

## Review Article

# Applications of the Phytomedicine *Echinacea purpurea* (Purple Coneflower) in Infectious Diseases

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Extracts of *Echinacea purpurea* (EP, purple coneflower) have been used traditionally in North America for the treatment of various types of infections and wounds, and they have become very popular herbal medicines globally. Recent studies have revealed that certain standardized preparations contain potent and selective antiviral and antimicrobial activities. In addition, they display multiple immune-modulatory activities, comprising stimulation of certain immune functions such as phagocytic activity of macrophages and suppression of the proinflammatory responses of epithelial cells to viruses and bacteria, which are manifested as alterations in secretion of various cytokines and chemokines. These immune modulations result from upregulation or downregulation of the relevant genes and their transcription factors. All these bioactivities can be demonstrated at noncytotoxic concentrations of extract and appear to be due to multiple components rather than the individual chemical compounds that characterize *Echinacea extracts*. Potential applications of the bioactive extracts may go beyond their traditional uses.

## 1. Traditional Uses of *Echinacea*

Herbal medicines derived from several species of the indigenous *Echinacea* genus were in use throughout the plains of North America long before the introduction of European medicines, primarily as treatments for various infectious diseases and wounds. Nine discrete species were classified subsequently by botanists, as indicated in Table 1, although medical records suggest that considerable interchange between uses of designated species occurred and consequently the association of a specific species with particular treatments has to be viewed with caution [1–3]. Even in recent years there have been revisions in the taxonomy of the genus [4, 5]. Nevertheless it is generally agreed that *Echinacea purpurea*, the purple coneflower, was widely used by Native peoples and later by the Eclectic practitioners of North America, possibly because it was so widespread, and also because it was apparently effective in a number of diseases. Current herbal preparations, which have become very popular in North America, Europe, and elsewhere, have tended to favor this species over the others, and the majority of the basic scientific studies have focused

on this one. Accordingly, this review is restricted primarily to discussion of *E. purpurea* (abbreviated EP), with occasional reference to alternative species.

The source material for scientific and clinical studies is usually an aqueous “pressed juice” or ethanol tincture/extract of aerial parts of the dried plant or roots. The chemical composition differs substantially between such preparations, at least in terms of the known “marker compounds”, such as caffeic acid derivatives, alkylamides, and polysaccharides, all of which have been claimed to contribute to the medicinal benefits [5–7]. However, the uncertainty in the identity of the principal bioactive compounds has made interpretation of basic and clinical studies difficult, and unfortunately the problem has been exacerbated by the frequent use of uncharacterized source material.

In an attempt to validate some of the traditional uses of *Echinacea*, numerous studies have been made recently on the effects of characterized EP preparations on viruses, bacteria and other organisms, inflammatory responses, and gene expression in infected and uninfected human cell cultures, as well as animal studies. These results are discussed in following sections. However I have omitted reference to

TABLE 1: Traditional uses of *Echinacea* (Coneflower) extracts.

Echinacea species	Traditional applications
	Respiratory infections: colds and ‘flu, bronchitis, strep throat, toothache
<i>E. purpurea</i> , <i>E. angustifolia</i> , <i>E. pallida</i>	Urinary tract infections: herpes sores, gonorrhea Skin disorders: staph infections, cold sores, ulcers, wounds, burns, insect bites, eczema, allergies Others: rheumatoid arthritis
<i>E. atrorubens</i> , <i>E. laevigata</i> <i>E. paradoxa</i> , <i>E. sanguinea</i> <i>E. simulata</i> , <i>E. tennesseensis</i>	Not frequently used

Adapted from [1].

studies that used combinations of EP with other herbs, for reasons explained below (Section 16).

## 2. Antiviral Activities

**2.1. Respiratory Virus Infections.** Acute respiratory infections in humans are usually caused by one or more of a group of well-known viruses, which includes over 100 rhinoviruses (“common cold” viruses), influenza viruses A and B, parainfluenza viruses, corona viruses, respiratory syncytial virus, and certain adenoviruses [8–10]. Influenza virus A deserves special consideration because of its unique capacity for genetic reassortment between animal and human strains and consequent production of epidemics (Section 2.3 below). The SARS virus (SARS-HCoV) also merits additional comments, as explained in Section 15. In addition the recent application of more sensitive molecular detection techniques has revealed the presence of other viruses, such as metapneumoviruses and bocaviruses, which might also be involved in the generation of respiratory symptoms [10]. However we do not know if these newly recognized viruses are really pathogenic or are simply “passengers” that eluded previous diagnostic techniques. Herpes simplex virus type 1 (HSV-1) also produces oral mucosal infections (“cold sores”), and these may be accompanied by symptoms reminiscent of respiratory viruses.

Clearly various families of viruses, with different structures and replication schemes, and consequently bearing different potential molecular targets, are involved in respiratory symptoms, and many of them are susceptible to *Echinacea* extracts, as indicated in Table 2. Among the possible viral targets are: (i) the virion itself (membrane components); (ii) cellular attachment or entry; (iii) one or more of the many stages in virus replication and development, particularly those that involve virus-specific enzymes; (iv) egress of progeny virus from infected cells. However, because of the variety of replication schemes among these viruses the chances of success for a single therapeutic drug are low, especially considering that in the majority of respiratory infections specific virus information is lacking.

Another problem with the specific antiviral target approach, especially in the case of compounds directed at specific viral genes or their products, is the inevitable emergence of virus resistant mutants [14, 15] and their subsequent spread through the community and environment. The conventional answer to this problem has been to use combinations of two or more antiviral drugs, with distinct molecular targets, notwithstanding the likely increase in undesirable side effects. However, a logical alternative approach is the use of a noncytotoxic agent that has the capacity to inhibit many different respiratory viruses simultaneously, and recent evidence indicates that certain herbal extracts could fulfill this requirement [15–17].

**2.2. Causes of Respiratory Symptoms.** “Colds”, “flu”, and “bronchitis” are terms that have been coined to describe various permutations of common symptoms, supposedly brought about by the actions of specific viral infections of the upper respiratory tract. These symptoms may include such familiar discomforts as sneezing, stuffy nose, irritation of mucous membranes, excess mucus production, sinusitis, cough, sore throat, malaise, and fever, as well as exacerbation of asthma and COPD (chronic obstructive pulmonary disease). In some cases symptoms may spread to include the lower respiratory tract and lungs and result in bronchiolitis or pneumonia [16, 18–20]. However these symptoms may not be a direct result of virus replication, which in many cases is minimal in differentiated airway tissues [21, 22], but rather an indirect consequence of virus-induced inflammatory responses [17, 23].

