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Profiling of reference and commercial *Echinacea* extracts via liquid and gas chromatography, *in vivo* and planar assays

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INTRODUCTION & AIM

Numerous *Echinacea* extracts are available on the market, all promoted for the same application, *i.e.* enhancing immune system function, but exhibiting different standardization profiles. This study aims to evaluate various techniques for the authentication and standardization of *Echinacea* reference materials and commercial samples. Additionally, a functional evaluation was conducted to confirm proper standardization.

METHOD

Echinacea raw materials (*Echinacea purpurea* L. leaves and roots, *n* = 13) were used as reference samples, along with their corresponding water extracts, and commercial *Echinacea purpurea* extracts (from whole herb, aerial parts and 100% leaves-ADM®, *n* = 15). These samples were analyzed using HPLC-DAD/MS [**1**], HPTLC-EDA-UV/Vis/FLD [**2**], and GC-FID/MS [**3**]. Additionally, the *Caenorhabditis elegans* "*in vivo*" model was used to evaluate antioxidant and antimicrobial activities [**4**].

RESULTS & DISCUSSION

The chromatographic analysis of *Echinacea purpurea* leaf and root samples (**Figures 1, 2**) was carried out by comparing them with their respective aqueous extracts, showing the same chromatographic profile for all samples at 330 nm. The major components identified were chicoric acid and caffeic acid derivatives, whose concentrations varied depending on the batch (**Table 1**).

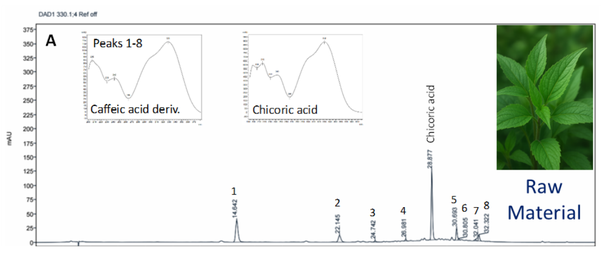

Figure 1. HPLC chromatogram of *Echinacea* leaves

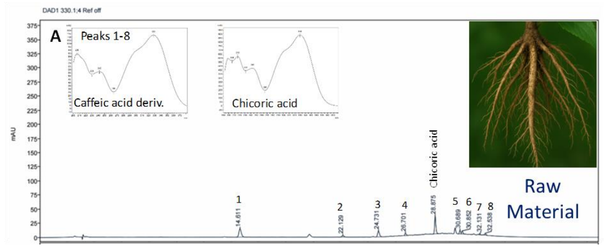

Figure 2. HPLC chromatogram of *Echinacea* roots

Different commercial extracts (N=15) from the whole herb, aerial parts or 100% leaves (ADM®) of *Echinacea purpurea* L exhibited similar chromatographic profiles. Nevertheless, a wide content range in chicoric acid and caffeic acids was observed (**Table 1)**.

Table 1. Phenolics (%, dry basis) in commercial extracts of *E. purpurea* (whole herb, aerial parts, or leaves) by means of HPLC.

Commercial samples	Caffeic acids	Chicoric acid
Whole Herb (roots, leaves, stems, flowers)		
PH1	1.47 ± 0.13	2.23 ± 0.02
PH3	2.55 ± 0.11	3.80 ± 0.01
PH4	0.69 ± 0.04	0.47 ± 0.01
PH5	1.78 ± 0.06	1.94 ± 0.01
Aerial Parts (leaves, stems,flowers)		
PH2	0.71 ± 0.00	1.40 ± 0.01
PH6	0.03 ± 0.01	0.24 ± 0.03
PH7	0.05 ± 0.01	0.25 ± 0.02
PH8	0.02 ± 0.02	0.21 ± 0.01
PH9	0.03 ± 0.02	0.25 ± 0.00
PH10	1.79 ± 0.03	0.31 ± 0.00
PH11	0.58 ± 0.13	1.01 ± 0.26
PH12	2.18 ± 0.09	5.59 ± 0.11
PH13	1.24 ± 0.00	2.36 ± 0.12
PH14	1.30 ± 0.04	3.34 ± 0.10
100% Leaves		
PH15	6.35 ± 0.81	4.17 ± 0.20

The *Echinacea* water extracts obtained at laboratory scale showed an average increase of 34% and 65% respectively for caffeic acid derivatives and chicoric acid (**Figures 3/4**).

