Echinacea tennesseensis ethanol tinctures harbor cytokine- and proliferation-enhancing capacities

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Abstract

Background—Members of the genus *Echinacea* are used medicinally to treat upper respiratory infections such as colds and influenza. The aim of the present investigation was to characterize the phytomedicinal properties of the American federally endangered species *Echinacea tennesseensis*.

Methods—Fifty-percent ethanol tinctures were prepared from roots, stems, leaves, and flowers and tested separately for their ability to influence production of IL-1β, IL-2, IL-10, and TNF-α as well as proliferation by young human adult peripheral blood mononuclear cells (PMBC) *in vitro*. Tincture aliquots were stored at three different temperatures (4°, −20°C, and −80°C) for 21 h before testing. At one-month post-extraction, tinctures stored at −20°C were tested again for cytokine modulation. Phytochemical analyses were performed using HPLC.

Results—Fresh root, leaf, and flower tinctures stimulated PBMC proliferation. Fresh root tinctures alone stimulated IL-1β, IL-10, and TNF-α production. No tinctures modulated IL-2 production. Stem tinctures showed no activity. Storage temperature did not influence any outcomes. Root tinctures maintained their ability to modulate IL-1β, IL-10, and TNF-α production after one month of storage at −20°C.

Conclusions—These results suggest *E. tennesseensis* harbors phytomedicinal properties that vary by plant organ, with roots demonstrating the strongest activities.

Keywords

Echinacea tennessensis; tincture; cytokine

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1. Introduction

Alternative medical therapies, including herbal preparations such as those from *Echinacea* spp., are gaining use in the United States [1,2]. The genus *Echinacea* is used most frequently for preventing or treating upper respiratory infections such as colds and influenza [3,4]; however, while some studies have supported its use in this capacity [5–8], others have suggested it may be ineffective [9–12]. Since *Echinacea angustifolia*, *E. pallida*, and *E. purpurea* are used commercially, these three species have received the overwhelming majority of scientific attention and much data exists regarding their genetics, phytochemistry, immunomodulatory activity, and efficacy in clinical settings [13]. By comparison, very little is known about the American federally endangered species *E. tennesseensis*.

Several classes of purported medicinal compounds have been identified from this genus, but current data suggest that no single molecule or class of molecules is responsible for *Echinacea*'s activities [13]. Known bioactive molecules include alkamides, caffeic acid derivatives, and polysaccharides. Present bioavailability studies suggest that alkamides most likely account for the majority of observed immunomodulatory effects [14,15].

Much of the published work has focused on the phytochemistry of *Echinacea tennesseensis*. Three diacetylenic isobutylamides (polyacetylenes) have been identified from *E. tennesseensis* seedlings; further, in a cross-genus comparison it was shown that *E. tennesseensis* harbors higher levels of one of these compounds (amide 5) as compared to other members of the genus [16]. In contrast, other bioactive molecules such as phenolic compounds (including caffeic acid derivatives) were found to be lower in *E. tennesseensis* root extracts than in extracts from other sister species [17]. Levels of alkenes, amides, and caffeic acid derivatives from *E. tennesseensis* root extracts in comparison to sister species have been reported [18]. In particular, this species harbors higher levels of several monoene and tetraene amides (phytochemicals with demonstrated antiviral activity [18]), but perhaps lower levels of caffeic acids and their derivatives, compared to other *Echinacea* species.

Previously, our lab reported that *E. tennesseensis* root extracts may harbor immunomodulatory properties, based on multiple studies from two different extracts sets. For the first extract set, we used freshly-harvested late autumn root material which was diced and then extracted at a ratio of 1:9 parts plant:solvent for 1 h in either 50% ethanol/50% sterile water (tincture), or hot or cold sterile water (infusions) [19]. We found that the ethanol tincture from this species had the capacity to enhance TNF-α production while suppressing IL-12 production by human peripheral blood mononuclear cells (PBMCs) cultured *in vitro* [19]. We also showed that the ethanol tinctures were more active than cold or hot water infusions made from the same plant stock [19]. In a follow-up investigation, identical aliquots that had been stored undisturbed at −20°C for 2 years were tested in the same assays [20]. This particular storage regimen was found to alter the immunomodulatory properties of *E. tennesseensis* extract such that it no longer had any impact on TNF-αor IL-12 production, yet instead acquired the ability to stimulate IL-10 production and PBMC proliferation [20]. When the frozen extracts were co-cultured with influenza virus using PBMCs isolated from individuals vaccinated against those same virus strains, extracts from *E. tennesseensis* did not modulate IL-2, IL-10, or IFN-γ [20]. For the second extract set, we used late autumn root material which was dried in a climate-controlled facility for 16 months prior to being diced and then extracted at the same ratio but for only 20 minutes [21]. In the context of influenza virus-stimulated PBMCs from older individuals vaccinated against influenza, we showed that *E. tennesseensis* root tinctures augmented IL-10 production, diminished IL-2 production, and had no effect on IFN-γ production [21]. When the same extract was tested alone using PBMCs from unvaccinated individuals, it was able to augment IL-1β and TNF-α, but not IL-2, production [22]. Only root

extracts were used in these studies, so at present, the immunomodulatory properties of extracts from other *E. tennesseensis* tissues are unclear.

The aim of the present work was to more fully characterize the immunomodulatory properties of *E. tennesseensis*. To achieve this aim, we prepared ethanol tinctures from roots, stems, leaves, and flowers separately and tested their abilities to modulate PBMC production of IL-1β, IL-2, IL-10, and TNF-α, as well as PBMC proliferation. We also investigated whether storage temperature conditions influenced outcomes.

2. Methods

2.1 Plant selection and extraction

A lone individual of *Echinacea tennesseensis* (PI631250) was harvested from a field population at the North Central Regional Plant Introduction Station in Ames, Iowa on June 21, 2005. The plant was processed into extracts less than 3 h after harvesting.

The plant was rinsed with Nanopure water. Roots, stems, leaves, and flowering tops were separately minced with a surgical scalpel and dissolved in 50% ethanol and 50% sterile water at a ratio of 1 part plant, 4 parts solvent to generate ethanol tinctures. Preparations steeped at room temperature for 1 h on a horizontal agitator before being filtered through sterilized tulle. Tinctures were fractionated into three aliquots for storage at 4°C, −20°C, and −80°C. To mimic the freeze/thaw cycles that laboratory extracts typically experience as a result of repeated handling for separate experiments, all aliquots were removed and brought to room temperature every 7 h for 21 h (total 3 cycles). Following initial testing of the fresh extracts, the −20°C aliquots were saved and tested again 1 month later. We chose the aliquots stored at this temperature because this is the typical storage temperature used in many laboratories. Extracts will be referred to as "Day 1" or "Day 31" in this report.

