Journal of Applied Pharmaceutical Science Vol. 3 (12), pp. 001-005, December, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.31201
ISSN 2231-3354

High-potency Anti-influenza Therapy by a Combination of *Echinacea purpurea* fresh herb and root tinctures

Vimalanathan, Selvarani[1]; Schoop, Roland[2]; Hudson, James[1]

[1]Department of Pathology & Laboratory Medicine, University of British Columbia, Vancouver, Canada.
[2]Medical Department, A. Vogel Bioforce AG, Roggwil (TG), Switzerland.

ARTICLE INFO

Article history:
Received on: 26/10/2013
Revised on: 27/11/2013
Accepted on: 14/12/2013
Available online: 30/12/2013

Key words:
Echinacea purpurea, influenza virus, antiviral, anti-inflammatory, extraction, dried & fresh herb.

ABSTRACT

The medicinal plant *Echinacea purpurea* (EP) contains potent antiviral and anti-inflammatory activities, which are believed to be responsible for the efficacy of such preparations in the treatment of colds and 'flu. To determine to what extent the processes of drying, composition and extraction could affect this efficacy, different parts of fresh and dried EP plants: herb, root, flower heads and petals, were separately extracted and evaluated for activity against Influenza virus. Maximal activity was obtained from freshly extracted herb, while root extracts showed no such activity. The observed antiviral activity did not correlate with the total dry mass, or the cichoric acid, rutoside, total phenols or alkylamide content. The latter however appears to be responsible for the anti-inflammatory effects of the root extracts. Thus, the parallel extraction of antiviral and anti-inflammatory substances from fresh EP herb and root could represent an optimized combination for the treatment of influenza infections.

INTRODUCTION

Acute respiratory infections in humans are usually ascribed to one or more of a group of well known viruses, including influenza, rhinoviruses, para influenza viruses, corona viruses, respiratory syncytial virus, and certain adenoviruses (Gwaltney, 2002). However the variety of replication schemes among these viruses reduces the chances that a single antiviral drug could be effective as a generic remedy for "colds and flu". In addition, the symptoms that accompany these infections are largely due to the viral induced inflammatory responses, which include substantial induction of cytokines and chemokines (Eccles, 2005; Fedson, 2009; Sharma *et al.,* 2009). This is particularly evident in serious influenza infections, which may give rise to "cytokine storms" and subsequent pathology (Oslund and Baumgarth, 2011). Consequently it is difficult to conceive of a single target-directed antiviral agent that could also provide appropriate antiinflammatory treatment (Johnston, 1995; Hudson and Vimalanathan, 2011).

* *Corresponding Author*
James Hudson, 1Department of Pathology & Laboratory Medicine, G-408, 2211, Wesbrook mall, University of British Columbia,, Vancouver, Canada, V6T 1Z1, Tel: 604 948 2131, Email;jbhudson@mail.ubc.ca

An alternative approach is the use of a non-toxic multi-component medication with the capacity to inhibit many different respiratory viruses simultaneously, and that is suitable for long-term intake during different phases of infection. Previous studies have shown that particular commercial ethanol tinctures of *Echinacea purpurea* (EP), but not all of them, contain potent antiviral and anti-inflammatory activities, which could explain the efficacy of these extracts in controlling respiratory infections *in vitro* and in clinical studies (Vimalanathan *et al.*, 2005; Chicca *et al.*, 2009; Vohra *et al.*, 2009; Hudson & Vimalanathan, 2011; Jawad *et al.*, 2012). However other studies have indicated that there is considerable variation in chemical composition of anatomically different parts of the plant, and among Echinacea plants grown and processed in different ways (Tobler *et al.*, 1994; Binns *et al.*, 2002). Such chemical differences are likely to influence the bioactivities and therefore the efficacy of the preparations (Hudson and Vimalanathan, 2011). In order to address this issue we compared the antiviral activity of ethanol tinctures prepared from different parts of both fresh and dried EP plants. Influenza virus was used as the indicator of antiviral activity since different strains of avian and human influenza viruses are known to be particularly sensitive to potent Echinacea extracts (Pleschka *et al.*, 2009).