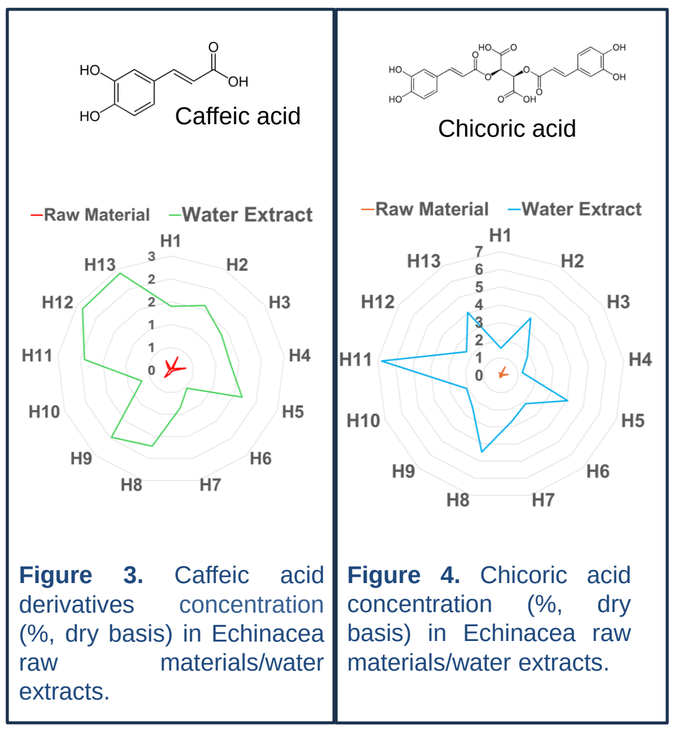

Figure 3. Caffeic acid derivatives concentration (%, dry basis) in Echinacea raw materials/water extracts.

Figure 4. Chicoric acid concentration (%, dry basis) in Echinacea raw materials/water extracts.

The volatile profile of the commercial extracts showed in their composition characteristic major volatile components depending on the origin of the extract (whole herb, aerial parts), already described [5]. However, different undesired components were identified in their composition, chiefly furfural, acetic acid, together with organic solvents (ethanol, ethyl acetate), which in most of the cases were not declared in the corresponding technical documentation (**Figure 5**).

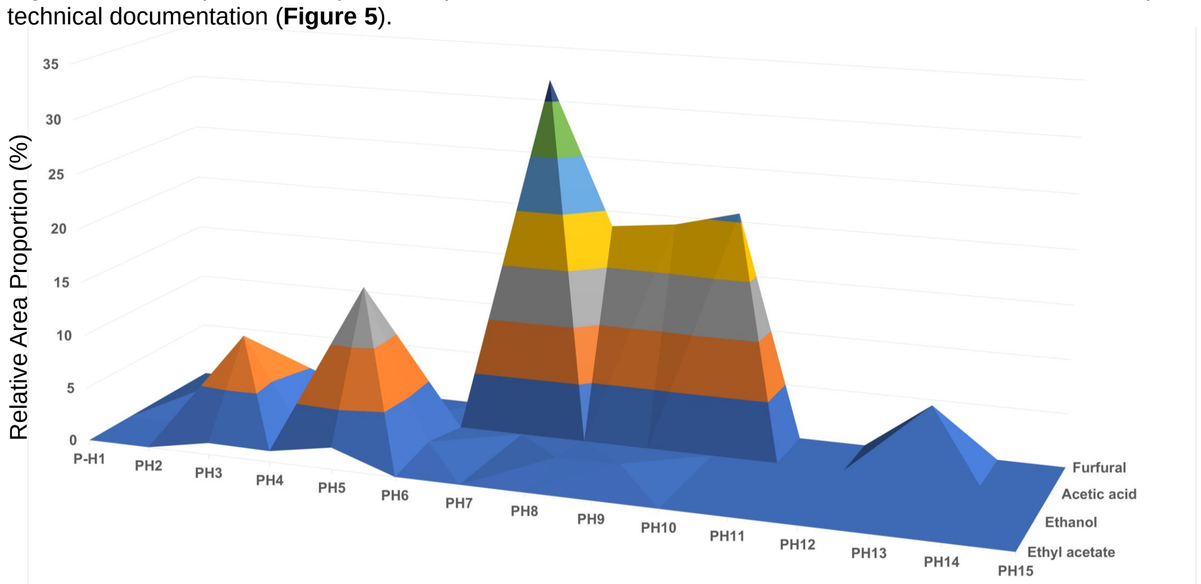

Figure 5. Gas chromatography profile of undesired volatiles (relative area percentage, %) in commercial *Echinacea* extracts from whole herb and aerial parts.