2.2 Phytochemical profiling

Phytochemical analysis was performed to detect alkamides and caffeic acid derivatives in the *Echinacea* extracts with the use of high performance liquid chromatography (HPLC). We have previously published a supplement showing the full names and chemical structures of these compounds (PDF freely downloadable from http://www.thieme-connect.com/bilder/planta/200613/supmat/324sup_10-1055-s-2006-947254.pdf) except for amides 16 and 17 which are undeca-2*Z*-ene-8,10-diynoic acid 2-methylbutylamide and dodeca-2*E*-ene-8,10-diynoic acid 2-methylbutylamide, respectively [23]. Before analysis, into 160 μl of *Echinacea* extracts, 15 μl (1 mg ml^{-1}) N-isobutylundeca-2-ene-8,10-diynamide ($C_{15}H_{21}O_2$) and 15 μl (1 mg ml^{-1}) 3,5-dimethoxy-4-hydroxy-cinnamic acid ($C_{11}H_{12}O_5$) were added as internal standards for quantification of lipophilic metabolites and hydrophilic metabolites, respectively. Fifteen microliters of each sample were injected into a Beckman Coulter HPLC with a 508 autosampler, 126 pump control and 168 UV-photodiode array detector controlled by 32karat^{TM} software (Version 5.0), and a YMC-Pack ODS-AM RP C18 (250 × 4.6 mm, 5 μm) analytical column (Waters, MA). The solvent system for lipophilic constituents was acetonitrile/H_2O at a flow rate of 1.0 ml/min following a linear gradient of 40–80% acetonitrile over 45 min. The solvent system for hydrophilic constituents consisted of acetonitrile/H_2O and 0.01% formic acid, at a flow rate of 1.0 ml/min following a linear gradient of 10–35% acetonitrile over 25 min. Online UV spectra were collected between 190–400 nm.

For compound identification, alkamides 8/9, cichoric acid, echinacoside, caftaric acid were purchased from Phytolab, Germany; chlorogenic acid was purchased from Sigma Aldrich, USA; alkamides 8, 10, 11, 12, 13, and 14 were synthesized by Dr. George Kraus, Department

of Chemistry, Iowa State University [16,24]. In the absence of standards, alkamides 1, 16, and 17 were identified by HPLC fractionation coupled with GC-MS analysis. Phytochemicals were quantified based on the internal standard with the limit of HPLC detection at approximately 0.02 μg/ml.

2.3 Human subjects and cell culture

All procedures involving human subjects were approved by the Institutional Review Board of Iowa State University. Sixteen subjects between the ages of 19–36 donated blood for the testing of the fresh extracts. Four of these same subjects donated blood for the testing of the one-month-old extracts. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque plus (Amersham Pharmacopia Biotech, Piscataway, NJ) gradient centrifugation. Cell counts were performed with a hemacytometer using Trypan Blue to assess viability; all cell suspensions were adjusted to 1.0×10^6 cells/mL in AIM-V media (GIBCO/Invitrogen, Carlsbad, CA).

For the cytokine assays, one milliliter of AIM-V media containing cells (1.0×10^6 cells) was plated per well in 24-well Costar tissue culture plates. Fifty microliters of one of the *Echinacea* preparations diluted 1:12.5 was added per treatment well; the control well received 50 microliters of AIM-V media. This extract concentration was determined from preliminary studies of human PBMC ethanol tolerability (data not shown). Cell cultures were incubated for 24 h at 37°C, 5.0% carbon dioxide in a humidified atmosphere. Supernatants were harvested and stored at −20°C until used in cytokine quantification assays for IL-1β, IL-2, IL-10, and TNF-α via ELISA (BD Biosciences Pharmingen, San Diego, CA). Due to experimental complications during testing of the fresh extracts, our sample size for IL-2 was n=14 and for IL-10 n=8.

For the proliferation assay, one hundred microliters of AIM-V media containing 1.0×10^6 cells were plated per well in 96-well flat-bottom Costar plates. Cells were stimulated with 5 microliters of *Echinacea* preparations diluted as above or AIM-V media (control). All trials were conducted in triplicate. Cells were incubated for 5 d at 37° C, 5.0% carbon dioxide in a humidified atmosphere and proliferation assessed via CellTiter (Promega, Madison, WI) per manufacturer's instructions and read in a plate reader (Bio-Rad, Hercules, CA).

All glassware used in the extract preparation was baked at 180°C for 16 h prior to use to minimize endotoxin contamination. Sterile water was obtained from Hospira, Inc., Lake Forest, IL and used for all tinctures. Endotoxin levels were determined from both sterile water (0 EU/mL) and all stock tinctures using Bio-Whitaker QCL 1000 kits (Cambridge, MA).

A univariate ANOVA was used to test for significant differences in immune effects by plant organ, storage temperature, and plant organ × storage temperature interactions for each cytokine or measure of proliferation using SPSS 15.0 (Chicago, IL). "Plant organ" and "storage temperature" were chosen as between-subjects factors and each immune outcome was chosen as a dependent factor. Whenever significant effects were revealed, pairwise comparisons were conducted to examine effects of individual extracts. An α level of 0.05 was used in all instances as a threshold for statistical significance, whereas 0.10 was used as a threshold for statistical trends.

3. Results

Endotoxin levels from all stock extracts were determined and are as follows (in EU/mL): root (23.611), stem (1.252), leaf (1.464), flower (9.708). As described in Methods, the extracts were diluted before being added to the wells. Thus, final endotoxin concentrations (EU/mL) in the cell culture wells were: root (0.086), stem (0.005), leaf (0.005), flower (0.035). We have

demonstrated elsewhere experimentally that these endotoxin levels are not likely to contribute to immune outcomes in our assays [21]; therefore, we may gauge that the effects observed in our assays are due to the *Echinacea* extracts themselves and not any contaminating endotoxin.

The phytochemical analysis of our extracts for alkamides and caffeic acid derivatives is presented in Table 1. Although tested for, none of the caffeic acid derivatives (caffeic acid, chlorogenic acid, cichoric acid, echinacoside) were detected in any of the samples. Root contained all amides tested, whereas the other organs did not contain all of the amides. Root showed the greatest whereas leaf showed the least diversity of amides. Quantities of each amide differed by organ.

The abilities of the Day 1 extracts to stimulate cytokine production by peripheral blood mononuclear cells (PBMCs) versus media alone (control) are shown in Figures 1–4. None of the analyses produced significant main effects of storage temperature, nor any significant organ-by-temperature interactions.

