Echinacea purpurea: Pharmacology, phytochemistry and analysis methods

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ABSTRACT

Echinacea purpurea (Asteraceae) is a perennial medicinal herb with important immunostimulatory and anti-inflammatory properties, especially the alleviation of cold symptoms. The plant also attracted scientists' attention to assess other aspects of its beneficial effects. For instance, antianxiety, antidepression, cytotoxicity, and antimutagenicity as induced by the plant have been revealed in various studies. The findings of the clinical trials are controversial in terms of side effects. While some studies revealed the beneficial effects of the plant on the patients and no severe adverse effects, some others have reported serious side effects including abdominal pain, angioedema, dyspnea, nausea, pruritus, rash, erythema, and urticaria. Other biological activities of the plant such as antioxidant, antibacterial, antiviral, and larvicidal activities have been reported in previous experimental studies. Different classes of secondary metabolites of the plant such as alkamides, caffeic acid derivatives, polysaccharides, and glycoproteins are believed to be biologically and pharmacologically active. Actually, concurrent determination and single analysis of cichoric acid and alkamides have been successfully developed mainly by using high-performance liquid chromatography (HPLC) coupled with different detectors including UV spectrophotometric, coulometric electrochemical, and electrospray ionization mass spectrometric detectors. The results of the studies which were controversial revealed that in spite of major experiments successfully accomplished using *E. purpurea*, many questions remain unanswered and future investigations may aim for complete recognition of the plant's mechanism of action using new, complementary methods.

Key words: Analysis methods, anti-inflammatory, clinical trial, cytotoxic, *Echinacea purpurea*, immunomodulatory, psychotic

INTRODUCTION

Echinacea purpurea (L.) Moench is one of the most important and well-known medicinal plants in the world, belonging to the Asteraceae (Compositae) family. The plant is the most widely cultivated medicinal plant in this species,[1] which has been mainly used in chemo-preventive and chemotherapy for infectious diseases in both upper and lower respiratory systems.[2,3] This species has been traditionally employed for the treatment of toothache, bowel pain, snake bite, skin disorders, seizure, chronic arthritis, and cancer.[2] Although the isolation and structural elucidation of its main compounds have been noticed by investigators, there is no affirmation about its mechanism of action. Alkamides, caffeic acid derivatives, and polysaccharides have been considered important constituents of the plant. A number of studies revealed that alkamides are involved in the immunomodulatory properties of *Echinacea* extracts *in vitro* and *in vivo*.[4,5] Additionally, caffeic acid is found in some species of *Echinacea* and could be applied toward authentication and quality control of the plant extracts. The polysaccharides play an important role in the anti-inflammatory effect of *Echinacea* preparations.[6] Taxonomic, chemical, pharmacological, and clinical characteristics of some species of the *Echinacea* genus including *E. angustifolia*, *E. pallida,* and *E. purpurea* were reviewed in previous papers.[1,7] Medicinal properties of the plant were also considered in a review paper, which suggested that more research is required for more definitive medicinal recommendations.[8] This paper is a review about *E. purpurea*: Its phytochemical contents and its pharmacological and biological activities, along with common methods of plant extract analysis. In addition, the psychoactive and mosquitocidal effects of the plant are mentioned in this paper.

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PHARMACOLOGICAL ACTIVITY

Immunomodulatory effects

The immunostimulant activity of the plant or its preparations is caused by three mechanisms: Phagocytosis activation, fibroblast stimulation, and the enhancement of respiratory activity that results in augmentation of leukocyte mobility.[9] There are numerous *in vivo* studies on the immunomodulatory and anti-inflammatory effects of *E. purpurea* that suggest that innate immunity is enhanced by administration of the plant and that the immune system is strengthened against pathogenic infections through activation of the neutrophils, macrophages, polymorphonuclear leukocytes (PMN), and natural killer (NK) cells.[7] For this reason, it can be suitable for prevention against and treatment of various infectious diseases such as infections of the upper and lower respiratory systems, wound infections, and chronic pelvic infections.[2,3] The complex chemical composition of the roots and herbs of *Echinacea* involves alkamides [Figure 1], ketoalkenes, caffeic acid derivatives, polysaccharides, and glycoproteins, which are believed to be responsible for noted immunostimulatory and anti-inflammatory activities.[7] Moreover, alkamides are demonstrated to be effective on cannabinoid receptor type 2 (CB2), and this is considered as a possible mechanism of their immunomodulatory properties.[10-12] Their possible molecular mechanism could be the increase of cyclic adenosine monophosphate (cAMP), p38 mitogen-activated protein kinases (p38/MAPK), and c-Jun N-terminal kinases (JNK) signaling, as well as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activating transcription factor 2/cAMP responsive element binding protein 1 (ATF-2/CREB-1) in primary human monocytes and macrophages.[5] Another study showed that N-alkamides from a root and herb tincture induce synergistic activity on CB2 and ultimately lead to immunomodulatory effects along with the superstimulation of interleukin-10 (IL-10) and the inhibition of tumor necrosis factor (TNF-α) *in vitro*.[12] They are also able to inhibit both cyclooxygenase enzymes (COX-1 and COX-2) and 5-lipoxygenase (F-LO), causing the inhibition of NK cells and anti-inflammatory activity.[13] The administration of the alkamide fraction of the plant in healthy rats, stimulated with bacterial lipopolysaccharide (LPS), caused a dose-dependent enhancement in nitric oxide (NO) and TNF-α release from alveolar macrophages as well as phagocytic activity. The mentioned effects may contribute to produce the first immune response mediators and to antiviral activity. However, different doses of alkamides, cichoric acid, and polysaccharides did not impact the release of TNF-α, interferon gamma (IFNγ), or IL-2 by splenocytes.[4] Additionally, *E. purpurea* is reported to stimulate the NO response of peritoneal exudate cells (PEC) to LPS 2-4 times higher compared to the control in Swiss mice.[3] Moreover, alkamides diffuse through Caco-2 monolayers and their diffusion is not affected by the presence of other constituents.[14] They are also characterized after oral administration of the plant using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in the blood stream.[15,16] Alkamides in lozenges made from *E. purpurea* have been rapidly absorbed through the buccal and esophageal membranes in

