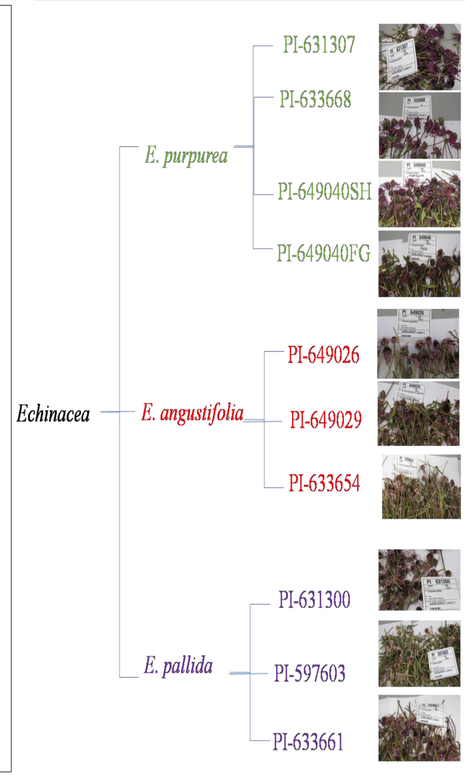
Chromatographic Quantitation of Echinacea Constituents that Mediate Free Radical Quenching Utilizing Chemometric Analysis

O. C. MCGEHEE[1], C. MUTHUMULA[1], H. A. HUSSIN[1], J. D. CARSTENS[2], C. V. LANDINGHAM[3], AND S. A. MEYER[1]. University of Louisiana at Monroe, MONROE, LA; North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA; and Ramboll Environ US Corporation, MONROE, LA.

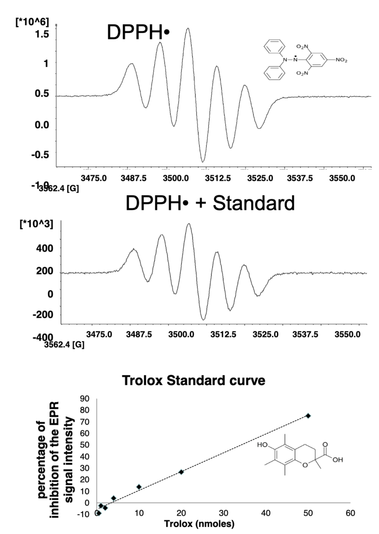
ABSTRACT

A continuing challenge of natural products research is to identify constituents that express a given bioactivity, *in situ*, in source material. An equivalent type of bioactivity may be intrinsic for multiple constituents of source material, but combined activity *in situ* may be altered by interactions. Thus, an analytical approach that distinguishes constituent activity *in situ* versus that in isolation is required for further understanding. Many studies have shown that *Echinacea* contains well-characterized phenolic compounds that possess strong antioxidant properties. We determined specific area under the curve of separate peaks from RP-HPLC chromatograms of extracts from aerial parts of 10 different USDA characterized *Echinacea* accessions and one commercial grown. We used electron paramagnetic resonance (EPR) to quantify specific activity of different polyphenols: gallic acid, cichoric acid, caftaric acid, echinacoside, cynarin, chlorogenic acid, and quercetin using reagent grade standards. RP-HPLC standard curves for the reagent grade standards were generated in parallel. Specific activity of each individual standard was quantified then compared to the quenching activity of the 11 accessions relevant to the amount of dry chemical present in the plant. Partial least squares regression (PLS) was performed using EPR quenching data against area under the curve from RP-HPLC chromatograms of extracts from aerial parts of the 11 different *Echinacea* accessions. This modeling enabled identification of peaks in the chromatograms and directed us to certain constituents that contribute best to the quenching activity present among different species of *Echinacea in situ*. Further studies will explore factors of the mixtures that alter constituent specific activities.