Results for IL-1β are shown in Fig. 1. A significant main effect of plant organ was found ($p <$ 0.001). In pairwise comparisons, IL-1β production was found to be significantly enhanced by root tinctures as compared to both control and all other tinctures (all $p < 0.001$). Although not statistically significant, the root tincture stored at −80°C elicited approximately 1.3× as much IL-1β as either of the root tinctures stored at 4°C or −20°C (−80°C = 11.67 ± 28.8 vs. 4°C = 84.2 ± 21.9 and −20°C = 85.9 ± 29.6). Storage temperature heterogeneously impacted on the IL-1β-modulating abilities of extracts produced from other organs.

Results for IL-2 are shown in Fig. 2. A significant main effect of plant organ was found ($p =$ 0.012). Pairwise comparisons indicated that none of the individual extracts significantly altered IL-2 production as compared to control (all $p \geq 0.242$). However, flower tinctures as a group were significantly different from leaf ($p = 0.002$), root ($p = 0.007$), and stem ($p = 0.051$) tinctures. No pattern could be discerned regarding storage temperature effects: for leaf and flower extracts, colder storage temperatures resulted in extracts that stimulated greater IL-2 production, whereas for root extracts the pattern was opposite.

Results for IL-10 are shown in Fig. 3. A significant main effect of plant organ was found ($p <$ 0.001). In pairwise comparisons, IL-10 production was found to be significantly enhanced by root tinctures as compared to control ($p = 0.001$) and all other tinctures (all $p < 0.001$). Generally, with the exception of the root extracts, warmer storage temperatures yielded extracts that stimulated greater IL-10 production.

Results for TNF-α are shown in Fig. 4. A significant main effect of plant organ was found ($p < 0.001$). Post-hoc analyses indicated that root tinctures significantly augmented TNF-α production as compared to both control ($p < 0.001$) and all other tinctures (all $p < 0.001$). Additionally, the flower tinctures showed a trend ($p = 0.079$) towards stimulating more TNF-α production than the stem extract. Storage temperature heterogeneously impacted on the TNF-α-modulating abilities of the extracts.

To examine possible effects of storage time, we re-tested the extracts stored at −20°C following 1 month of storage (Day 31), using the same experimental conditions as at Day 1. Extracts were left undisturbed during the 1 month. Results from both Day 1 and Day 31 are displayed in Table 2 (the data for Day 1 is a numerical expression of the same data found graphically in Figs. 1–4, given again here for comparative purposes). For both IL-1β and TNF-αat Day 31, a significant main effect of organ (both $p \leq 0.019$) was found; post-hoc analyses indicated that root tinctures alone significantly enhanced cytokine production as compared to control (both $p < 0.005$) and all other tinctures (all $p \leq 0.007$). For IL-10 at Day 31 there was a trend towards a main effect of plant organ ($p=0.086$) such that the root tinctures significantly augmented

IL-10 production as compared to both control ($p = 0.02$) and all other tinctures (all $p \leq 0.039$). No significant main effects were found for IL-2 at Day 31. When Day 1 and Day 31 data were compared statistically, main effects of time (Day 1 vs. Day 31) were seen for IL-1β ($p = 0.021$) such that Day 31 extracts elicited greater IL-1β production than Day 1 extracts, and for IL-2 ($p < 0.001$) such that Day 31 extracts elicited less IL-2 production than Day 1 extracts. Main effects of time were not observed for TNF ($p = 0.703$) or IL-10 ($p = 0.160$) despite the differences seen in Table 2.

The abilities of the 4°C Day 1 extracts to stimulate PBMC proliferation versus media alone (control) are presented in Figure 5. A significant main effect of organ was found ($p < 0.001$). Follow-up analyses indicated that the flower, leaf, and root tinctures were able to significantly enhanced PBMC proliferation as compared to control ($p < 0.001$), whereas the stem tincture did not ($p = 0.237$).

4. Discussion

Results from this study suggest that tinctures prepared from freshly harvested *Echinacea tennesseensis* harbor phytomedicinal properties *in vitro*, but that these abilities vary by plant organ. Production of the cytokines IL-1β, IL-10, and TNF-α were significantly enhanced by *E. tennesseensis* root tinctures as compared to control, but not by flower, leaf, or stem tinctures at both Days 1 and 31 (Figs. 1, 3, 4; Table 2). None of the tinctures modulated IL-2 production at either time point (Fig. 2; Table 2). Considered together, these results suggest that *E. tennesseensis* does harbor cytokine-modulating properties, but that these properties are more pronounced in extracts generated from root tissues versus other organs.

Day 1 extracts stored at 4°C were also tested for their ability to modulate PBMC proliferation (Fig. 5). Tinctures made from flower, leaf, and root were able to significantly enhance proliferation (in contrast to what we observed with cytokine modulation), suggesting that all organs except for stem harbor the phytochemicals or phytochemical combination responsible for this property.

The immunomodulatory results presented here are congruent with findings published previously from our laboratory. Generalizing from studies reviewed in the Introduction [19–22], in *in vitro* studies using human PBMCs, *E. tennesseensis* root tincture has been reported to increase IL-1β, decrease or have no effect on IL-2, increase or have no effect on IL-10, increase or have no effect on TNF, and increase or have no effect on PBMC proliferation. Comparisons between these studies should be made with the understanding that the studies differed in extraction processes, laboratory culture procedures, participant characteristics, and sample sizes. The extracts being investigated in this study differ from preceding batches in 2 major ways: (1) use of a 1:4 parts plant:solvent extraction ratio, and (2) use of material harvested in the summertime. Despite these experimental design differences, the results from this paper confirm and extend those of previous reports by demonstrating that *E. tennesseensis* root tinctures, and root tinctures alone, produced via these methods are able to augment IL-1β, IL-10 and TNF-α (but not IL-2) production. Previously we found that *E. tennesseensis* root tinctures did not significantly enhance PBMC proliferation [19], whereas in this study we report that flower, leaf, and root tinctures harbor this ability. Differences in extraction procedure methods most likely explain these differences. This is the first study to examine immunomodulatory properties of stem, leaf, or flower tissues from *E. tennesseensis*.

Chemical analysis demonstrated that amide content differed between extracts made from various organs of *Echinacea tennesseensis* (Table 1). Our research team has previously reported a phytochemical analysis of *Echinacea tennesseensis* extracts generated during a prior study [21]. Phytochemical results between this study and the previous study were quite

different; as just one example, amides 1 and 8–11 were absent in our previous analysis, whereas chlorogenic acid and echinacoside were present. Three main factors likely explain these discrepancies, including the two enumerated in the preceding paragraph and also that, in the aforementioned study [21], the *E. tennesseensis* specimen was dried for 16 months prior to extraction, whereas in this study, the specimen was harvested and extracted within 3 h.