In respiratory infections, the invading virus initially encounters epithelial tissues, which are composed largely of epithelial cells and occasional dendritic cells and macrophages. These cells respond by means of the various antimicrobial strategies that make up the innate immune system, including defense peptides (antimicrobial peptides) and the secretion of various proinflammatory cytokines and other mediators of inflammation [24–26]. Other molecules such as kinins are released and are probably responsible for some of the early symptoms. Rhinoviruses have also been shown to induce kinin gene expression [27], although this effect is more likely a delayed effect of virus infection. In response to all these mediators phagocytic cells and various types of inflammatory cell may then be attracted to the site of infection [26]. In addition the redox balance of the cells may be adversely affected, either by the virus infection itself or as a consequence of the proinflammatory response [28].

Since most of the symptoms reflect this common non-specific host response to infecting agents, rather than to the direct cytolytic or cytopathic effects of a specific virus [15–17], then a more rational therapeutic approach would be the application of anti-inflammatory agents, especially if the intention of the therapy is to ameliorate symptoms. If a potential safe anti-inflammatory agent also contains multiple antiviral activities, then this would provide a bonus. Several herbal extracts have been shown to possess a combination of bioactivities that could be useful in the control of respiratory infections [17, 29, 30], and these *Echinacea* extracts have

become very popular, although, because of the variation in their chemical composition (as mentioned in Section 1), not all of them are necessarily beneficial.

**2.3. Influenza Virus Type A.** Influenza viruses are ubiquitous and produce significant annual morbidity and mortality throughout the world, with potentially devastating consequences for human and animal health, and the global economy [31, 32]. There are three types of influenza virus, A, B, and C, the latter two being confined mainly to humans, in which they produce relatively mild seasonal outbreaks. However the greatest impact derives from Influenza A virus, which has been associated with several well-known human pandemics during the last century and an increasing number of epidemics (epizootics) in domestic birds [31–34]. It is believed that influenza A virus originated in wild birds, possibly waterfowl such as ducks and geese and that these birds act as reservoirs and vectors for the many known subtypes (strains) of influenza A virus [35].

The classical symptoms of human influenza include cough, malaise, and fever, often accompanied by sore throat, nasal obstruction, and sputum production, which resolve spontaneously in most healthy individuals, although immune compromised and elderly individuals tend to be more vulnerable. Complications may include bronchitis and pneumonia, and exacerbation of asthma, and chronic obstructive pulmonary disease (COPD) [16, 18, 20].

More serious disease in healthy individuals, especially during pandemics, is often accompanied by excessive over-reaction of the innate immune response with the secretion of dangerous levels of cytokines (“cytokine storms”) and other inflammatory mediators [32–34]. Also the importance of concurrent bacterial infection cannot be overlooked, since this may lead to more serious outcomes [36]. Thus an ideal control agent should be able to prevent or reduce the replication and spread of the virus, as well as any potentially pathogenic bacterial infection, and also counteract the overproduction of inflammatory mediators.

Vaccines are generally advocated for routine application during each influenza seasonal outbreak, based on the prevailing strain of the previous season; but because of the unpredictable nature of influenza epidemics one cannot be sure of the success of any vaccine, and several researchers have questioned the wisdom of widespread vaccination [15, 31, 37, 38].

Numerous antiviral drugs for use in infected patients have been tested experimentally, in animal models and in humans, but none has proven satisfactory [30, 39]. The most recent synthetic compounds are the neuraminidase inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza), but drug-resistant strains of human and avian Influenza viruses have been documented with increasing frequency [40, 41].

**2.4. Antiviral Activities of *Echinacea Purpurea* (EP).** Early studies showed that only certain *Echinacea* extracts possessed significant antiviral activity. *E. purpurea* (EP) aerial parts and roots contained potent antiviral activities (virucidal) against

influenza virus, herpes simplex virus, and coronavirus, and these were distributed among more than one solvent derived fraction, probably reflecting the presence of more than one antiviral compound [11, 12]. However there was no correlation between antiviral activity and composition of the recognized marker compounds, that is, caffeic acids, polysaccharides, and alkylamides, and in fact a purified polysaccharide fraction from EP possessed no significant activity, while cichoric acid and several caffeic acid derivatives showed only weak to moderate activity, insufficient to account for the potent activities of EP [42]. In addition the antiviral activity of commercial ethanol tinctures from EP can remain stable for at least several years at ambient temperatures, which would seem to rule out many potential candidate bioactive compounds. Furthermore some, but not all, of the antiviral activities were due to photosensitizers, which again limits the number of prospective candidates [12, 43].

Recent studies with the standardized preparation Echinaforce (EF, comprising ethanol extracts of *E. purpurea*, 95% aerial parts plus 5% roots) showed that this preparation was a very potent virucidal agent against several viruses with membranes, as indicated in Table 2. In addition to HSV-1 and respiratory syncytial virus, all tested human and avian strains of influenza A virus, as well as influenza B virus, were susceptible [13, 44]. In addition rhinovirus and feline calicivirus were also equally susceptible at the relatively high concentrations of EF recommended for oral consumption [45]. Thus EF at 1:10 dilution (equivalent to 1.6 mg/mL dry weight/volume) was capable of killing at least  $10^5$  infectious viruses by direct contact. Adenoviruses however were resistant.

In further studies EP was found to be much less effective against intracellular virus [13, 44]. Consequently virus already present within a cell could be refractory to the inhibitory effect of EP, whereas virus particles shed into the extracellular fluids should be vulnerable. Therefore EP can act during initial contact with the virus, that is, at the inception of infection and also during transmission of virus from infected cells.

Additional experiments showed that continuous passage of influenza A virus in cell cultures in the presence of EP did not result in the emergence of resistant strains, whereas passage of the virus through successive cultures in the presence of Tamiflu rapidly generated Tamiflu resistance. Furthermore Tamiflu-resistant virus remained fully susceptible to EP [13]. Therefore continuous usage of EP in the population would be less likely to generate resistant strains of virus than Tamiflu or other anti-influenza compounds currently in the market.