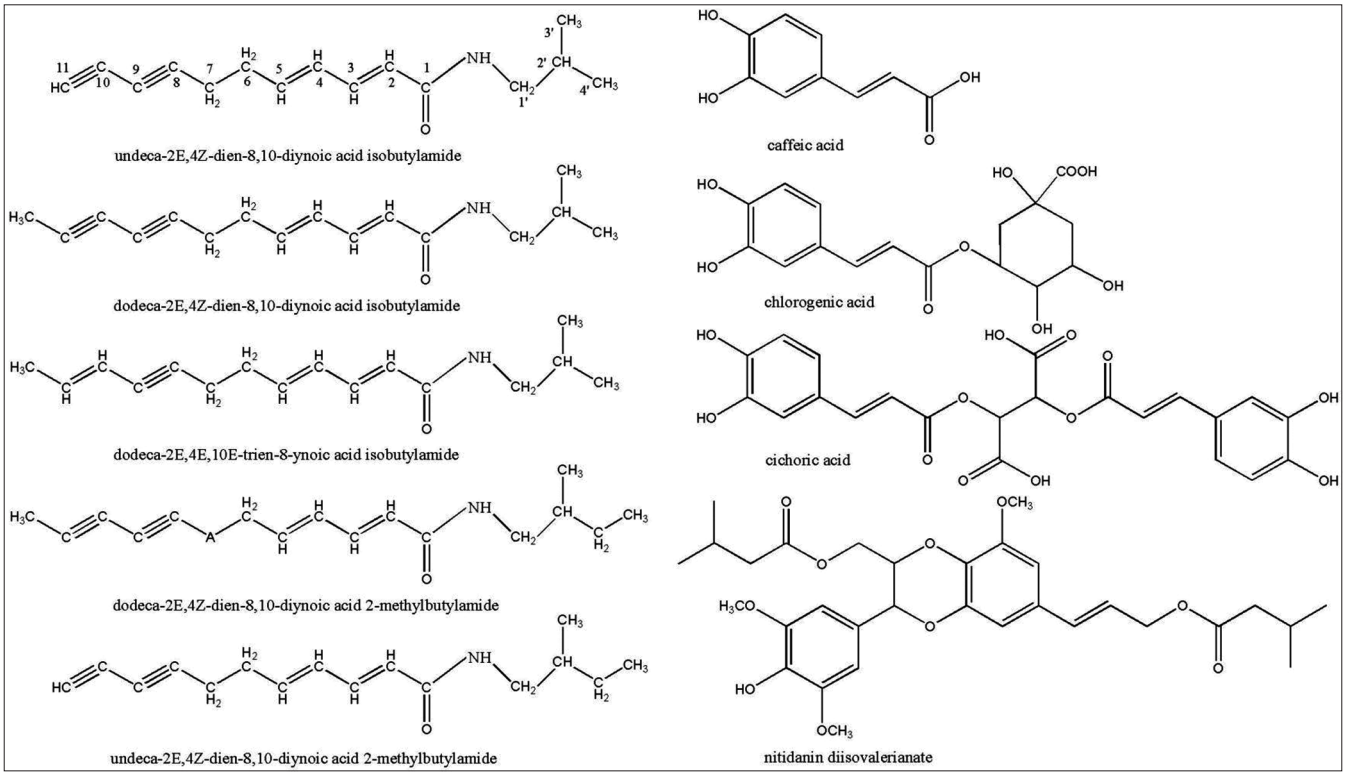

Figure 1: Chemical structure of some secondary metabolites of *E. purpurea*

six healthy volunteers. All the doses significantly decreased proinflammatory cytokines including IL-12p70, IL-8, IL-6, and TNF-α. It seems that this effect is independent of the alkamide doses, suggesting synergistic effects of the plant compounds.[17] Some pharmacological activities of alkamides are summarized in Figure 2.