Interestingly, amides 14, 16, and 17 were present only in root extract, suggesting that these phytochemicals may be partially responsible for the ability of root extract to modulate IL-1β, IL-10 and TNF-α production. In contrast, amides 8, 9, and 12 were present in extracts from all organs, suggesting that these phytochemicals may be partially responsible for the ability of all extracts to modulate PBMC proliferation.

Our analyses indicated that there were no significant differences between the behaviors of tinctures aliquoted from the same stock batch but stored at different temperatures for 21 h before being tested for immunomodulatory properties. Previously, we investigated the effects of 4 days storage at 4°C on the immunomodulatory properties of root infusions and tinctures from several different *Echinacea* spp. to mimic the storage conditions that lay herbalists typically employ [19]. We discovered that extract abilities changed over time, but this was sometimes due to endotoxin effects. The results presented here suggest that laboratory investigators wishing to study *Echinacea* extracts as lay herbalists produce them might be able to store the extracts at freezing temperatures (i.e., −20°C, −80°C) for short-term purposes without significantly altering their active properties as may be observed if they were stored at 4°C.

Results from Figs. 1–4 and Table 2 showed that a month of storage did not significantly alter the cytokine-modulating properties of the tinctures tested in this investigation, at least in statistical comparisons of extracts versus relevant controls. These results suggest that storage of *Echinacea* tinctures for a short time period (one month) does not significantly change their immunomodulatory behaviors from a statistical standpoint. However, storage time appeared to increase the ability of the extracts to stimulate IL-10 production, and may have had heterogeneous effects the ability of the extracts to influence TNF production.

The genus *Echinacea* is comprised of nine traditionally recognized species [25]. Of these nine, only *E. angustifolia*, *E. pallida*, and *E. purpurea* are used to any appreciable extent in the herbal supplement industry. *Echinacea tennesseensis* exhibits immunomodulatory properties similar to some but not all other species of *Echinacea*. Other investigative teams as well as our own have shown that *E. angustifolia*, *E. pallida*, and *E. purpurea* differ in both activity and/or phytochemical composition [13,16–19,21,26–32]. In a previous phenetic analysis [22], we established that *Echinacea tennesseensis* appears to exhibit *in vitro* immunomodulatory characteristics more similar to *E. purpurea* than to *E. angustifolia* or *E. pallida*. Results presented in this paper are consistent with previous findings and provide further support for that analysis. The findings presented here, alongside previous antiviral [18], immune [19–22], and phytochemical [16–18] data, suggest that *E. tennesseensis* may be of use in modern herbalism.

Acknowledgments

Literature Cited

1. Gilroy CM, Steiner JF, Byers T, Shapiro H, Georgian W. *Echinacea* and truth in labeling. Archives of Internal Medicine 2003;163:699–704. [PubMed: 12639203]

2. Barnes PM, Powell-Griner E, McFann K, Nahin RL. Complementary and alternative medicine use among adults: United States, 2002. Adv Data 2004;343:1–19. [PubMed: 15188733]

3. Barrett BP. Medicinal properties of *Echinacea*: a critical review. Phytomedicine 2003;10:66–86. [PubMed: 12622467]

4. Caruso TJ, Gwaltney JM. Treatment of the common cold with *Echinacea*: a structured review. Clinical Infectious Diseases 2005;40:807–810. [PubMed: 15736012]

5. Goel V, Lovlin R, Chang C, Slama JV, Barton R, Gahler R, Bauer R, Goonewardene L, Basu TK. A proprietary extract from the echinacea plant (*Echinacea purpurea*) enhanced systemic immune response during a common cold. Phytotherapy Research 2005;19:689–694. [PubMed: 16177972]

6. Goel V, Lovlin R, Barton R, Lyon MR, Bauer R, Lee TD, Basu TK. Efficacy of a standardized echinacea preparation (Echinilin) for the treatment of the common cold: a randomized, double-blind, placebo-controlled trial. Journal of Clinical Pharmacology and Therapeutics 2004;29:75–83.

7. Schulten B, Bulitta M, Ballering-Bruhl B, Koster U, Schafer M. Efficacy of *Echinacea purpurea* in patients with a common cold. A placebo-controlled, randomised, double-blind clinical trial. Arzneimittelforschung 2001;51:563–568. [PubMed: 11505787]

8. Lindenmuth GF, Lindenmuth EB. The efficacy of *Echinacea* compound herbal tea preparation on the severity and duration of upper respiratory and flu symptoms: a randomized, double-blind placebo-controlled study. Journal of Alternative and Complementary Medicine 2000;6:327–334.

9. Barrett BP, Brown RL, Locken K, Maberry R, Bobula JA, D'Alessio D. Treatment of the common cold with unrefined *Echinacea*. Annals of Internal Medicine 2002;137:939–946. [PubMed: 12484708]

10. Turner RB, Bauer R, Woelkart K, Hulsey TC, Gangemi JD. An evaluation of *Echinacea angustifolia* in experimental rhinovirus infections. New England Journal of Medicine 2005;353:341–348. [PubMed: 16049208]

11. Yale SH, Liu K. *Echinacea purpurea* therapy for the treatment of the common cold: a randomized, double-blind, placebo-controlled clinical trial. Annals of Internal Medicine 2004;164:1237–1241.

12. Sperber SJ, Shah LP, Gilbert RD, Ritchey TW, Monto AS. *Echinacea purpurea* for prevention of experimental rhinovirus colds. Clinical Infectious Diseases 2004;38:1367–1371. [PubMed: 15156472]

13. Barnes J, Anderson LA, Gibbons S, Phillipson JD. *Echinacea* species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L) Moench): a review of their chemistry, pharmacology and clinical properties. Journal of Pharmacy and Pharmacology 2005;57:929–954. [PubMed: 16102249]

14. Woelkart K, Marth E, Suter A, Schoop R, Raggam RB, Koidl C, Kleinhappl B, Bauer R. Bioavailability and pharmacokinetics of Echinacea purpurea preparations and their interaction with the immune system. International Journal of Clinical Pharmacology and Therapeutics 2006;44:401–408. [PubMed: 16995328]

15. Matthias A, Addison RS, Agnew LL, Bone KM, Watson K, Lehmann RP. Comparison of Echinacea alkylamide pharmacokinetics between liquid and tablet preparations. Phytomedicine 2007;14:587–590. [PubMed: 17289362]

16. Wu L, Bae J, Kraus G, Wurtele ES. Diacetylenic isobutylamides of *Echinacea*: synthesis and natural distribution. Phytochemistry 2004;65:2477–2484. [PubMed: 15381412]

17. Pellati F, Benvenuti S, Melegari M, Lasseigne T. Variability in the composition of anti-oxidant compounds in *Echinacea* species by HPLC. Phytochemical Analysis 2005;16:77–85. [PubMed: 15881113]

18. Binns SE, Hudson J, Merali S, Arnason JT. Antiviral activity of characterized extracts from *Echinacea* spp. (Heliantheae:Asteraceae) against *Herpes simplex* virus (HSV-I). Planta Medica 2002;68:780–783. [PubMed: 12357386]

19. Senchina DS, McCann DA, Asp JM, Johnson JA, Cunnick JE, Kaiser MS, Kohut ML. Changes in immunomodulatory properties of *Echinacea* spp. root infusions and tinctures stored at 4°C for four days. Clinica Chimica Acta 2005;355:67–82.