MATERIALS AND METHODS

Preparation of Tinctures

In July 2012 fifty (50) kilograms of aerial plant parts of *E. purpurea* were freshly harvested and dissected into stem + leaves, flower heads (without petals) and the petals. Each individual plant part, as well as the total herb, were freshly processed (within 24h), chopped and subsequently extracted with 65% (V/V) ethanol using a drug to extraction solvent ratio (DER) of 1:11 to 1:12 to give batches FE120704, FE120705, FE120706 and FE120703 (Table 1). 10 kg of the freshly harvested *E purpurea* herb was dried using a convection oven at 45°C prior to chopping and alcoholic extraction with 65% (V/V) to yield tincture FE120802.

In September 2012 30kg of *E. purpurea* roots were either freshly processed or subjected to drying at 45°C prior to alcoholic extraction to yield batches FE120901 and FE120902 (Table 1).

Echinaforce® tincture (FE120905) was manufactured by combining fresh herbal tincture FE 120703 and fresh root tincture FE 120901 at a ratio of 95:5. A corresponding combination was produced from dried herb tincture (FE120802) and root tincture (FE120902) to yield FE120906 (Echinaforce dried).

Cells and virus

MDCK canine kidney cells were acquired originally from ATCC (American Type Culture Collection, Rockville, MD), and were cultivated in Dulbecco MEM (DMEM), in cell culture flasks, supplemented with 5% fetal bovine serum, at 37°C in a 5% CO_2 atmosphere (cell culture reagents were obtained from Invitrogen, Ontario CA). No antibiotics or anti-mycotic agents were used.

Influenza virus type A, strain H3N2 was acquired from BC Centre for Disease Control, Vancouver, and was grown and assayed, by plaque formation, in MDCK cells, the standard cell line for growth and measurement of Influenza viruses (WHO manual, 2011; Vimalanathan and Hudson, 2012).

Antiviral assays

The assay technique was based on our standard techniques for the evaluation of plant extracts for antiviral activity (Vimalanathan *et al.*, 2005; Vimalanathan and Hudson, 2012). The experimental procedure consisted of incubating two-fold dilutions of the extract in phosphate buffered saline, in 96-well trays, with a known amount of the virus (10^3 plaque-forming units, pfu) for 60 min at 22ºC (in triplicate reactions). The reaction mixtures were then assayed for residual infectious virus (plaques) in monolayers of freshly confluent MDCK cells, in 6-well culture trays. Reduction in the number of virus plaques represents the degree of antiviral activity. Controls consisted of virus in medium without extract.

Statistical analysis

The results were expressed as mean ± SD for three independent experiments. Statistical differences between different samples were determined using one-way analysis of variance (ANOVA) with Turkey's post-hoc test. The statistical significance of two samples (herb (fresh) and Herb dried) was determined by Student's t-test with Welch correction. Differences were considered significant when *P* value was less than 0.05.

RESULTS

Tinctures were prepared from separated herb, roots, flower heads and petals of both fresh and dried EP plants. Each tincture was then tested for antiviral activity against a standard amount of influenza virus (10^3 pfu). The results are summarized in Fig. 1 and 2.

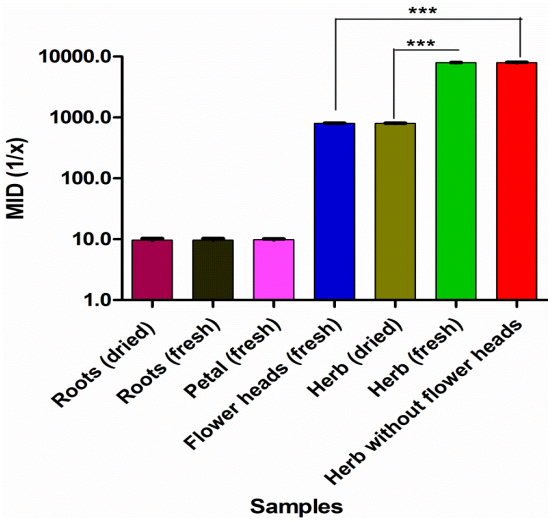

Fig. 1: Logarithmic maximal inhibitory dilution (MID) of extracts made from freshly harvested or dried *E. purpurea* roots, petals, flower heads, herb (total aerial parts) and the herb without flower heads. The maximal dilution gives the amount of extract sufficient to inactivate 100% of influenza virus (10^3 plaque-forming units). Two-fold dilutions were made of each sample and plaque assays were done in triplicate. *P* values were determined using one-way analysis of variance (ANOVA) with Turkey's posttest, ***, $P<0.0001$.