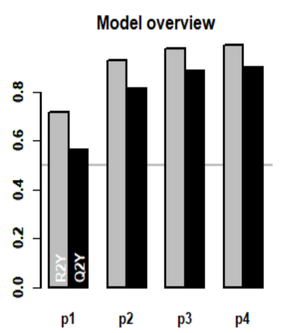
USDA PLANTS



EPR Spectra



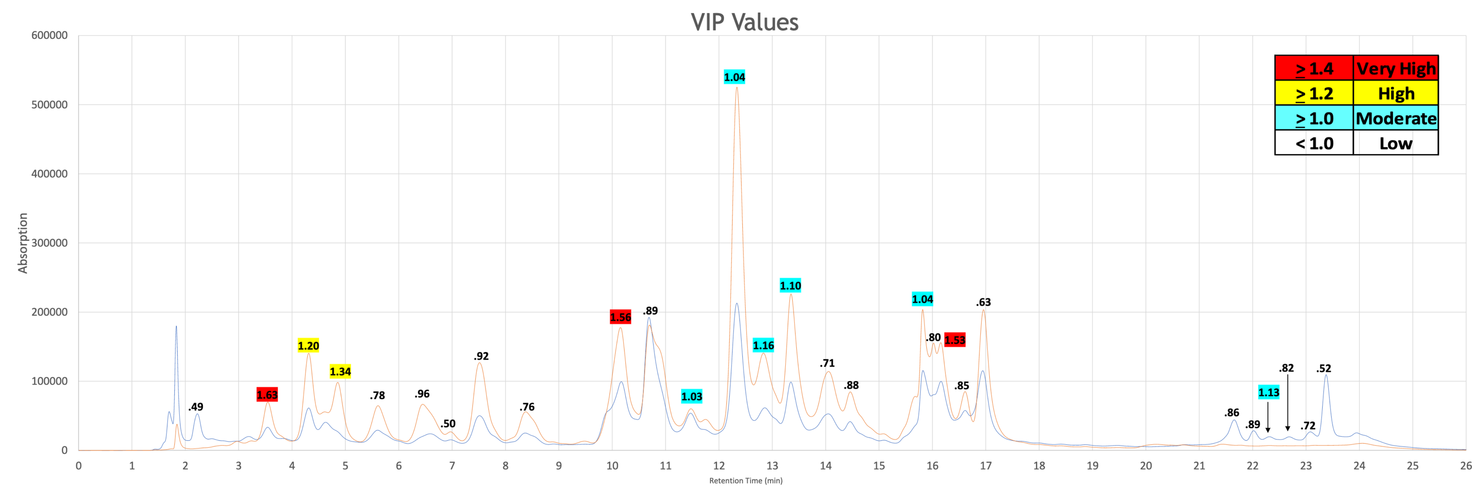
PLS Model

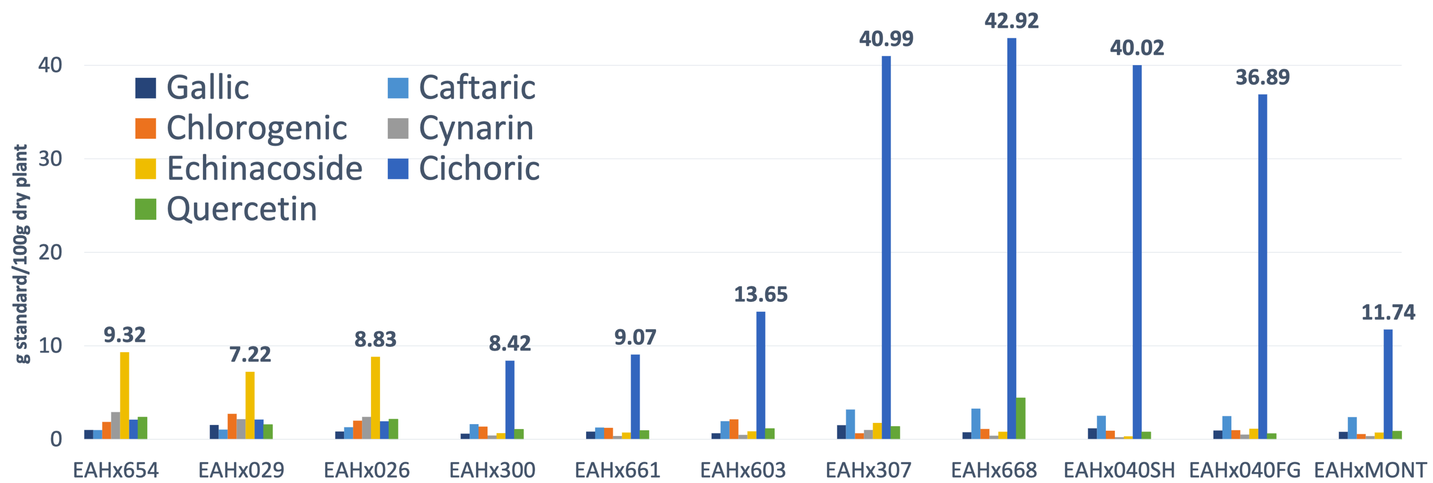


Model fits with 4 principle components

R2Y explains 99% of the variation in Y(EPR TEAC) and Q2Y explains 83% in X(HPLC AUC)