It was shown by hemagglutination assays that EP inhibited the receptor-binding activity of influenza A viruses, over a range of EP concentrations, suggesting that EP interfered with viral entry into the cells, thus effectively rendering the virus noninfectious [13]. EP also inhibited neuraminidase activity *in vitro* (unpublished results), suggesting that the active compounds could block influenza virus entry and spread by acting on at least two virion targets. However, the susceptibility of other viruses, which do not rely on HA or

TABLE 2: Antiviral activities of EP at noncytotoxic concentrations.

virus	Relevant properties	Susceptible to EP ethanol extracts (+ or -)	Susceptible to EP aqueous extracts (+ or -)
rhinoviruses	ss RNA, no membrane	+ only at high conc.	+ only at high conc.
Influenza viruses	segmented RNA, + membrane & 2 target virion proteins (HA, NA)	+	+
Respiratory syncytial virus	ss RNA, + membrane & fusion protein	+	nt
Coronavirus	ss RNA + membrane	+ (mouse virus)	nt
Calicivirus	ss RNA, no membrane	+ high conc.	+ high conc.
Poliovirus	ss RNA, no membrane	-	-
Herpes viruses	ds DNA + membrane	+	+

Data from [11–13].

NA functions, to EP, indicates that there must be additional molecular targets.

### 3. Effects of EP on Virus-Infected Cells: Involvement of Cytokines and Chemokines

Rhinovirus-infected human bronchial and lung epithelial cells were used to study the effects of EP on cellular gene expression and secretion of cytokines and chemokines, principal mediators of inflammatory responses. Rhinoviruses stimulated the secretion of many different cytokines [46, 47], including the proinflammatory IL-1, IL-6, IL-8, and TNF $\alpha$ , which are known to be collectively involved in many of the symptoms common to respiratory infections, such as sneezing, fever, sore throat, nasal discharges, and inflammation in various respiratory tissues. Several EP preparations were able to completely or partly reverse this stimulation [48–50]. EP could be added before or after virus infection, with similar success, and also the results were not affected by virus dose or the time of exposure to EP [49].

It is noteworthy that RV14 and RV1A, which are known to use distinct cellular receptors (ICAM-1 and LDL, resp.), both stimulate cytokine secretion that is reversed by EP, suggesting the involvement of multiple signaling pathways. This concept was supported by the demonstration that many transcription factors (TF's) known to be associated with cytokines and chemokines were also stimulated by RV14, and these TF's were modulated by EP treatment [50, 51].

Other viruses, including HSV-1, influenza A virus, adenovirus type 3 and 11, and respiratory syncytial virus, stimulated the secretion of pro-inflammatory cytokines, and in each case the stimulation was reversed by EP (Table 3, and [44]). However only live infectious viruses were able to do this, for infection by equivalent doses of ultraviolet-inactivated viruses failed to elicit the responses. This suggests that the virus may have to enter the cells and undergo some degree of gene expression in order to stimulate the cytokine expression or secretion. It is also interesting that viruses such as adenoviruses, which are not vulnerable to direct attack by *Echinacea*, but could nevertheless stimulate cytokine secretion, were still susceptible to cytokine reversal.

In a more recent study with influenza A-infected mouse macrophage-like cells, the viral induced production of

cytokines and chemokines was also suppressed to various degrees by EP extracts and some of their constituent alkylamides (details below).

Several conclusions can be derived from these results. Probably the most important is that we could not correlate cytokine inhibitory effects, that is, anti-inflammatory properties, with specific individual compounds or groups of marker compounds, namely, alkylamides, polysaccharides, and caffeic acid derivatives. In the case of EP, all the fractions derived from roots, leaves plus stems, and flowers, were anti-inflammatory according to IL-6 and IL-8 measurements [52]. Numerous viral and bacterial infections, as well as wounds, result in substantial stimulation in levels of proinflammatory cytokines, especially IL-6 and IL-8, which are consequently considered to represent useful markers of an inflammatory condition [23, 29]. Thus any compound or herbal extract that inhibits or reverses this elevation in IL-6/8 and so forth, could be considered as a potential anti-inflammatory agent. Consequently many preparations derived from roots or aerial parts of EP would be expected to possess anti-inflammatory properties.

### 4. Mucin Secretion

Secretion of excessive mucus is one of the more annoying symptoms of colds and other respiratory infections and occurs frequently in chronic pulmonary infections. Many pharmaceuticals have been designed to relieve this feature of an infection, usually with the accompaniment of undesirable side effects.

Mucins, the products of at least 18 mucin genes in humans, are highly glycosylated macromolecules that constitute part of the innate defense system against respiratory pathogens [53]. But in some chronic conditions, and in response to certain airway infections such as rhinovirus [54], one or more genes may be induced to hypersecrete mucins. In studies on bronchial epithelial cells in culture, and in organotypic bronchial tissue cultures, rhinovirus induced the secretion of excess MUC5A, the dominant respiratory mucin, and EP reversed this secretion [55], suggesting that this could be an additional benefit of EP treatment. Table 4 shows a representative result.

TABLE 3: Cytokines/chemokines induced by viruses (+) and reversed by EP.

cytokine	RV1A	RV14	FluV	RSV	Ad 3
IL-1a	+	+	+	+	+
IL-5	+				+
IL-6	+	+	+	+	+
IL8 (CXCL-8)	+	+	+	+	+
TNF $\alpha$	+	+	+	+	+
GRO $\alpha$		+	+	+	
CCL-3				+	+
CCL-4				+	+

Adapted from [44]. RV: rhinovirus (1A/14); FluV: influenza virus (H1N1/H3N2); RSV: respiratory syncytial virus; Ad 3: adenovirus type 3.

### 5. 3-D Tissues of Human Airway Epithelium

It is important that the cell culture models used to evaluate anti-infectious agents reflect conditions *in vivo* as far as possible [56]. This condition was confirmed for EP by means of a commercial source of normal human airway epithelial tissue (EpiAirway tissue, a 3-D organotypic model), which could be propagated *ex vivo* under defined conditions such that tissue architecture and differentiation patterns were preserved, according to standard histological examination [57]. Under these conditions the effects of rhinovirus infection, and EP, on various parameters of tissue integrity and cytokine induction were evaluated [55]. Individual replicate tissue samples, maintained as inserts in culture for three days or three weeks, were infected with rhinovirus type 1A (RV1A), EP alone, a combination of the two, or medium only. None of the treatments affected the histological appearance or integrity of the tissues, all of which maintained a high level of cell viability and preservation of cilia, with the exception of the conspicuous virus-induced mucopolysaccharide inclusions in the goblet cells. There was no evidence of virus replication, although the RV infected tissues secreted substantial amounts of the proinflammatory cytokines IL-6 and IL-8, and this response was reversed by EP treatment (Table 5). The goblet cells also appeared normal and free of inclusions in the EP-treated tissues. Therefore these results confirmed the findings derived from studies of bronchial and lung epithelial cell lines (above), namely, that RV infection resulted in a substantial inflammatory response and mucin secretion in the absence of virus replication.