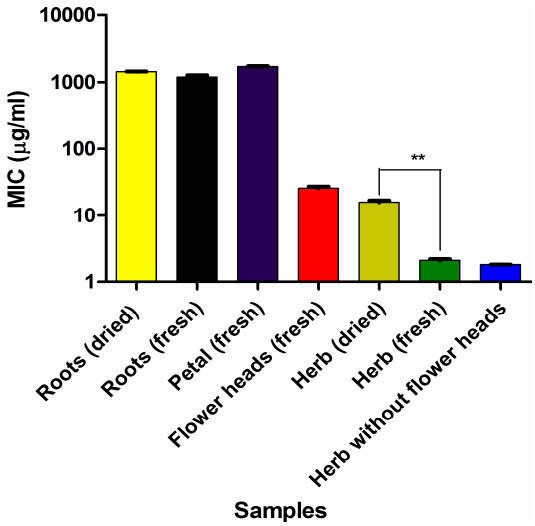

Fig. 2: Logarithmic minimal inhibitory concentration (MIC, anti-influenza virus) with respect to dry mass. The greatest antiviral activity was observed in the freshly harvested aerial parts of *E. purpurea*. *P* value for Herb (fresh) versus Herb (dried) for antiviral activity (MIC) was calculated by Student t-test with Welch's correction: **, $P=0.0016$.

The relative antiviral activity is expressed as the maximal inhibitory dilution of the respective extracts (MID 1/x) (Fig. 1) and the required minimal inhibitory concentration (MIC_{100}, dry mass in µg/ml) to fully inhibit influenza virus growth (Fig. 2). The tinctures prepared from freshly harvested or dried EP roots were largely devoid of antiviral activity even at a dilution of 1:10 (corresponding to MIC > 1 mg/ml, Fig's 1 and 2).

Extracts prepared from fresh flower heads (without petals) contained moderate activity with MID = 1:800 (MIC = 26.4 µg/ml), but the separated petals were inactive (MID > 1:10).

The most potent activity was obtained with extracts from freshly harvested *Echinacea purpurea* herb (aerial parts), which were still active at dilutions of 1:8,000. Depending on the dry mass content the minimal inhibitory concentrations fell in the low µg/ml range ($p<0.01$). In contrast, tinctures made from the dried above-ground plant parts showed substantially less antiviral activity than those prepared from the freshly harvested parts (1:800 versus 1:8,000 $p<0.0001$), indicating that the active ingredient/s were unstable during the drying process.

High levels of potency were observed in a combination product consisting of 95% of above-ground plant parts supplemented with 5% of *E. purpurea* roots (standardized Echinaforce®, EF). Again, usage of fresh plant material significantly ($p<0.0001$) increased the antiviral potency of the preparation (Fig. 3), and evidently the herb component contributed to the difference in activity (Fig. 2 and 3). In an attempt to correlate the observed antiviral effects with the known marker substances of EP, alkylamides, cichoric acid, rutoside, total phenols and the total dry mass were measured. However, no correlation was observed (Table 2). Extracts with the highest antiviral activity (fresh herb) demonstrated lower total dry mass than samples with lowest activity (petals or dried roots). Moreover, the alkylamide and cichoric acid rich samples from the roots and flower heads proved to have less activity in the antiviral assay. Phenols and rutoside were mainly found in the petals without any apparent link with bioactivity. Interestingly, although the content in phenols increased during the drying process, the antiviral activity diminished, which further substantiated the observation that the antiviral principle of *E. purpurea* does not belong to this substance group.