20. McCann DA, Solco A, Liu Y, Macaluso F, Murphy PA, Kohut ML, Senchina DS. Cytokine- and interferon-modulating properties of *Echinacea* spp. root tinctures stored for two years at −20 degrees C. Journal of Interferon & Cytokine Research 2007;27:425–436. [PubMed: 17523874]

21. Senchina DS, Wu L, Flinn GN, Konopka DN, McCoy JA, Widrlechner MP, Wurtele ES, Kohut ML. Year-and-a-half old, dried *Echinacea* spp. roots retain cytokine-modulating capabilities in an in vitro human older adult model of influenza vaccination. Planta Medica 2006;72:1207–1215. [PubMed: 17021999]

22. Senchina DS, Flagel LE, Wendel JF, Kohut ML. Phenetic comparison of seven *Echinacea* species based on immunomodulatory characteristics. Economic Botany 2006;60:207–215.

23. Bauer R, Remiger P. TLC and HPLC Analysis of Alkamides in Echinacea Drugs. Planta Medica 1989;55:367–371. [PubMed: 17262436]

24. Kraus GA, Bae J. Synthesis of *N*-(2-methylpropyl)-2-undecene-8,10-diynamide, a novel constituent of *Echinacea angustifolia*. Tetrahedron Letters 2003;44:5505–5506. [PubMed: 16971964]

25. McGregor RL. The taxonomy of the genus *Echinacea* (Compositae). The University of Kansas Science Bulletin 1968;48:113–142.

26. Binns SE, Arnason JT, Baum BR. Phytochemical variation within populations of *Echinacea angustifolia* (Asteraceae). Biochemical Systematics and Ecology 2002;30:837–854.

27. Chen Y, Fu T, Tao T, Yang J, Chang Y, Wang M, Kim L, Qu L, Cassady J, Scalzo R, Wang X. Macrophage activating effects of new alkamides from the roots of Echinacea species. Journal of Natural Products 2005;68:773–776. [PubMed: 15921428]

28. Pellati F, Benvenuti S, Magro L, Melegari M, Soragni F. Analysis of phenolic compounds and radical scavenging activity of Echinacea spp. Journal of Pharmaceutical and Biomedical Analysis 2004;35:289–301. [PubMed: 15063463]

29. Laasonen M, Wennberg T, Harmia-Pulkkinen T, Vuorela H. Simultaneous analysis of alkamides and caffeic acid derivatives for the identification of Echinacea purpurea, Echinacea angustifolia, Echinacea pallida and Parthenium integrifolium roots. Planta Medica 2002;68:572–574. [PubMed: 12094312]

30. Sloley BD, Urichuk LJ, Tywin C, Coutts RT, Pang PK, Shan JJ. Comparison of chemical components and antioxidants capacity of different Echinacea species. Journal of Pharmacy and Pharmacology 2001;53:849–857. [PubMed: 11428661]

31. Hu C, Kitts DD. Studies on the antioxidant activity of Echinacea root extract. Journal of Agricultural and Food Chemistry 2000;48:1466–1472. [PubMed: 10820044]