Another important type of bioactive compound of this plant is polysaccharides, which have been reported to increase production of interleukin-1 (IL-1), interleukin-6 (IL-6), and TNF-α by macrophage, along with the enhancement of their phagocytosis, microbicidal activity (both *in vitro* and in vivo), and the production and secretion of cerebrospinal fluids (CSFs).[18-21] The results of some studies revealed that polysaccharides from *E. purpurea* cell culture are able to provide protection against *Listeria monocytogenes* and *Candida albicans*, mainly through macrophages and PMN) in normal mice.[22] Moreover, a polysaccharide treatment of immune-deficient mice by cyclophosphamide or cyclosporin A made them resistant toward *L. monocytogenes* and *C. albicans*, which are macrophage- and granulocyte-dependent infections, respectively. They also enhanced cytotoxicity against WEHI 164 tumor cells in macrophages *in vitro*, along with increasing the number of leukocytes and neutrophil granulocytes in immune-deficient mice.[23] It has been demonstrated that the oral administration of the root extract of *E. purpurea* increases the NK cells in normal, leukemic, and aging mice as well as neutrophils and eosinophils in rabbits.[7,24] The effects of both a polysaccharide-rich aqueous extract (of root) and an alkamide-rich ethanolic extract (of leaf) were assessed on dendritic cells (DCs). Investigation of the effects of the plant on DCs is important, since these cells play a role in both innate and adaptive immunity. The results of this investigation revealed that alkamides may suppress the function of DCs of murines; however, polysaccharides in the aqueous extract may activate DCs.[25] It is revealed that the expression of CD83 marker a marker of DC maturation, is significantly enhanced by flower and root extracts of the plant, while stem and leaf extracts can greatly decrease CD83 levels. Moreover, the extracts from root and aerial parts of the plant are reported to play a role in up- and down-regulation of insulin-like growth factor 1 receptor (IGF1R), respectively. In addition, the root extract can up-regulate some genes involved in immune cell activation or function including: Chemokine (C-C motif) ligand 4 (CCL4); interleukin-7 receptor (IL7R); nuclear factor of activated T-cells, cytoplasmic 2 (NFATC2); T-box transcription factor (TBX21); cytohesin-interacting protein (PSCDBP); integrin, alpha E (ITGAE); and intercellular adhesion molecule-1 (ICAM1), while CD34 and integrin beta-1 (ITGB1) were down-regulated by aerial part extract in DCs. It is implied that aerial parts of the plant except flowers decrease the adhesion, chemotaxis, and activation function of specific immune cell types such as DCs. Down-regulation of other immunomodulatory genes such as: Signal transducers and activators of transcription (STAT); tumor necrosis factor ligand superfamily, member 6 (FASLG); β2-microglobulin; elongation factor; proto-oncogene protein Wnt-1 (WNT1); and transcription factor ETV5 was observed with the root extract. The augmentation of gene expression by root extract can be attributed to the higher content of alkamides in it compared to extract of the aerial parts.[26]