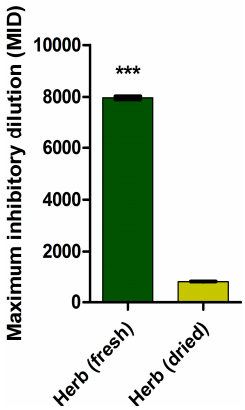

Fig. 3: Echinaforce® (EF fresh) is produced from 95% *Echinacea purpurea* herb and 5% roots while using fresh plant material. The manufacturing process (drying) evidently has a critical influence on the antiviral activity of the preparation.

Table. 1: *Echinacea purpurea* plants were dissected into the individual plant parts (stem + leaves, flowerheads, petals and roots). Alcoholic tinctures were prepared from both the freshly harvested and the dried plant material.

Batch No.	Plant species	Plant parts	Processing	Extraction solvent
FE120703	*E. purpurea*	Aerial parts (herb)	fresh	65% ethanol (V/V)
FE120704	*E. purpurea*	Stem + leaves	fresh	65% ethanol (V/V)
FE120705	*E. purpurea*	Flower heads (w/o petals)	fresh	65% ethanol (V/V)
FE120706	*E. purpurea*	Petals	fresh	65% ethanol (V/V)
FE120901	*E. purpurea*	Roots	fresh	65% ethanol (V/V)
FE120802	*E. purpurea*	Aerial parts (herb)	dried	65% ethanol (V/V)
FE120902	*E. purpurea*	Roots	dried	65% ethanol (V/V)
FE120905	*E. purpurea*	Echinaforce (EF fresh) 95% Aerial parts [FE120703] 5% Roots [FE120901]	fresh	65% ethanol (V/V)
FE120906	*E. purpurea*	Echinaforce (EF dried) 95% Aerial parts [FE120802] 5% Roots [FE120902]	dried	65% ethanol (V/V)

Table. 2: Composition of known marker compounds in the tinctures. No correlation with antiviral activity (MID's) was observed.

Extract	Dry mass [%]	Dodecatetraene [mg/100g]	Cichoric acid [mg/100g]	Rutoside [mg/100g]	Phenols [mg/100g]	MID [1/x]
Dried root s	1.6	15.87	136.53	1.06	195.2	10
Fresh roots	1.34	15.06	25.97	1.05	64.8	10
Fresh petals	1.93	0.96	170.68	37.94	313	10
Fresh flower heads	2.35	11.61	29.36	2.53	95.4	800
Dried herb	1.5	1.98	79.1	5.87	150.5	800
Fresh herb	1.9	3.14	19.91	4.11	78	8000
Fresh Herb w/o Flower heads	1.63	1.95	25.42	3.99	82.3	8000

DISCUSSION

Originally, Echinacea was discovered as a medicinal plant by the First Nations people of the Great Plains of North America. According to anecdotal reports the therapeutic and medicinal benefits were mainly obtained from fresh plants and roots or the sap and crushed pulp derived from them. Contemporary Echinacea extracts show a wide variation in regard to the species and their plant parts used, and in the details of processing (e.g. manufacturing of fresh or dried plant material). Such variation leads to significant differences in chemical composition and activity (Binns *et al.,* 2002; Barnes *et al.,* 2005). However, the production of effective cold and influenza remedies relies on optimized isolation of antiviral and anti-inflammatory principles (Johnston, 1997; Hudson and Vimalanathan, 2011). The characterization of these activities from Echinacea is important since to date no chemical marker is known for the antiviral principle which could guide the processing.

Isolation of the antiviral activity

It has often been noted that the process of drying (generally the exposure to temperature) can adversely affect the chemical composition of medicinal plant extracts, with loss of certain chemicals and consequent decrease in bioactivity (Tobler *et al.,* 1994). In the case of Echinacea extracts we do not know which ingredients are vital for the antiviral activity but they appear to be lipophilic since ethanol provides an efficient solvent for their isolation (Vimalanathan *et al.,* 2005). The present data show that, at least for retention of the highly potent anti-influenza activity freshly extracted herb is important. The fresh plant material used in our experiments was chopped and immediately exposed to alcoholic extraction within 24h post harvesting. Enzymatic degradation of ingredients was thus minimal. Preliminary chromatographic fractionation indicated that several compounds appear to be antiviral (data not shown). This could explain the low tendency of influenza viruses to develop resistance against such extracts (Pleschka *et al.,* 2009).