The analysis by HPTLC–FLD and non-target effect-directed HPTLC–EDA–Vis of *Echinacea* plant parts *versus* extracts revealed various components in quite differing amounts, and with antioxidant capacity as depicted below (**Figures 6, 7**).

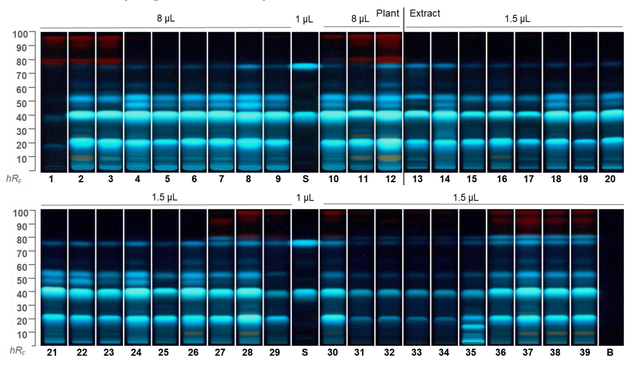

Figure 6. HPTLC–FLD 366 nm, PEG derivatization

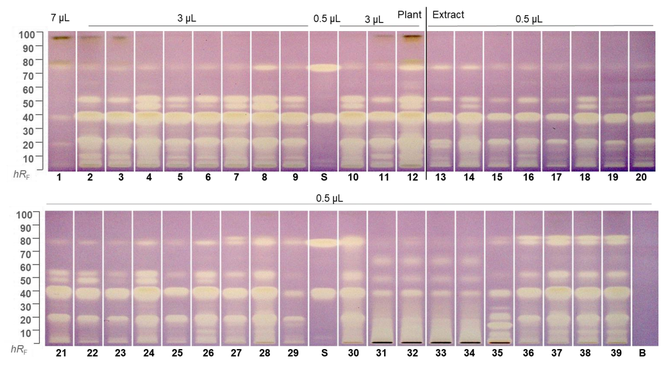

Figure 7. HPTLC–DPPH-Vis → antioxidants

The antioxidant activity of Echinacea leaves extract was evaluated in *C. elegans*. The nematode viability after an acute oxidative stress (2 mM H_2O_2) in the presence of different doses of Echinacea leaves extract was performed according to Martorell *et al*. 2011 (4). **Figure 8** indicates that worms' viability was significantly increased in the presence of the extract comparing with standard media (Nematode Growth Medium, NGM). Dose of 0.1 mg/mL exhibited the highest survival effect (47.3% versus 28.7% in control fed population).

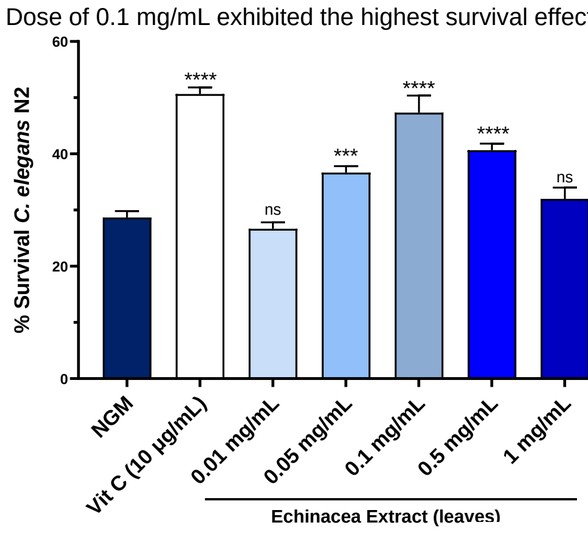

Figure 8. Antioxidant effect of Echinacea leaves extract (PH15). Percentage of survival of *C. elegans* N2 worms after the acute oxidative stress (H_2O_2). N2 strain was synchronized in *E. coli* OP50 NGM plates (standard media) and NGM supplemented with Echinacea extract (0.01 to 1 mg/mL). Viability was scored at day 5 of adult worms. Vitamin C (Sigma-Aldrich,St. Louis, MO) was used as a positive control. One-way ANOVA was applied. . **** *p-value*<0.0001; *** *p-value*<0.001; ns: not significant. Data are the average of three independent experiments. n=150 worms.