32. Mazza G, Cottrell T. Volatile components of roots, stems, leaves, and flowers of Echinacea species. Journal of Agricultural and Food Chemistry 1999;47:3081–3085. [PubMed: 10552612]
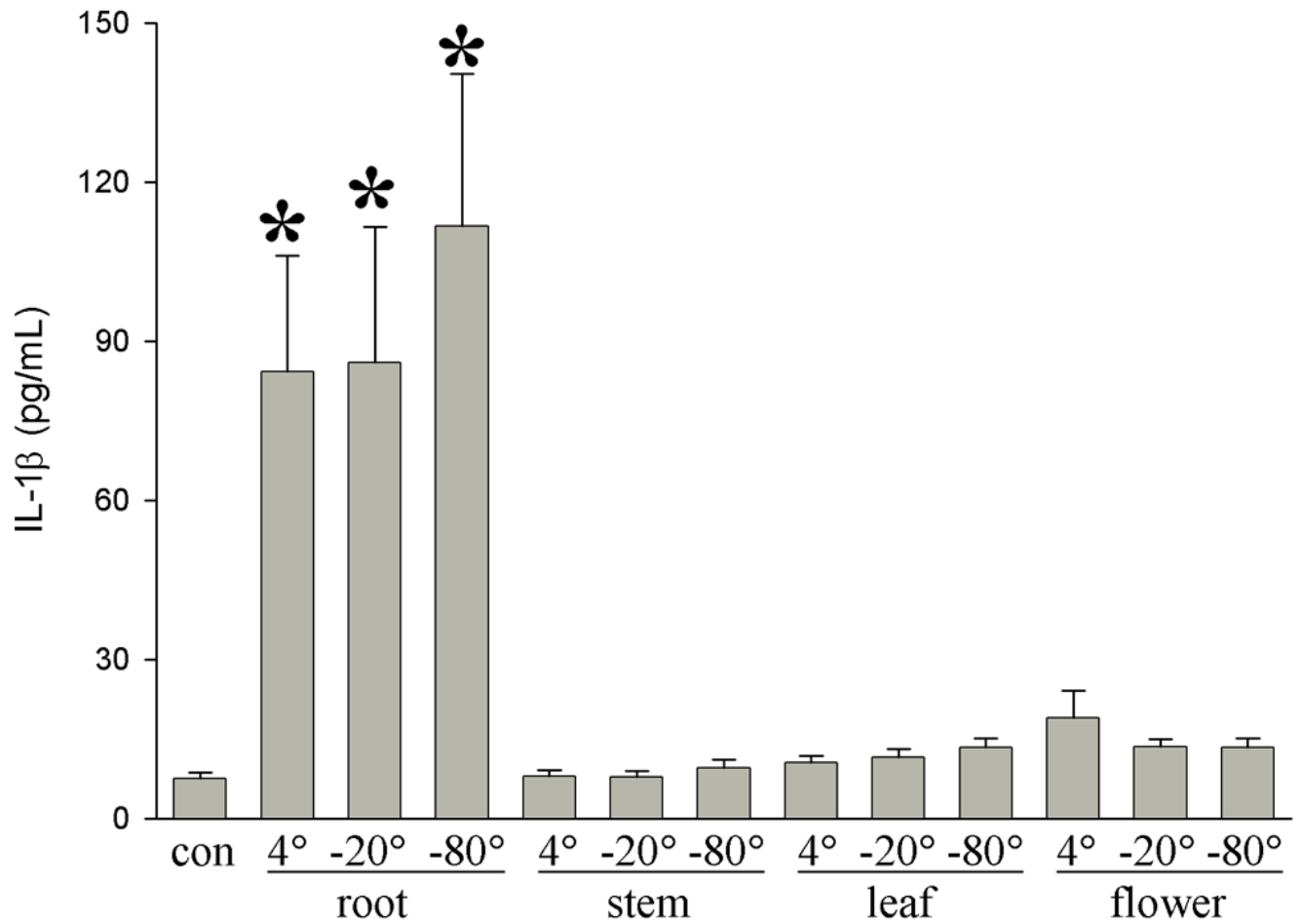
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Figure 1.
Interleukin-1β production by human PBMC cultures stimulated with *Echinacea tennesseensis* extracts. Bars represent means ± standard errors. Asterisks indicate statistical significance compared to control ($p \leq 0.05$).
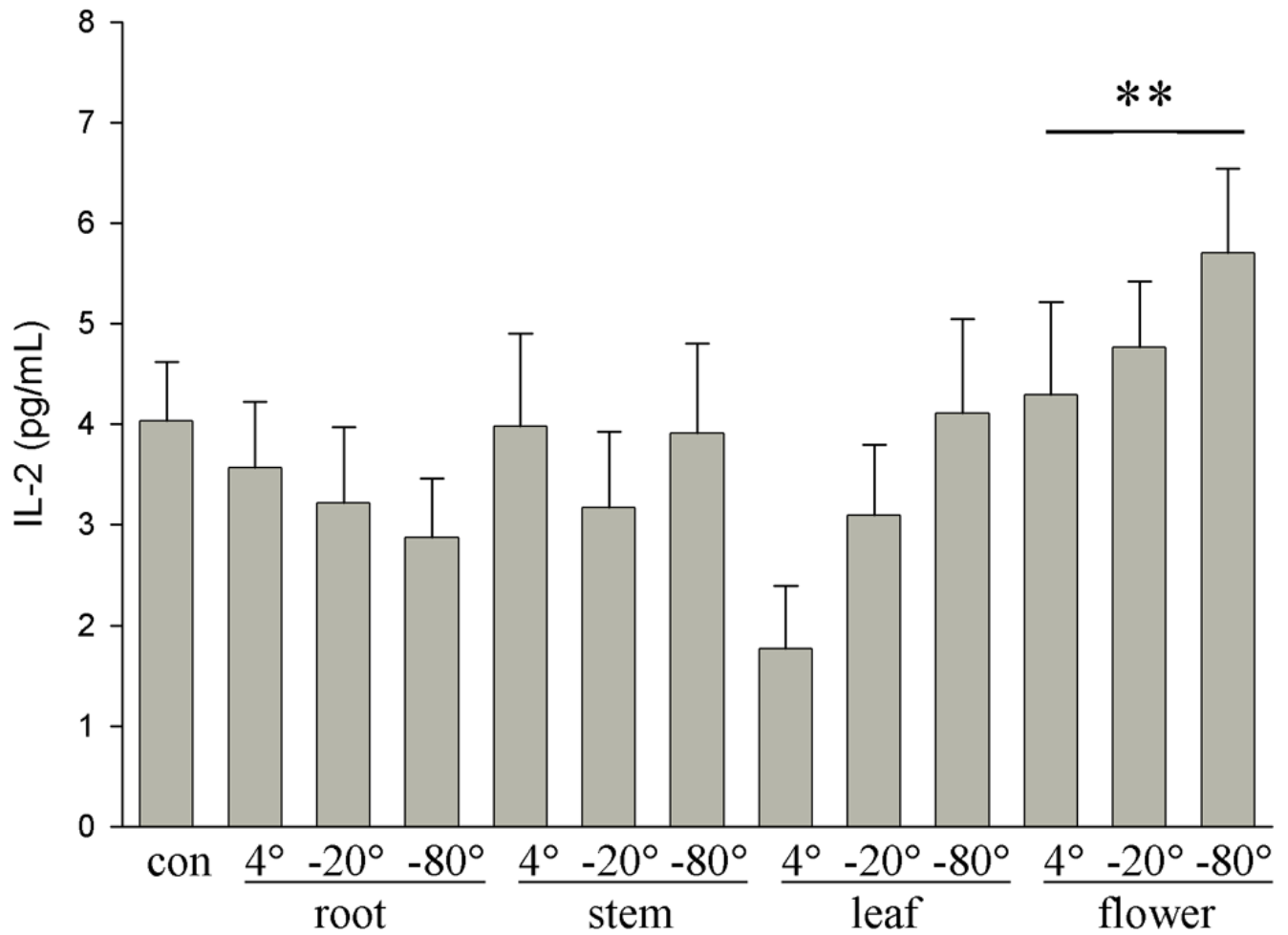
Figure 2.
Interleukin-2 production by human PBMC cultures stimulated with *Echinacea tennesseensis* extracts. Bars represent means ± standard errors. Double asterisks indicate that flower extracts as a group were significantly different than root, stem, or leaf extracts as groups (all $p \leq 0.051$).
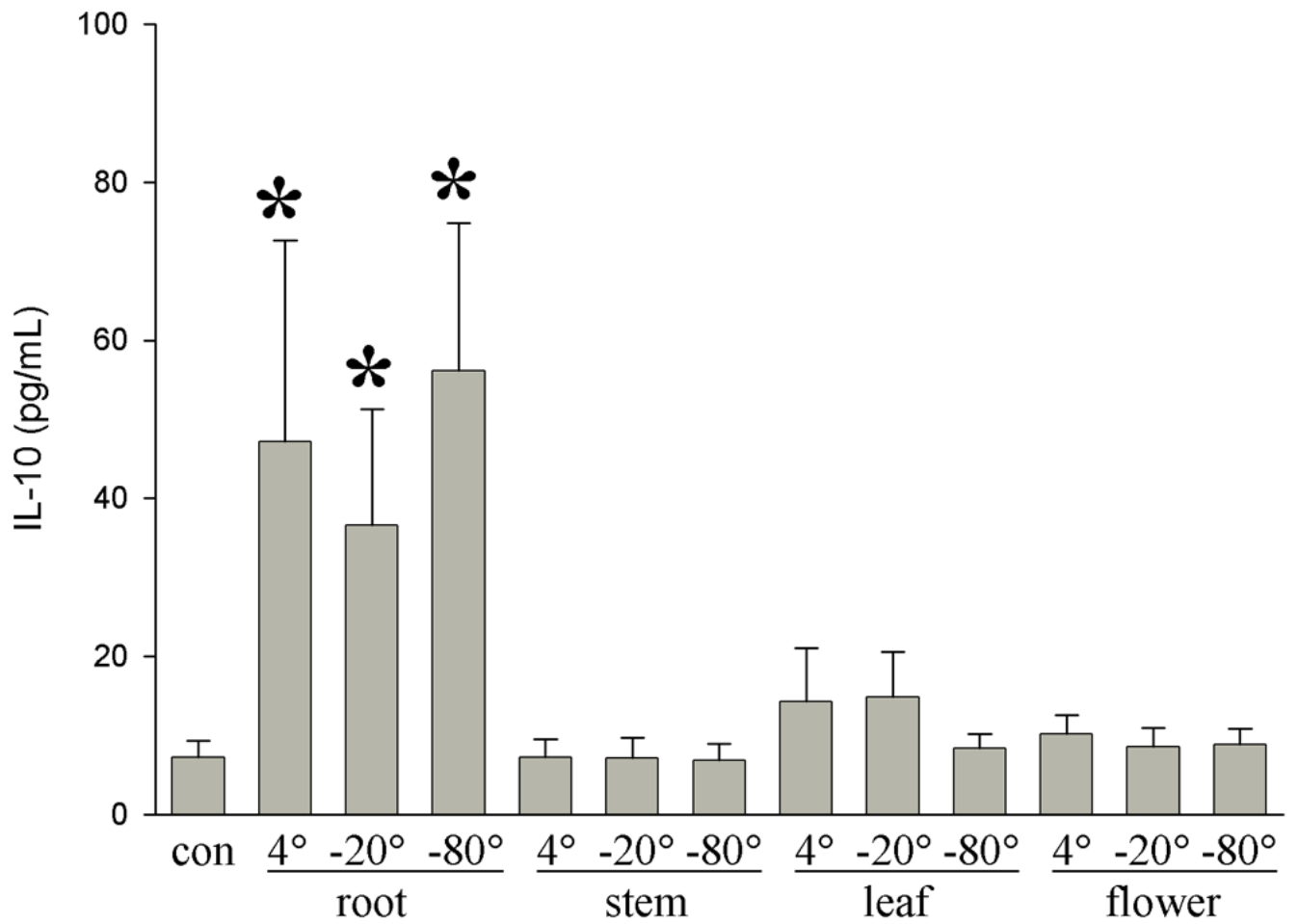
Figure 3.
Interleukin-10 production by human PBMC cultures stimulated with *Echinacea tennesseensis* extracts. Bars represent means ± standard errors. Asterisks indicate statistical significance compared to control ($p \leq 0.05$).