### 6. Effects of EP on Gene Expression in Rhinovirus-Infected Cells

Gene expression in human bronchial cells was analyzed by means of DNA microarrays, following treatment by one of two EP preparations, an aqueous extract and an ethanol extract, with or without infection by rhinovirus type 14 [58, 59]. Both extracts influenced the expression of many genes, including cytokine genes, although the pattern of expression was different for the two extracts. In addition the virus itself induced numerous changes, mostly increases in expression,

TABLE 4: Mucin secretion in human epithelial cell cultures.

Treatment	Ratio, treatment/control
None (control)	1.00
<i>Echinacea</i> (EP) only	0.76
Rhinovirus (RV1A) infection	2.18
<i>Echinacea</i> (EP) + RV1A	0.64

Bronchial epithelial cell cultures (BEAS-2B) were treated with combinations of EP and rhinovirus RV1A, as indicated, and supernatants were assayed for secreted MUC5A by ELISA (details in [55]).

and the extracts tended to decrease (i.e., restore to normal levels) these expression levels. Further analysis of the effects revealed that some of the changes in cytokine expression were interconnected through a specific transcription factor, C/EBPb (CAAT/enhancerbinding protein b, [59]). Since numerous transcription factors were known to be affected by EP extract in this same cell-virus system [50], it is tempting to conclude that many gene expression effects of *Echinacea* extracts could be due to changes in expression or activation status of multiple transcription factors. This in turn could be brought about by interaction with surface receptors or their intracellular modulators.

Analyses of IFN $\alpha$  (interferon alpha) gene expression, by DNA microarray analysis and PCR assays, revealed only a few changes in cells treated by different EP extracts, in uninfected bronchial epithelial cells, or in rhinovirus-infected cells, although certain interferon stimulated genes (ISG's) were upregulated by the virus and downregulated by the EP extracts [59]. Interferon bioassays in bronchial cells failed to detect IFN, in contrast to cells stimulated by the known IFN inducer poly I:C (Vimalanathan and Hudson, unpublished data). Negative findings were also reported recently in a mouse macrophage cell line infected with HSV-1 [60], from which the authors concluded that EP induced little if any IFN alpha or beta, and consequently antiviral effects of EP are not likely to involve the interferon system.

### 7. Antibacterial Activities

Several potentially pathogenic bacteria have the capacity to cause respiratory symptoms, resulting from initial interactions with epithelial cells of the oral and nasal mucosa and various parts of the lung. General features of infection by these organisms include proliferation and spread of the bacteria with resultant cellular damage, often aided by products of bacterial virulence genes, and the induction of excessive proinflammatory cytokines, which can lead to chronic inflammation. A herbal medicine with bactericidal and anti-inflammatory properties could provide benefits to individuals suffering from respiratory symptoms, and certain preparations of EP possess these activities, in addition to their antiviral activities described above. Results are summarized in Table 6.

*Streptococcus pyogenes* (Group A streptococcus, or GAS) is responsible for widespread infections, ranging from hundreds of millions of relatively mild cases of pharyngitis

globally, to more severe toxic shock syndromes and necrotizing fasciitis (“flesh-eating disease”), the more severe symptoms being ascribed to inflammatory cytokines (“cytokine storms”). In addition several *Streptococcal* gene products or virulence factors have been described which aid the bacteria in persistence in oral epithelia and saliva and dissemination to other tissues [63–66]. Consequently the dual actions of EP in killing the bacteria and reversing their proinflammatory activities could be a significant aid in combating such infections.

*Hemophilus influenzae* is part of the normal nasopharyngeal flora. Recently additional pathogenic strains have been associated with otitis media, chronic bronchitis, and pneumonia. Initial interaction with epithelial cells can result in proinflammatory cytokine secretion, via toll receptors and other mediators [66, 67]. EP can kill this organism readily and also inhibit the cytokine induction in bronchial epithelial cells (Table 6).

*Legionella pneumophila*, associated with Legionnaires’ disease and sometimes more severe cases of pneumonia, is widely distributed in water and soil, from which the organism can be inhaled as an aerosol and once inside alveolar macrophages localizes in a relatively resistant vacuole, in which it replicates [68, 69]. The organism is however very sensitive to EP, and its induction of proinflammatory cytokines is inhibited by EP treatment (Table 6).

*Staphylococcus aureus* has long been recognized as part of the normal skin flora, but Methicillin-resistant (MRSA) strains have been associated in recent years with increased frequency of hospital-acquired infections, resulting in severe pneumonia [70, 71]. Preparations of EP had relatively little effect on growth of MRSA or MSSA (methicillin sensitive *S. aureus*) but were very effective in inhibiting the proinflammatory response to the bacteria, indicating at least partial benefits in counteracting the detrimental effects of MRSA infection. *Klebsiella pneumoniae*, often associated with antibiotic resistant pneumonia, was also relatively resistant to the bactericidal effects of EP, as were *Mycobacterium smegmatis* and several other bacterial opportunists [62].

Several conclusions were drawn from these results: (i) EP and other *Echinacea* extracts were selective in their antibacterial activities; (ii) different organisms showed significant differences in their patterns of sensitivity; (iii) there were no correlations between chemical composition of extracts, in terms of known marker compounds, and their corresponding antibacterial activities; (iv) different preparations of *Echinacea* showed markedly different effects on bacteria, indicating that EP has distinct mechanisms of action against each bacterium; (v) in general EP can reverse the stimulation of proinflammatory cytokines regardless of the inducing bacterium or virus.

In addition to the studies with live bacteria, several groups have examined the effects of EP on the stimulation of inflammatory mediators by bacterial lipopolysaccharide (LPS usually derived from *Escherichia coli*) in various human and mouse cell cultures (see below, Section 12). Such models can serve as useful tests for anti-inflammatory agents [23, 29], although they do not necessarily represent live bacteria. These studies together indicate that EP is effectively a general

TABLE 5: Effects of EP on cytokines/mucin in 3-D tissues (ratio, treatment/control).

