USE OF CHEMICAL MARKERS FOR IDENTIFICATION OF SPECIES IN THE GENUS *ECHINACEA*

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ABSTRACT

Highly-priced seed and root of two commercially important species of echinacea, *Echinacea angustifolia* and *Echinacea pallida,* are very difficult to distinguish macro- and micro-scopically. In support of high standards of quality and in effort to clear the confusion on the medicinal plant market, we have developed a chemical fingerprint test for authentication of echinacea seed using chromatographic techniques (TLC and HPLC). This test is able to reveal whether seed is of true species, or a cross between the species. Reference will also be made to authentication of *Echinacea* species by chemical fingerprinting of lipophylic root extract.

Over last three years echinacea was the best selling medicinal plant and herbal immunostimulant in North America. In response to a worldwide demand for echinacea as a cold and flue remedy and unspecific enhancer of the body's defense mechanism, there has been an increased interest in cultivation of this plant across Canada. In Saskatchewan at present, is has been estimated that there is about 100 acres under echinacea, predominantly ***Echinaceaangustifolia.***

The genus ***Echinucea*** is endemic to the Great Plains between the Appalachian Mountains in the east and the Rocky Mountains in the west of United States. The northern tip of its natural habitat is southern Saskatchewan, where wild stands of *E. **angustifolia*** can be found. The first comprehensive study on the taxonomy of the genus ***Echinucea*** was reported by Dr. McGregor in 1968. The species known up to date are reported in Table 1. At least three species are used medicinally and are in large scale cultivation around the world: *E. angustifolia, E. purpurea, and E. pallida.*

It is interesting to know that in North America *E. angustifolia* is by far the most popular species (particularly root), while in Germany, *E. purpurea* (expressed juice of aerial parts) and *E. pallida* (root) are in highest demand. Considering likely differences in pharmacological activity of various *Echinacea* species and the various plant parts within the species, as well as the differences in economics of production of various

species [(for example seed cost of *E. angustifoIia* $1,4OO/kg, *E. pallida* $390/kg, *E. purpurea $11* O/kg) and sale price of dry root *(E. angustifolia* $28O/kg, *E. pallida* $155/kg)], it became very important to be able to authenticate the species in a practical and objective way. Of particular importance to developing Saskatchewan as a reliable place for production of high quality echinacea, is authentication of propagative material, namely seed, prior to large scale planting.

Table 1. Known Species in the Genus *Echinacea*

Echinacea angustifolia DC. var. *angustifolia*
Echinacea angustifolia DC. var. *strigosa McGregor*
Echinacea atrorubens NUTT.
Echinacea laevigata BLAKE
Echinacea *pallida* NUTT .
Echinacea paradoxa BRITTON var. *paradoxa*
Echinacea paradoxa BRITTON var. *neglecta* McGREGOR
Echinacea ***purpurea*** MOENCH
Echinacea simulata McGREGOR
Echinacea sanguinea NUTT .
Echinacea tennesseensis SMALL

The different species in the genus *Echinacea* can be distinguished phytochemically by their typical constituents: moderately polar phenolic compounds such as caffeic acid derivatives (e.g. echinacoside, cynarin, chicoric acid), and lipophilic compounds such as isobutylamides and polyacetylenes. Recent studies on polar compounds, polysaccharides and glycoproteins, more readily extractable in aqueous solutions, have suggested potential application of these compounds as chemotaxonomical markers.

Table 2. Typically Used Chemical Markers for *Echinacea* Species Authentication

Fraction	*E. purpurea*	*E. angustifolia*	*E. pallida*
Lipophilic	Isobutylamides	Isobutylamides	Polyacetylenes
Hydrophilic	Chicoric acid	Echinacoside Cynarin	Echinacoside

Our studies have suggested that the above mentioned lipophilic and hydrophilic markers could also be used for authentication of at least two more *Echinacea* species: *E. paradoxa*

and *E. simulata*. The chemical structures of three typical phenolic markers in the genus *Echinacae are* shown in Figure 1.