Figure 4.
Tumor necrosis factor-α production by human PBMC cultures stimulated with *Echinacea tennesseensis* extracts. Bars represent means ± standard errors. Asterisks indicate statistical significance compared to control ($p \leq 0.05$).
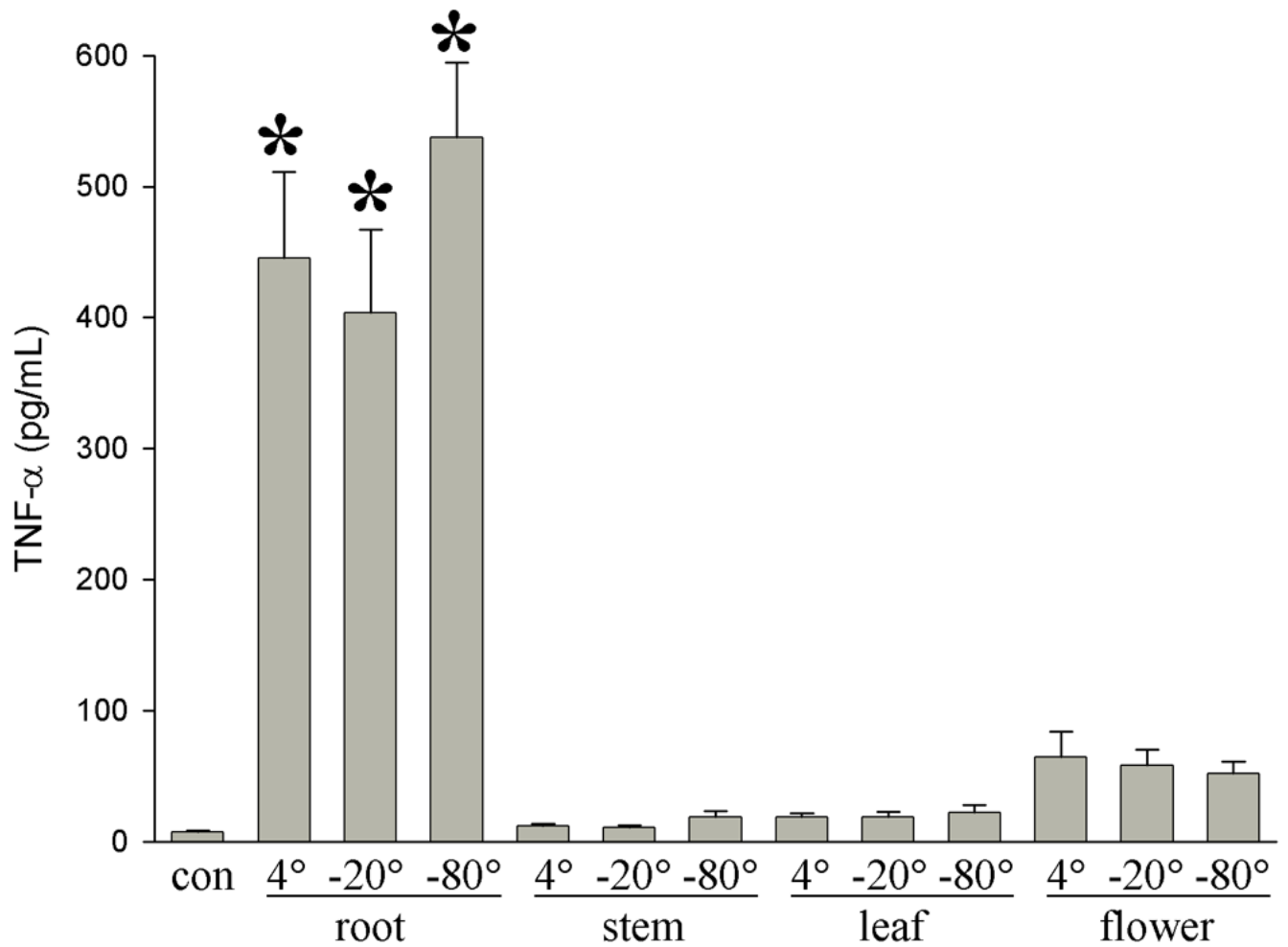
Figure 5.
Proliferation of human PBMC stimulated with fresh *Echinacea tennesseensis* extracts. Bars represent means ± standard errors. Asterisks indicate statistical significance compared to control ($p \leq 0.05$).
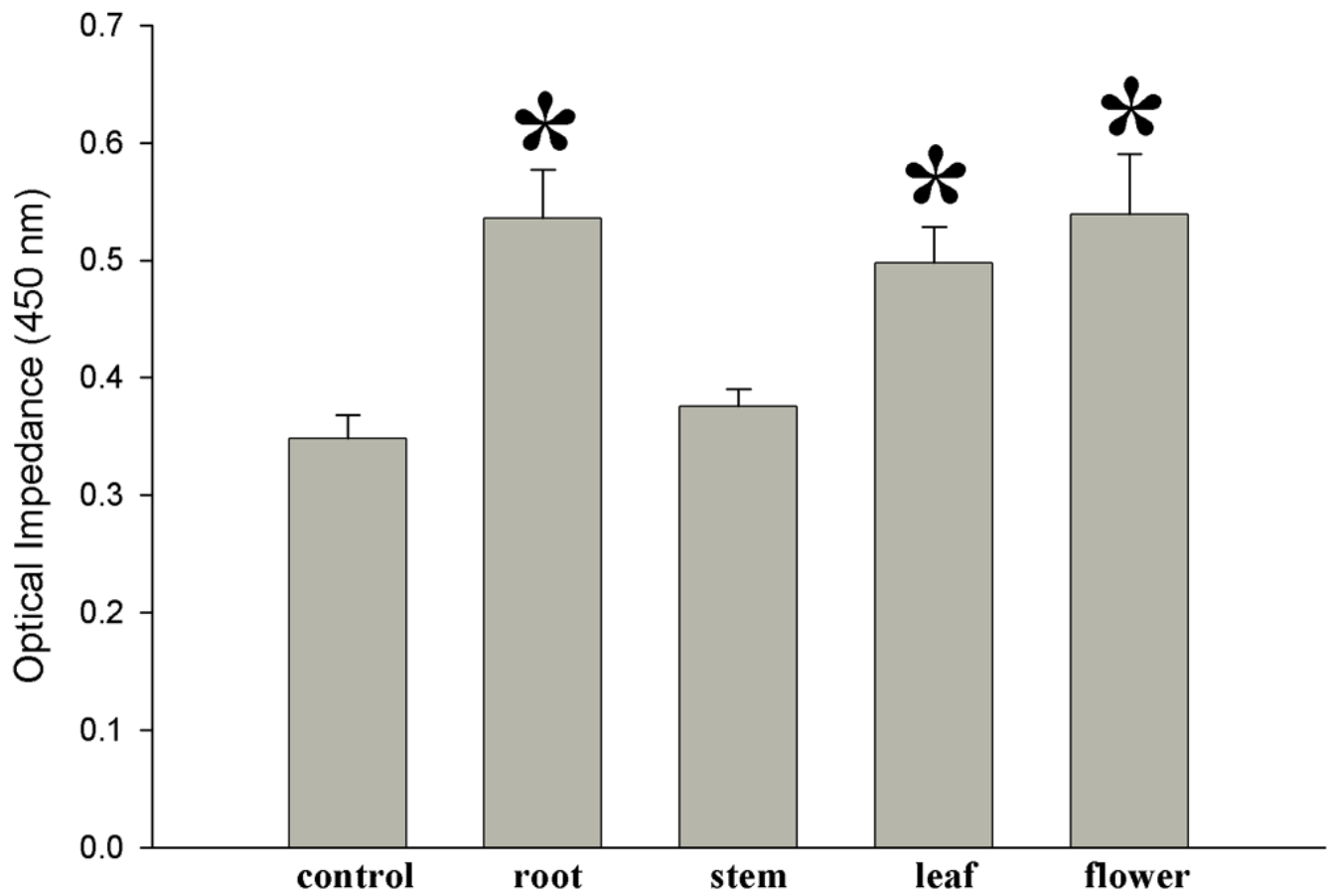
Table 1