Treatment	IL-6	IL-8	MUC5A
EP	0.7	1.5	0.82
Rhinovirus	3.1	22.0	2.0
EP + rhinovirus	0.5	2.0	0.76

Data from [55].

anti-inflammatory agent and should be capable of ameliorating many of the symptoms of respiratory infections.

## 8. Coinfections with Viruses and Bacteria

Many authors have commented on the importance of coinfections of the airways between a respiratory virus and bacterial opportunists, and the possible enhancement of pulmonary diseases [36]. This has already been alluded to above, but a number of interesting studies have suggested possible mechanisms. Rhinoviruses, respiratory syncytial virus, and influenza virus, all altered signaling pathways in cultured epithelial cells, leading to cell membrane changes, including enhancement of attachment of certain bacteria, such as *H. influenzae* and *S. pneumoniae*, and resulting in cytopathic effects and possible colonization [72–75]. The reverse process was also demonstrated; prior infection of epithelial cells with *H. influenzae* increased the level of ICAM-1 receptors and resulted in enhanced rhinovirus infection [76]. Since EP can inactivate these viruses and bacteria on contact, as well as inhibiting their induction of proinflammatory cytokines, it is conceivable that such coinfections could be halted by EP oral administration in the form of tinctures or sprays.

## 9. Skin Infections

**9.1. Herpes Simplex Viruses.** Many skin infections are caused by viruses and microorganisms, and some of these could be amenable to topical treatment with EP. Herpes simplex virus types 1 and 2 (HSV-1/2) are common infections of oral and genital mucosa (“cold sores” and “genital sores”) and may become chronic infections with painful recurrent eruptions of the skin. Keratitis, an infection of the corneal epithelium and stromal tissue, which may also be recurrent, is a major cause of blindness. Fortunately most of these infections are accessible to topical agents, and not surprisingly many antiviral drugs have been marketed for their control, with variable success; but since most of these were designed to target viral genes, then emergence of resistant mutants is always a threat. However many plant extracts have been evaluated as anti-HSV agents [77, 78], and some of these have shown promising results. Several EP preparations showed potent virucidal activity against HSV, and these results have already been summarized in Section 2.1 above, in connection with respiratory viruses. The advantages of EP are its broad spectrum of activity, which minimizes the chances of resistant mutants arising, its high potency, and its relative lack of cytotoxicity.

9.2. *Acne*. *Acne vulgaris* is a multifactorial chronic disorder of the pilosebaceous follicles of the skin. *Propionibacterium acnes*, the dominant microbe in the sebaceous glandular regions, and inflammation, possibly initiated by this bacterium, appear to be the two main instigators and drivers of the disease, although other factors also appear to be involved, such as hormones and host nutritional status [79]. One explanation for the chronic nature of the disease is that the *P. acnes* induces the production of proinflammatory cytokines and chemokines, as well as other inflammatory mediators, which attract leukocytes to the site of infection and thereby set up a cascade of inflammatory responses, which also involve production of reactive oxygen species and other radicals, all of which in combination lead to the development of the acne lesion [79, 80].

Conventional therapy targets the development of the lesions, by means of retinoic acid analogues and other compounds and antibiotics directed against the bacterium [80]. Needless to say that the continued application of antibiotics entails the risk of resistant bacteria emerging. As an alternative approach, several recent reports have indicated the possibility of using plant extracts to counter the growth of the bacteria and/or the inflammatory response, although these have yet to be evaluated *in vivo* (e.g., [81]).

The organism itself, including laboratory and clinical isolates, was readily inactivated by dilutions of EP well below the normal recommended dose for topical treatment or for oral consumption in the control of colds and flu symptoms. Furthermore, the bacterial induction of proinflammatory cytokines, evident in three human cell lines examined (bronchial epithelial, lung epithelial, and skin fibroblasts) was also inhibited by EP, which suggests that this extract could offer dual benefits to acne patients [82]. Results are summarized in Table 7. The bacterial-induced cytokines included IL-6 and IL-8 (CXCL8), and to a lesser extent TNF $\alpha$ , which are hallmarks of inflammatory responses and would be expected to lead to influx of various inflammatory leukocytes. In addition the secretion of GRO $\alpha$  normally results in attraction of monocytes. Such a combination of cytokines could well explain the production of inflammation at the site of the infection; consequently an agent capable of safely reversing this effect should be beneficial to the patient.

9.3. *Fungi*. Fungi are the causative agents of numerous acute and chronic infections of the skin in many parts of the body and are often inaccessible to immunological attack. A limited number of studies have been reported on antifungal activity of various *Echinacea* extracts, including some EP extracts, against *Candida* species and some dermatophytes, but these were essentially qualitative by design and merely indicated that certain extracts could inhibit growth of some fungi [83, 84]. Since EP is formulated for oral and superficial applications, a more comprehensive evaluation of their fungistatic and fungicidal activities could support and validate some of the traditional uses and anecdotal reports of success in controlling chronic fungal infections of the skin [1–3].

## 10. Other Organisms

10.1. *Clostridium Difficile*. *C. difficile* is a Gram positive spore-forming gut anaerobe, which has been associated increasingly in recent years with epidemics of diarrhea (*Clostridium difficile*-associated diarrhea, CDAD) and pseudomembranous colitis, especially in health care institutions where patients may be subjected to chronic administration of antibiotics with resulting disruption of the normal balance of gut flora [85, 86]. Certain preparations of EP are capable of killing the organism [61], as shown in Table 8. This suggests that oral consumption of appropriate extracts, and possibly some teas made from EP, could be beneficial in infected patients. It has been reported that EP supplementation to the diet can apparently cause changes in gut bacterial flora [87], but it is not clear how significant such changes are and whether they are beneficial or not.

10.2. *Parasites*. Leishmaniasis and trypanosomiasis are diseases caused by protozoans *Leishmania* and *Trypanosoma* species belonging to the trypanosomatidae family. *Leishmania* species are mainly transmitted by the bite of an infected female phlebotomine sandfly [88]. *Trypanosoma* species are transmitted to humans by the bite of an infected tsetse fly (*Glossina* Genus), causing sleeping sickness, or an infected Assassin bug (sub-family *Triatominae*), causing Chagas disease in humans.