The "fingerprint" chromatograms of phenolic derivatives and alkylamides, present in the chloroform extracts of the root of three *Echinacea* species of commerce: *E. angustifolia, E. pallida* and *E. purpurea* are shown in Figure 2 and Figure 3, respectively. In our experience, "fingerprint test" of the lipophilic extract is reliable and often necessary tool for positive identification of *E. angustifolia* and *E. pallida* The "fingerprint test" will also reveal whether the plant is a true species, or some form of a hybrid, which results from voluntary cross-pollination between the two or more *Echinacea* species grown in proximity. These chromatographic profiles also provide sufficient information to reach a reasonable conclusion about the phytochemical quality of crude drugs and could be used for comparative assessment of echinacea samples.

Seed of *E. angustifolia* and *E, pallida* are difficult to tell apart based on appearance, colour, size and average weight/density, even for an experienced eye. Also, microscopic observations of cross sections of seeds of these two species could not provide reliable species identification. Therefore, seed of *E. angustifolia* and *E. pallida* are for all practical purposes indistinguishable, which often creates a problem in the industry.
Seed companies sometimes sell seed that has been collected in native wild stands where natural hybridization between the species is likely to occur. Also, novice growers, often ignorant about the cross-pollination possibilities between *Echinacea* species, sell seeds collected from various *Echinacea* species grown in close proximity. Considering all the above and high price of the seed, buying echinacea seed and particularly seed of *E. angustifolia, involves* significant risk and is of paramount importance for the profitability of echinacea production.
Recognizing the potential benefit of seed species verification for the herb industry, we were able to establish a chromatographic test (TLC/HPLC) by which a unique marker compound for *E. pallida, E. purpurea, E. paradoxa and E. simulata seed* could be detected. This compound was not, however, present only in *E. angustifolia* By the absence of this compound, whose complete structure elucidation is in progress, we are able with high certainty to authenticate the *E. angustifolia seed.*

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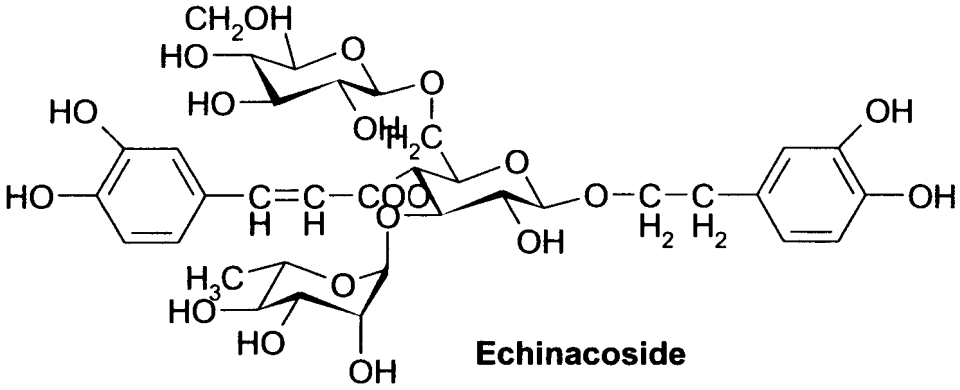
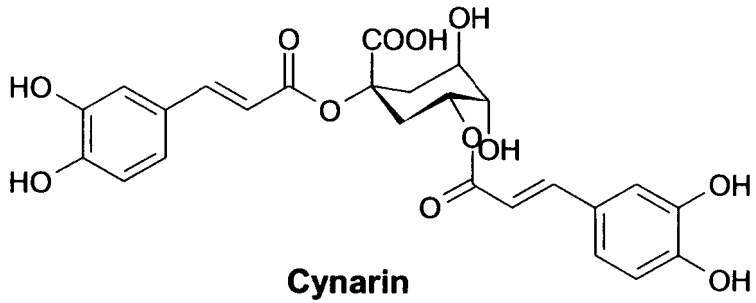
Figure 1. Phenolic Markers in Echinacea Species