Isolation of anti-inflammatory activity

More is known about the anti-inflammatory principles in Echinacea. In previous studies, Chicca et al. (2009) evaluated a standard *E. purpurea* ethanol tincture, derived from herb and root of fresh plant material, for anti-inflammatory activity against TNFα in cultured human peripheral blood cells. This activity was ascribed to the alkylamides, which are known to be prevalent in EP roots and which function through the endocannabinoid system. The isolated alkylamides influence anandamide transport, and the specific compounds undeca- and dodeca-2E, 4Z-diene-8,10-diyonic acid isobutylamides were considered to play an important role at this stage. Furthermore the isomers dodeca-2E, 4E, dienoic acid isobutylamides are likely to increase the efficacy of 2-arachidonoylglycerol (2-AG) and are also strong inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of anandamide and 2-AG (Fowler, 2000). Alkylamides were demonstrated to be bioavailable after per-oral application of alcoholic E purpurea extracts, and the effects on inflammatory mediators were confirmed in *ex vivo* experiments (Ritchie *et al.,* 2011)

As already demonstrated by Tobler et al. (1994) alkylamides appear to be temperature sensitive and consequently drying reduces their content in herb and roots significantly.

Combination of antiviral and anti-inflammatory activities

Virus infection, replication and subsequent spread are central to the pathogenesis of respiratory tract infections. Substances which block the viral life-cycle are therefore medicinally very important. The herb and roots of *Echinacea purpurea* provide an arsenal of substances that block both the virus and the inflammation respectively. Depending on the processing techniques, the resulting products comply with the actual requirement for cold and flu remedies.

Evidence for the therapeutic value of fresh EP herb and root combinations comes from clinical trials as well. Jawad et al.(2012) investigated the therapeutic and preventive benefits of a root/herb combination product in a large clinical trial. A 4-month consumption of Echinaforce® significantly reduced the frequency of recurrent infections, the number of days with colds, and the need for co-medication during acute episodes (Jawad *et al.,* 2012). In agreement with our observations, fewer patients tested positive for respiratory viruses than in the placebo group. In several other clinical studies combination products that employed freshly manufactured EP herb and roots proved effective in acute treatment of respiratory tract infections (Brinkeborn *et al.,* 1999; Goel *et al.,* 2004). Clinical evidence for the anti-inflammatory effects of Echinacea in rhinovirus infection studies finally comes from meta-analyses. Products devoid of ethanolic EP herb extracts could not directly prevent viral infections. Nevertheless they prevented the symptomatic development of infections into clinical colds by lowering the inflammatory reaction (Schoop *et al.,* 2006).

CONCLUSION

The most potent antiviral extracts of *Echinacea purpurea* were tinctures derived from freshly extracted herb. This antiviral activity however did not correlate with content of dry mass, caffeic acids, rutoside, total phenols or alkylamides. In order to obtain maximum potential bioactivities from the medicinal plant, the presence of root extract is also desirable, providing anti-inflammatory alkylamides. Such an optimized preparation containing herb and roots from fresh Echinacea plants, delivers effective relief from the symptoms associated with influenza and other respiratory virus infections, as well as inactivation of the virus.

Acknowledgement: The authors wish to acknowledge Urs Buehler (A. Vogel Bioforce AG) for the harvesting, separation, and extractions of the plants.

REFERENCES

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. J Pharm Pharmacol. 2005;57:929-54.

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 Med. 2002;68:780-3.

Brinkeborn RM, Shah DV, Degenring FH. Echinaforce and other Echinacea fresh plant preparations in the treatment of the common cold. A randomized, placebo controlled, double-blind clinical trial. Phytomedicine. 1999;6:1-6.

Chicca A, Raduner S, Pellati F, Strompen T, Altmann KH, Schoop R, Gertsch J. Synergistic immunomopharmacological effects of N-alkylamides in Echinacea purpurea herbal extracts. Int Immunopharmacol. 2009;9:850-8.