Both parasites cause widespread disease, with hundreds of thousands of new cases each year. Although drugs are available for the treatment of different stages of the diseases, they are frequently associated with severe side effects [88, 89]. However some recent studies have examined antiparasitic properties of several plant extracts, with promising results [89, 90].

Three different preparations of EP, one an ethanol extract and the others aqueous extracts of aerial plant parts, were evaluated for their ability to inhibit growth of the organisms *in vitro* and antiinflammatory activity [90].

All three EP preparations exhibited dose-dependent anti-leishmanial and trypanocidal activities after 24, 48, and 72 h incubation, although their relative potencies varied somewhat between extracts and target species. In general the ethanol extract was the most effective.

The mode of action of EP on these parasites is not known. It may differ between species, as it does for bacterial species (Section 8, above). Microscopic observations on the parasites indicated that EP slowed or eliminated their motility and caused cell rounding at high EP concentrations.

*L. donovani* also showed proinflammatory activity by stimulating the secretion of IL-6 and IL-8 (CXCL8) in two different human cell lines a bronchial epithelial line and a skin fibroblast line, similar to the stimulation shown in these cell lines by a variety of other viral and bacterial pathogens (as described above). In both cell types, the selected EP ethanol extract inhibited these *Leishmania*-induced responses (Table 9). Thus certain *Echinacea* preparations are capable of controlling growth of these parasites, and can inhibit the inflammatory activity induced by them.

TABLE 6: Antimicrobial effects of EP.

Species	Relative microbicidal activity of EP (- to ++)	Anti-inflammatory response by EP (+ or -)
<i>Streptococcus pyogenes</i>	++	+
<i>Hemophilus influenzae</i>	++	+
<i>Legionella pneumophila</i>	++	+
<i>Staphylococcus aureus</i>	+/-	+
<i>Klebsiella pneumoniae</i>	+/-	nt
<i>Propionibacterium acnes</i>	++	+
<i>Mycobacterium smegmatis</i>	+	+
<i>Clostridium difficile</i>	++	+
<i>Candida albicans</i>	+	nt
<i>Leishmania donovani</i>	+	+
<i>Trypanosoma brucei</i>	+	nt
Bacterial lipopolysaccharide (LPS)	n/a	+

Data from [61, 62]. nt = not tested; n/a = not applicable.

TABLE 7: EP reversal of *P. acnes* induced cytokines/chemokines.

Cytokine/chemokine	Ratio, <i>P. acnes</i> /control	Ratio, <i>P. acnes</i> + EP/control
IL-6	7.0	2.7
IL-8 (CXCL8)	3.0	0.35
GRO $\alpha$	1.9	0.56
TNF $\alpha$	1.2	0.52

Data from [82].

## 11. Antioxidant Properties

Many plant extracts have been shown to contain antioxidant properties, according to several standard tests, and phenolic components have often been incriminated [5, 29, 84, 91]. In addition to the generally accepted nutritional and health benefits of adding antioxidants to the diet, various acute and chronic infections have been associated with negative effects on the intracellular redox balance, including decreases in reduced glutathione and other important cellular antioxidants, which could have adverse effects on cellular metabolism [28].

Several reports have demonstrated the anti-oxidant property of EP preparations [84, 92]. Therefore supplementation with EP during infections could provide additional host protection by restoring the normal redox status.

## 12. Effects of EP on Immune Cell Functions

Several studies have reported the effects of *Echinacea* preparations on cellular gene expression in uninfected human cells relevant to the immune system. Changes in NK cells and cell surface antigens were described for blood cells taken from humans treated with EP [93, 94]. Randolph et al. [95] described changes in levels of expression (in terms of mRNAs and proteins) of several cytokine genes in human blood samples taken at different times after treatment with a commercial *Echinacea* product, and Brovelli et al. [96] found that the expression of several cytokine genes in cultured

human monocytes was influenced by the nature of the *Echinacea* preparation (stage of development and plant part used), presumably a reflection of their different chemical compositions.

Wang et al. [97] described the effects of a butanol fraction, derived from aerial parts of *E. purpurea*, on gene expression of immune-related molecules in human dendritic cells, which are part of the adaptive immune response. Many dendritic cell genes were affected, either upregulated or downregulated. Modulation of several cytokines by EP extract was also reported for cultured mouse dendritic cells [98]. None of these studies included infected cells, but they clearly confirmed that EP produces multiple gene effects in immune cells.

Many groups during the last 20 years have investigated the effects of *Echinacea* preparations on immune functions in cell cultures of mouse origin. In general, incubation of peritoneal, alveolar, or spleen monocyte/macrophage/lymphocyte preparations with EP resulted in stimulation of phagocytosis and cytokine secretion (reviewed in [5, 6]). This was in contrast to the studies described above for infected airway epithelial cells and fibroblasts, in which EP generally acted as an anti-inflammatory agent, via numerous changes in gene expression. In studies with partially purified EP polysaccharides (derived from *in vitro* EP plant cultures), stimulation of phagocytic activity in macrophages was also observed [99, 100], whereas isolated *Echinacea* alkylamides, individually or in groups, invariably displayed immune suppression in various cultured cells [101–105].

Unfortunately the earlier studies gave rise to a popular belief that *Echinacea* acted as a general “immune stimulant” or “immune booster”, statements that persist today on many commercial labels and web site descriptions. More recent studies however refer more appropriately to immune modulation rather than generalized immune stimulation (e.g., [47, 101–105]). Thus the net result of interactions between EP and cells *in vivo* will likely be influenced by the composition of the extract and the nature and location of the cell type.



TABLE 8: Effect of EP on *Clostridium difficile*.

EP extract	<i>C. difficile</i> (cfu/2.5 $\mu$ L)	$\log_{10}$ decrease
None	$1.5 \times 10^4$	—
EP ethanol (moderate alkylamides; no polysaccharide)	<10	>3
EP aqueous (+ polysaccharide; no alkylamides)	$4.0 \times 10^3$	0.6

Data from [61].

Rininger et al. made a different series of observations [106]. They reported that several preparations of EP showed minimal effects on production of cytokines in mouse cultures, unless the extracts were first exposed to a simulated gastric and intestinal digestion protocol, whereupon they acquired the ability to stimulate cytokine secretion. This result is clearly relevant to normal consumption of *Echinacea*, but remains unexplained.