The ability of Echinacea leaves extract to provide protection to the host against pathogens was tested. Nematodes were cultured in NGM plates containing Echinacea extract (0.1, 0.5 and 1 mg/mL) and the pathogen *Staphylococcus aureus* (ATCC25923) or *Salmonella thyphimurium* (ATCC 14028). Experiments were performed according to Martorell *et al*. 2021 [6]. **Figure 9** indicates that Echinacea leaves extract exerts protection against bacterial pathogens infections (*S. aureus* and *Salmonella*) in *C. elegans.* Nematodes fed with Echinacea showed a significant higher survival in comparison to control infected population (*p-value* < 0.0001), being dose of 1 mg/mL the most effective in both pathogens.

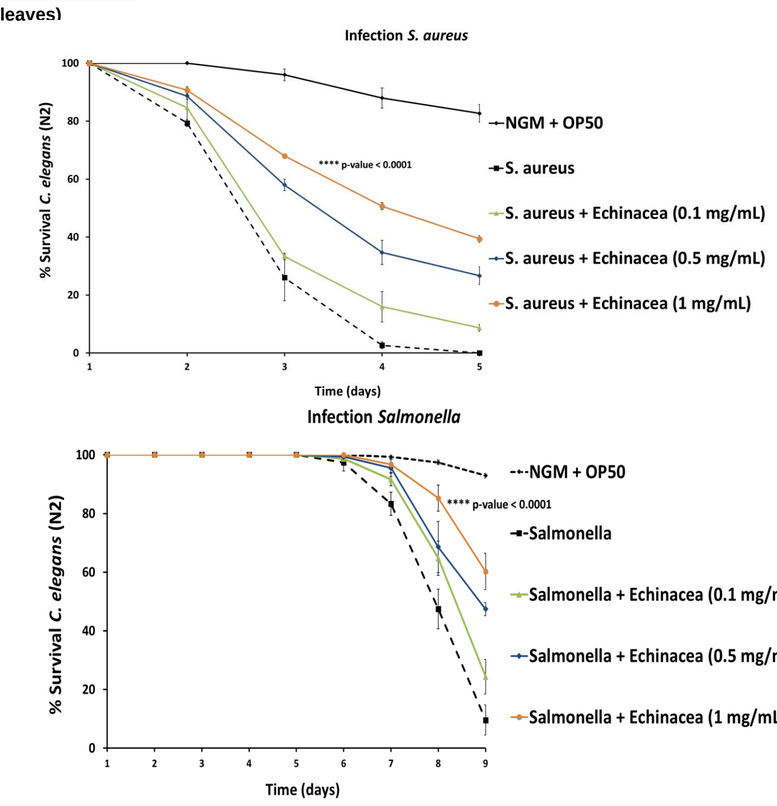

Figure 9. Protective effect of Echinacea leaves extract against pathogen infection. Percentage of survival of *C. elegans* N2 infected with bacterial pathogens *S. aureus* (ATCC25923) or *S. thyphimurium* (ATCC 14028) and fed with Echinacea leaves extract at doses of 0.1, 0.5 and 1 mg/mL. A condition without pathogen (NGM+OP50) was included. Log Rank T-test was applied. **** *p-value* < 0.0001. Data are the average of three independent experiments. n=150 worms.

CONCLUSION

Liquid and gas chromatography techniques are fundamental for evaluating quality of commercial Echinacea purpurea extracts. The standardized commercial Echinacea water extract from ADM®, without undesired volatiles, and exclusively obtained from 100% leaves demonstrates antioxidant activity and protective effects against pathogenic infections in *Caenorhabditis elegans* models.

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