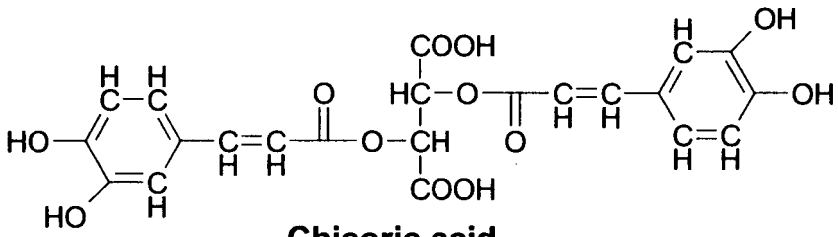
Echinacoside

Echinacea angustifolia
Echinacea pallida



Cynarin

Echinacea angustifolia



Chicoric acid

Echinacea purpurea

Figure 2. HPLC Fingerprints of Hydrophilic Extracts of Echinacea Root

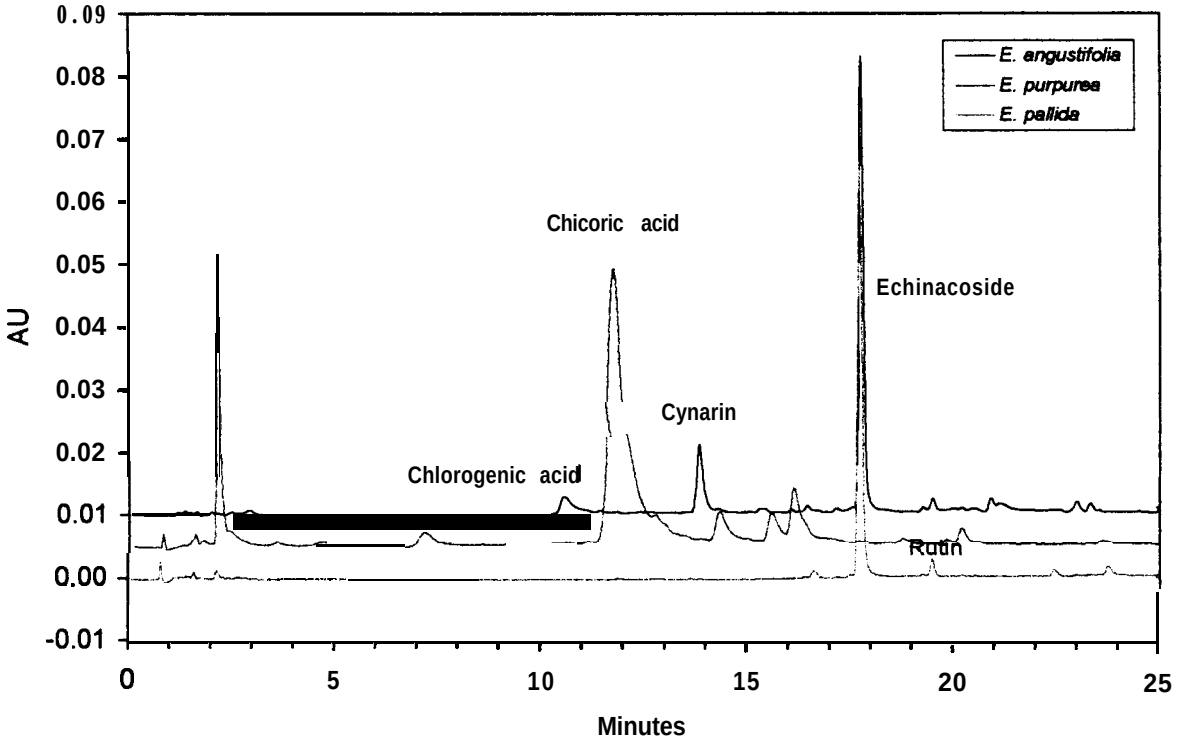


Figure 3. HPLC Fingerprints of Lipophilic Extracts of Echinacea Root