Mouse macrophage cell line RAW 274 responded to LPS (bacterial lipopolysaccharide) treatment by stimulating the production of the inflammatory mediator nitric oxide, and EP was able to inhibit this effect [92], in accordance with the anticytokine effects described above. In our preliminary studies (Sharma and Hudson, unpublished results) rhinovirus also stimulated NO production in these same cells, and EP reversed this effect. Thus although potential phagocytic cells can be stimulated by EP, similar cells that have already been stimulated by LPS (or possibly by live bacteria) are inhibited by EP, indicating the tendency of EP to restore normal immune functions.

### 13. Studies in Rodents

EP-treated rats were assessed for spleen and lung macrophage-related functions, including phagocytic activity and cytokine stimulation, both of which were stimulated in the treated animals [107], in agreement with the studies on mouse cells cultured *in vitro*.

In the studies with EP polysaccharides derived from plant cultures, treated normal and immunosuppressed mice could be protected from a lethal dose of either *Listeria monocytogenes* or *Candida albicans*, apparently as a result of reduced titers of the organisms in target tissues [108]. A similar beneficial effect of standard EP extract was also observed in a recent study in which mice were infected with *Listeria monocytogenes* [104]. Whether the protection in these cases was due to enhanced phagocytic activity and clearance of the organisms in various tissues, or to direct contact of EP components with the organisms, or a combination of these and other factors, is not clear from these studies.

A recent report described the successful use of a polysaccharide-enriched aerial EP extract in controlling the course of influenza virus A infection (a well characterized H1N1 strain) in mice. There was no apparent effect on lung virus titers, but there were significant effects on various cytokine levels in lungs and sera [109]. The authors

concluded that the benefits of EP treatment resulted from modulation of inflammatory cytokines, rather than direct antiviral activity, and this concurs with the studies indicating anti-inflammatory properties of EP. Thus EP could still be beneficial in systemic infections in which EP components are unlikely to make direct contact with the virus.

Miller and colleagues [110–112] have made several studies of the effects of dietary EP extract on the aging and survival of mice. Several parameters of growth indicated possible benefits of the EP diet, and a noteworthy positive effect was the lifelong stimulation of NK cell activity and function, which could theoretically help the animals deal with infectious diseases.

### 14. Veterinary Applications

Most domestic animals, including pets, livestock, and fish, require treatment at some point in their lives for viral and microbial diseases, and the causative organisms are usually analogous to the corresponding human counterparts that have already been discussed, for example, avian influenza viruses, animal herpes viruses, various respiratory viruses and bacteria, and many fungal and parasitic infections. Consequently some of them should be responsive to *Echinacea* treatment, either as direct antivirals, antimicrobials, or as an anti-inflammatory agent. In addition some of these organisms, especially bacteria such as *Salmonella* and *Campylobacter* species, are also important sources of contaminated foods. Furthermore some commentators have pointed out the need to evaluate herbal preparations as replacements for at least part of the antibiotic onslaught that farmed animals often receive.

Certain herbs, including *Echinacea*, have a modern tradition of veterinary applications [113, 114], in North America and Europe, and although relatively few reports have described basic studies analogous to those described for human diseases, or even controlled trials in animals, invariably the treatments were concluded to be safe and free of significant side effects. This conclusion is also supported by the studies in mice and rats described in the previous section, in which toxic effects were not observed.

In regard to infections, a study in chicks infected with the protozoan parasite *Coccidia* concluded that dietary supplementation with EP root extract significantly decreased lesion scores and improved the health of the animals, in comparison with animals raised on a normal diet [115], although immune parameters were not measured; consequently it is not clear if the effect of EP was directed against the parasite itself or on the immune system. Nevertheless an effective treatment for coccidiosis would be welcome in the poultry industry.

On the other hand, in a study in young pigs [116], dietary EP was found to offer no protection against the porcine reproductive and respiratory syndrome virus (PRRS virus). Since this virus is a member of the arterivirus family (related to coronaviruses) and possesses a membrane, it would be expected to be susceptible to direct contact with EP. However the systemic nature of the infection could render it

TABLE 9: Effect of EP on *Leishmania*-induced secretion of cytokines.

Cells	Treatment	IL-6 (pg/mL)	IL-8 (pg/mL)
Bronchial epithelial cells	None, control	19.8	42.8
	+ EP	29.2	45.7
	+ Ld ( <i>Leishmania donovani</i> )	63.1	147.5
	+ Ld + EP	25.8	18.1
Human skin fibroblasts	None, control	29.8	62.0
	+ EP	38.2	74.4
	+ Ld	207.8	320.0
	+ Ld + EP	9.6	42.8

Data from [90], (standard deviations removed for simplicity).

inaccessible to dietary *Echinacea* components. Alternatively, the treatment protocol might have been inadequate.

In addition to controlling infections in domestic animals, herbal preparations have been advocated for such things as immune stimulation, growth promotion, and performance enhancement. Studies carried out in uninfected horses [117] and fish (Tilapia, [118]) suggest possibilities for *Echinacea* preparations. Again safety was not considered a problem for the animals. Fish, like other farmed animals, are always potentially vulnerable to viral and microbial infections, especially under conditions of stress; consequently alternative treatments to synthetic antimicrobials could be useful.

### 15. Emerging Infections: The Example of Severe Acute Respiratory Syndrome (SARS)

In case we needed to be reminded of the unpredictable nature of microbial epidemiology, the SARS epidemic erupted in China in late 2002 and quickly spread to many other countries over the next year and a half, resulting in more than 8,000 seriously infected individuals, of whom nearly 10% died. The epidemic was officially recorded as the first pandemic of the 21st century. As a result of emergency global public health measures the disease was halted and appears to have been controlled, although continued vigilance is necessary in case the causative virus “returns” [119].

The novel coronavirus responsible (SARS CoV), believed to be of animal origin, was isolated and its genome quickly sequenced [119]. However no satisfactory antiviral therapy was or is available. A study on autopsy lung tissues revealed that viral nucleoprotein and RNA were present mainly in alveolar epithelial cells, the probable initial site of virus replication, and to a lesser extent in macrophages, which could be the mode of systemic transmission [120]. A more recent review summarized studies on SARS CoV infection in cell cultures and emphasized the role of innate immune responses to the virus infection [121]. This virus, like other coronaviruses and other respiratory viruses, stimulates inflammatory cytokine secretion, although coronaviruses generally do not induce antiviral interferon. Thus modulation of cytokines and evasion of the innate immune defences could be important contributors to SARS virus

pathology. Therefore a herbal preparation like EP, with anti-inflammatory and virucidal properties, could be useful if the SARS were to return.