Qualitative and Quantitative Analysis



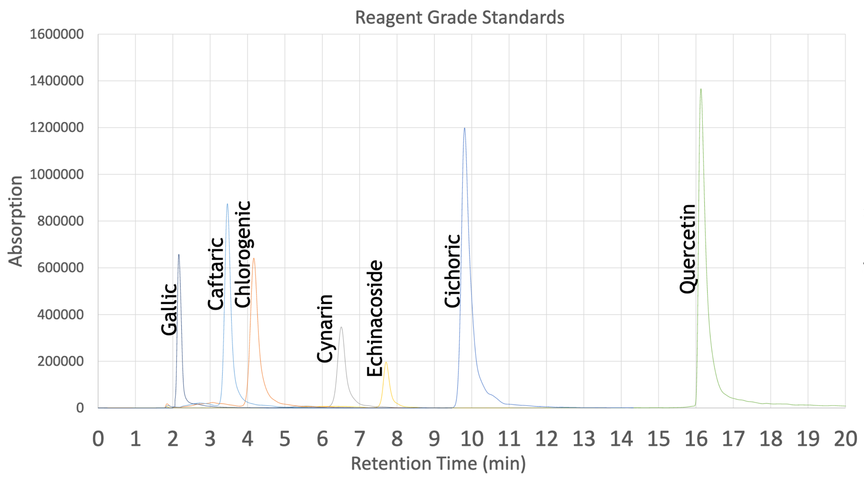


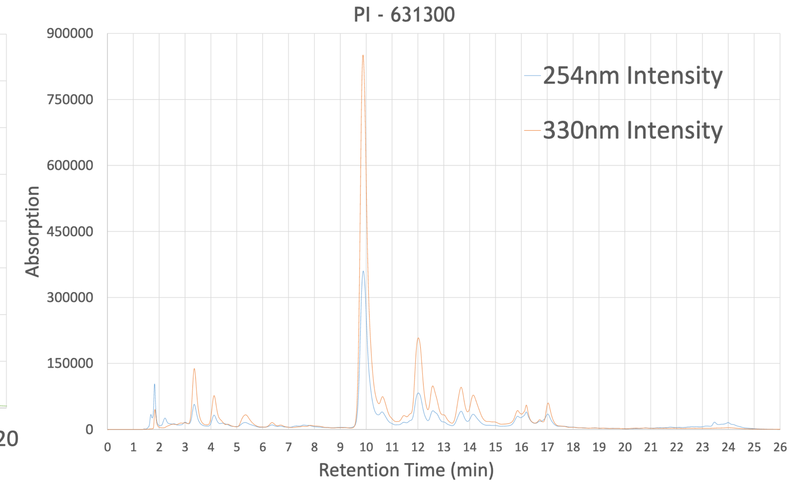
EAHx is a Hexane washed/Ethyl Acetate extracted sample of the 11 accessions developed in our lab by Chandra Mohan Reddy Muthumula