Phytochemical profiles of *Echinacea tennesseensis* extracts utilized in this investigation as determined by HPLC. Values are mg/mL and represent means of three separate analyses of the same extract. ND = non-detectable.

	Flower	Leaf	Stem	Root
Amide 1	0.0056	ND	ND	0.0066
Amide 8	0.0709	0.0068	0.0169	0.0117
Amide 9	0.1422	0.0041	0.0226	0.0058
Amide 10	0.0031	ND	0.0023	0.0074
Amide 11	0.0013	ND	0.0006	0.0041
Amide 12	0.0403	0.0009	0.0091	0.0907
Amide 13	ND	ND	0.0008	0.3654
Amide 14	ND	ND	ND	0.0227
Amide 16	ND	ND	ND	0.0204
Amide 17	ND	ND	ND	0.0058

Table 2

Comparison of cytokine production from human PBMC treated with *Echinacea tennesseensis* extracts stored at $-20°C$ on Day 1 and Day 31. Values are expressed as pg/mL, mean ± standard error. Asterisks indicate statistical significance ($p \leq 0.05$) compared to control. Daggers indicate trends towards statistical significance ($0.05 < p < 0.1$).

	Time	IL-1β	IL-2	IL-10	TNF-α
Control	Day 1	7.54 ± 1.14	4.03 ± 0.59	7.2 ± 2.09	7.7 ± 0.77
	Day 31	9.14 ± 0.83	1.24 ± 0.5	7.7 ± 0.26	20.2 ± 0.08
Root	Day 1	85.93 ± 25.57*	3.22 ± 0.76	36.61 ± 14.66*	403.96 ± 63.46*
	Day 31	78.34 ± 41.4*	1.49 ± 0.35	355.27 ± 141.03[†]	184.22 ± 79.11*
Stem	Day 1	7.85 ± 1.0	3.17 ± 0.75	7.16 ± 2.51	10.96 ± 1.22
	Day 31	9.17 ± 0.55	1.54 ± 0.46	11.86 ± 2.71	20.32 ± 0.19
Leaf	Day 1	11.55 ± 1.55	3.1 ± 0.7	14.91 ± 5.72	18.56 ± 4.48
	Day 31	18.1 ± 7.31	0.92 ± 0.52	35.03 ± 20.01	26.28 ± 5.35
Flower	Day 1	13.47 ± 1.44	4.77 ± 0.65	8.6 ± 2.34	58.08 ± 12.04
	Day 31	12.21 ± 2.89	1.90 ± 0.18	33.12 ± 8.26	24.76 ± 2.53