### 16. Standardization and Herb Mixtures

The need for standardization of EP has already been stressed in this paper, and a few studies have reinforced the concept of variation among different preparations from different suppliers [45, 106, 122]. This is a common problem with herbs, since the exact chemical composition can vary with different parts of a plant, the age and season of harvest, and the exact method of extraction [6, 7, 45, 96]. On the other hand we found in our studies that a standardized preparation of EP retained its full antiviral activity over several years, provided that storage conditions were appropriate (unpublished results). Similarly, preparations of EP maintained their cytokine-stimulating activity after storage for two years [123].

The presence of multiple antiviral activities among different extracts and fractions suggests that many kinds of *Echinacea* preparation, such as tinctures, sprays, tablets, teas, and so forth could be beneficial in the treatment of infections, although not all preparations are likely to be effective, and a recent study of 10 commercial preparations highlighted the variability of antiviral activity between different preparations, although lot-to-lot variation was less evident [45]. In general ethanol-based extracts had greater antiviral activities than aqueous extracts; but it was not possible to identify a specific component responsible for the activity. Furthermore there was no correlation between antiviral and anti-inflammatory activities.

Some commercial preparations of *Echinacea*, including those with EP, comprise mixtures of EP with other non-*Echinacea* herbs, the rationale being that two herbs are better than one, and three are even better, etc. In this respect such mixtures may be considered analogous to the complex mixtures often advocated in Chinese traditional medicine (TCM) and Ayurvedic medicines. However, this basic assumption is not necessarily valid, and in preliminary studies we examined a number of mixtures of EP with other standard herbs, for antiviral activities. In the majority of cases the mixtures were much less effective than the EP by itself and none were better than EP (unpublished data).

However since we do not know the exact mechanisms of the EP antiviral activity, or the nature of the active “compounds”, then we cannot explain these phenomena. But the lesson is obvious, namely, that some EP preparations by themselves can be potent antiviral agents and should not be mixed with unknowns.

## 17. Relevance of Bioactivities to Normal Consumption

*Echinacea* extracts intended for treatment of colds and flu, and sore throats are normally marketed for oral consumption. The active ingredients therefore acquire immediate exposure to the mucosal epithelia. According to our studies with standardized preparations (as described above), the recommended applications ensure that physiologically relevant amounts, that is to say, adequate local antiviral, antibacterial, and anti-inflammatory concentrations, are achieved under normal conditions of consumption. Subsequent absorption and metabolism of the various components, however, are less relevant to this application, since the sites of infection and inflammation are at the level of airway epithelial tissues.

Nevertheless, it has been demonstrated that alkylamides (and possibly additional components) can be absorbed quickly into the blood and remain in the circulation for some time, at least following *E. angustifolia* administration [124], although EP extracts tend to have relatively few alkylamides [6, 7]. Therefore, depending on the exact chemical composition of the *Echinacea* extract, there could be benefits to the consumer in addition to the initial interactions with the oral mucosa.

## 18. Clinical Trials

Numerous studies have been carried out in humans, with the objective of preventing or treating common colds, and these have been critically summarized [3, 5, 124, 125]. Unfortunately some of them used inadequately characterized *Echinacea* preparations derived, with various extraction protocols, from different species and plant parts. It is likely that the chemical composition of the extracts, and consequently their bioactivities, differed substantially. Attempts to rationalize the interpretation of results by the use of meta-analyses encountered the same problem, namely, the resulting variability in test materials. Consequently it is no surprise that the issue is “controversial”, and many popular scientists and pseudoscientists have jumped to conclusions about the efficacy or lack of efficacy of *Echinacea* in preventing or treating the common cold. In spite of these reservations there was an overall trend towards beneficial effects of the *Echinacea*, but equally importantly the safety of the preparations was confirmed repeatedly, including trials in children [125–127]. A more comprehensive trial that also includes influenza virus and other respiratory viruses, incorporating the use of a well-characterized bioactive *Echinacea* extract, is needed to confirm the benefits of oral *Echinacea* use.

Until that has been done, we are left with overwhelming anecdotal experience, in addition to the very encouraging results from the cell and tissue culture studies and animal studies, but no definitive conclusion regarding clinical efficacy in humans.

## 19. Mechanisms of Action

The results described have indicated that some *Echinacea* extracts evidently contain compounds, or combinations of compounds, with the ability to interact specifically with viral and microbial targets [13, 17, 62, 128]. In addition, these extracts can affect various signaling pathways of epithelial cells and inhibit the virus/bacterium-induced secretion of cytokines/chemokines and other inflammatory mediators that were responsible for the pulmonary symptoms. Since many signaling pathways can be affected by *Echinacea* in different cell types, including immune cells [47, 59, 97], it is conceivable that the overall beneficial effects are due to a particular combination of compounds acting synergistically. Examples of synergism in herbal medicine have been described and in some cases validated experimentally [23, 129, 130], and it is likely that certain *Echinacea* preparations also display synergism. However, in spite of our attempts to correlate bioactivities of *Echinacea* preparations with recognized chemical markers, that is, polysaccharides, caffeic acid derivatives, and alkylamides [5–7], we have not succeeded in doing so. In contrast, preliminary evidence in our laboratory has implicated other classes of compounds (unpublished data).

## 20. Conclusions

These studies on EP indicate multiple actions of the herbal preparation, resulting either from the individual activities of several compounds or the synergistic effect of different compounds. The resulting benefits are: (1) direct virucidal activity/activities against several viruses involved in respiratory infections, at concentrations which are not cytotoxic; (2) direct bactericidal actions against certain potentially pathogenic respiratory bacteria; (3) inactivation of other microbial pathogens relevant to humans and their domesticated animals; (4) reversal of the proinflammatory response of epithelial cells and tissues to various viruses and bacteria; (5) modulation of certain immune cell functions; (6) reversal of the excessive mucin secretion induced by rhinovirus. These bioactivities result from changes in gene expression. Thus a combination of these beneficial activities could reduce the amount of prevailing viable pathogens, and their transmission and also lead to amelioration of the symptoms of the infection.

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