Eccles R. Understanding the symptoms of the common cold and influenza. The Lancet infectious diseases. 2005;5:718-25.

Fedson DS. Confronting the next influenza pandemic with anti-inflammatory and immunomodulatory agents: Why they are needed and how they might work. Influenza Other Respi Viruses. 2009; 3(4):129-142.

Fowler CJ, Borjesson M, Tiger G. Differences in the pharmacological properties of rat and chicken brain fatty acid amidohydrolase. Br J Pharmacol. 2000;131:498-504.

Goel V, Lovlin R, Barton R, Lyon MR, Bauer R, Lee TDG, Basu TK. Efficacy of a standardized echinacea preparation (EchinilinTM) for the treatment of the common cold: a randomized, double-blind, placebo-controlled trial. J Clin Pharm Ther. 2004;29:75-83.

Gwaltney JM. Clinical significance and pathogenesis of viral respiratory infections. Am J Med. 2002;112 Suppl 6A:13S-8S.

Hudson J, and Vimalanathan S. Echinacea—A source of potent antivirals for respiratory virus infections. Pharmaceuticals. 2011;4:1019-31.

Jawad M, Schoop R, Suter A, Klein P, Eccles R. Safety and Efficacy Profile of Echinacea purpurea to Prevent Common Cold Episodes: A Randomized, Double-Blind, Placebo-Controlled Trial. Evid Based Complement Alternat Med. 2012;2012:841315.

Johnston SL. Problems and prospects of developing effective therapy for common cold viruses. Trends Microbiol. 1997;5:58-63.

Johnston SL. Natural and experimental rhinovirus infections of the lower respiratory tract. Am J Respir Crit Care Med. 1995;152:S46-52.

Oslund KL, Baumgarth N. Influenza-induced innate immunity: regulators of viral replication, respiratory tract pathology & adaptive immunity. Future Virol. 2011;6:951-62.

Pleschka S, Stein M, Schoop R, Hudson JB. Anti-viral properties and mode of action of standardized Echinacea purpurea extract against highly pathogenic avian influenza virus (H5N1, H7N7) and swine-origin H1N1 (S-OIV). Virol J. 2009;6:197.

Ritchie MR, Gertsch J, Klein P, Schoop R. Effects of Echinaforce(R) treatment on ex vivo-stimulated blood cells. Phytomedicine. 2011;18:826-31.

Schoop R, Klein P, Suter A, Johnston SL. Echinacea in the prevention of induced rhinovirus colds: a meta-analysis. Clin Ther. 2006;28:174-83.

Sharma M, Anderson SA, Schoop R, Hudson JB. Induction of multiple pro-inflammatory cytokines by respiratory viruses and reversal by standardized Echinacea, a potent antiviral herbal extract. Antiviral Res. 2009;83:165-70.

Tobler M, Krienbühl H, Egger M, Maurer C, Bühler U. Characteristics of whole fresh plant extracts. Schweizerische Zeitschrift für GanzheitsMedizin. 1994;5:257-66.

Vimalanathan S, Hudson J. Anti-Influenza Virus Activities of Commercial Oregano oils and their Carriers. Journal of Applied Pharmaceutical Science. 2012;2:214-8.

Vimalanathan S, Kang L, Amiguet VT, Livesey J, Arnason JT, Hudson J. Echinacea purpurea Aerial Parts Contain Multiple Antiviral Compounds. Pharm Biol. 2005;43:740-5.

Vohra S, Adams D, Hudson JB, Moore JA, Vimalanathan S, Sharma M, Burt AJ, Lamont E, Lacaze N, Arnason JT, Lee TD. Selection of natural health products for clinical trials: a preclinical template. Can J Physiol Pharmacol. 2009;87:371-8.

WHO Global Influenza Surveillance Network: Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza, section 2.C. 2011. whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf

How to cite this article:

Vimalanathan Selvarani, Schoop Roland, Hudson James. High-potency Anti-Influenza Therapy by a Combination of *Echinacea purpurea* fresh herb and root tinctures. J App Pharm Sci, 2013; 3 (12): 001-005.