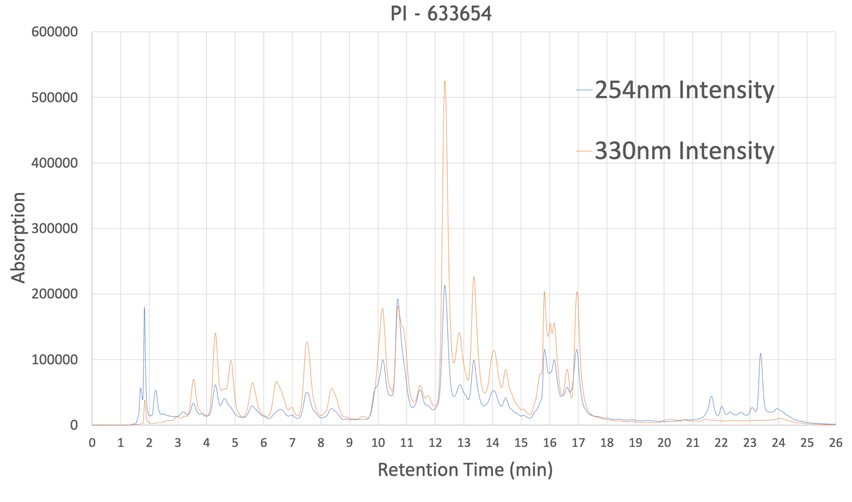
Objectives

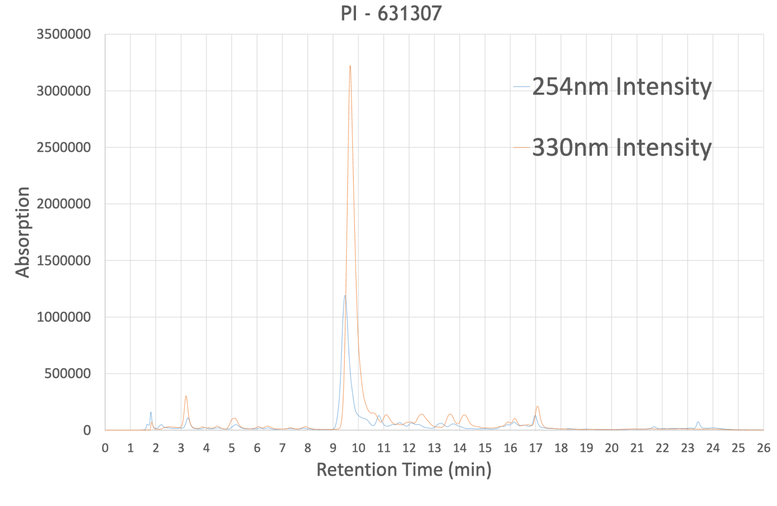
1. Quantify phenolic constituents of three separate *Echinacea* species that possess antioxidant activity.
2. Quantify the anti-oxidant activity of the identified *Echinacea* constituents *in situ* and compare with individual standards.
3. Observe if constituents in *Echinacea spp*. have synergistic or masking effects towards free radical quenching activity *in situ*.

RP-HPLC





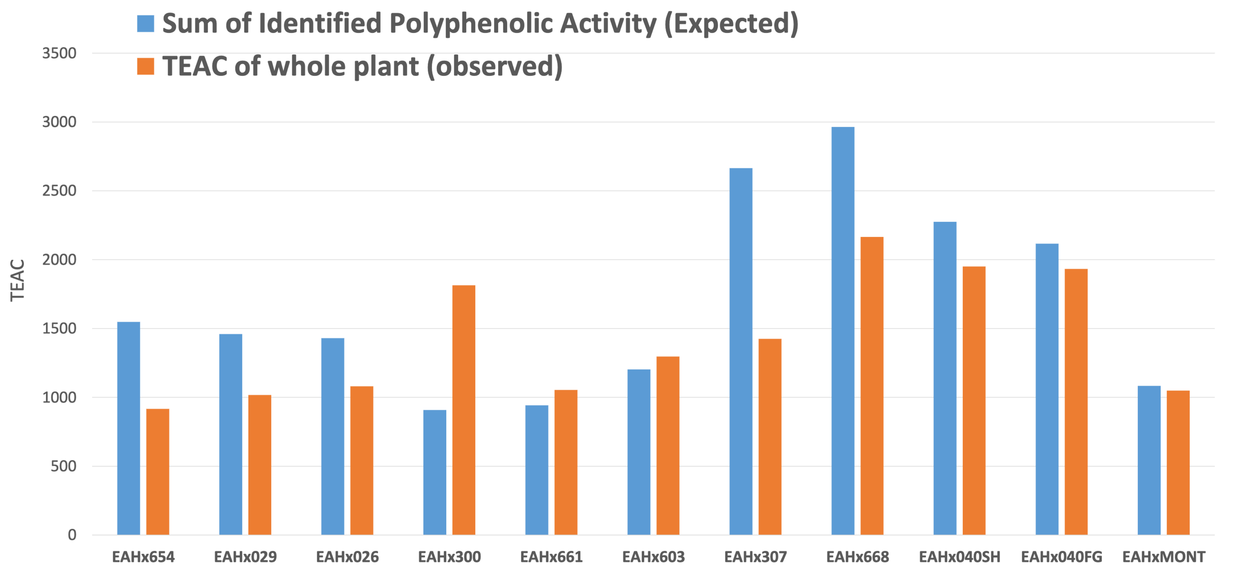




Quenching Free Radical Activity

Standards	Avg TEAC (nmoles)/mg Standard
Caftaric	7164.11
Chlorogenic	3958.87
Cichoric	3901.58
Echinacoside	5152.27
Cynarin	5457.31
Gallic acid	29139.72
Quercetin	16453.93

Expected Vs. Observed TEAC



Conclusions

1. VIPs generated by PLS model suggest peaks identified as polyphenols are drivers of free radical quenching *in situ*.
2. For 8 of the 11 accessions the expected sum of the measured quenching activity (TEAC) of the identified standards was greater than the TEAC of the whole plant.
3. This suggests that some constituents in *Echinacea spp*. possess inactivity or masking effects towards phenolic free radical quenchers *in situ*.