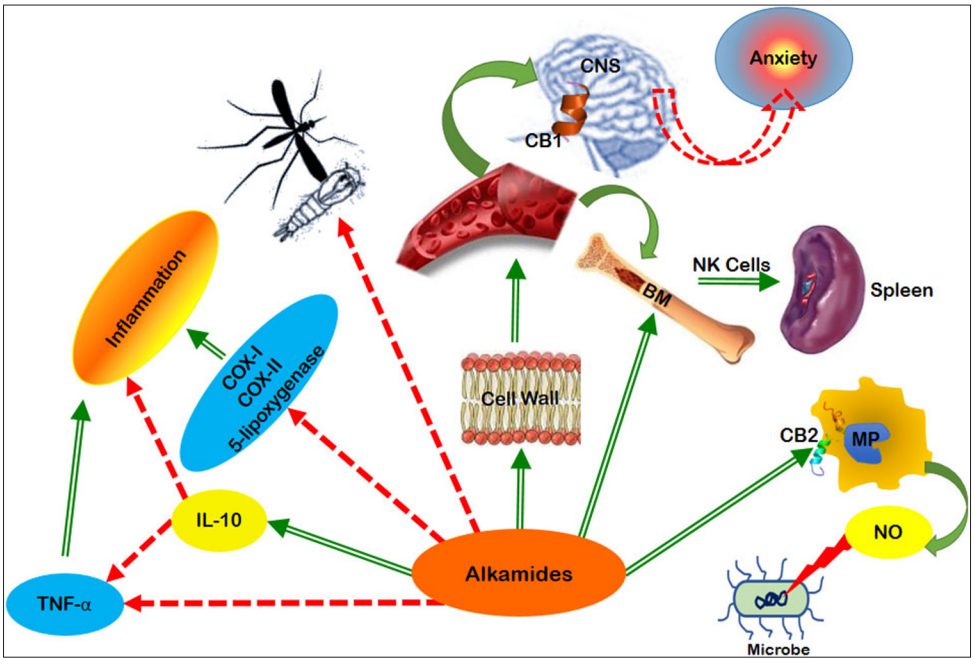

Figure 2: Schematic profile of some biological and pharmacological activities of alkamides isolated from *E. purpurea*. BM = Bone marrow, CB = Cannabinoid receptor, CNS = Central nervous system, COX = Cyclooxygenase enzyme, IL-10 = Interleukin-10, MP = Macrophage, NK = Natural killer cells, NO = Nitric oxide, TNF-α = Tumor necrosis Factor-α

When the plant root powder was administered to the male rats (in two doses: 30 and 100 mg/kg, for 3 and 15 days, respectively, to prolactin secretion), regulation of the immune system was observed. The low dose for 3 days did not affect prolactin secretion, while the higher dose for 15 days significantly inhibited prolactin secretion. A possible mechanism for this activity may be attributed to dopaminergic activity of the plant and/or structural similarity between chlorogenic acid and dopamine.[27] The activity of the combined preparation of *E. purpurea* and *Glycyrrhiza glabra* has been examined both *in vitro* and in vivo, and the results suggested that this mixture has a potential of synergic effects on immunostimulation, unlike the single plant therapy.[28]

Arabinogalactan-proteins from the suspension culture of *E. purpurea* did not influence the proliferation of mouse lymphocytes and only showed low activity on nitrite- and IL6 production in alveolar mouse macrophage culture and in the immunoglobulin M (IgM) production of mouse lymphocytes.[29] The root extract of the plant enhances NK cell numbers by increasing their production at the site of bone generation, causing an increase in their numbers in the spleen and also an increase in their antitumor and cytolytic functions in the aged mice.[30] Treatment with *E. purpurea* caused a significant increase in monocytes, neutrophils, and white blood cells (WBC) as well as in total protein and gamma globulin in rats. Besides, the plant extract and levamisole showed synergistic effects on phagocytic activity, and on monocyte and gamma globulin levels.[31]

Anti-inflammatory effects

The *Echinacea* preparation, interestingly, has reversed the inflammation caused by some bacteria in a culture of epithelial cells by reducing cytokines.[32] Carrageenan-induced paw edema in experimental animals is a test for the evaluation of anti-inflammatory effects of plants extracts.[33] Dried root powder of the plant, administered to the mice (30-100 mg/kg), inhibited carrageenan-induced paw edema similar to indomethacin.[34] This effect may be attributed to the inhibition of COX-1 and to a lesser extent COX-2 by alkamides.[35] Also, it is reported that the ethanol extracts of both aerial parts and roots of the plant have inhibited fibroblast-induced collagen concentration.[36] The effects of *Echinacea* and some of its compounds on NF-κB expression by Jurkat cells (a human T-cell line) were assessed in the presence and absence of stimulation by LPS and phorbol 12-myristate 13-acetate (PMA). NF-κB is a nuclear transcription factor stimulating the expression of some genes such as key factors of inflammation, e.g., TNF-α, IL-1, chemokines, adhesion molecules, and COX-2. In the absence of stimulants, all root extracts and compounds did not show a significant effect on NF-κB expression. In fact, LPS decreased NF-κB expression, while this effect was significantly reversed in the presence of root extracts, cichoric acid, and the alkamides fractions. Cichoric acid and a 2,4-diene alkamide are demonstrated to significantly increase NF-κB levels, while a 2-ene alkamide caused a significant inhibition, indicating considerable diversity in the bioactivity of this plant.[37]

Psychoactive activity

The anxiolytic activity of *Echinacea* drugs was determined in experimental animals with lower doses than those used in traditional indications.[38] Alkamides of *Echinacea* have been reported to have cannabinomimetic properties on both cannabinoid CB1 and CB2 receptors, which may be attributed to their structural similarity to the endogenous cannabinoid receptor ligand anandamide.[39] Anandamide's effects are mediated in the brain and periphery by CB1 and CB2 receptors. The activation of the former with endogenous ligands plays a considerable role in controlling anxiety and the latter is mainly involved in immune system activities.[40] Plants rich in alkamides induce paresthesia and were applied by Native Americans traditionally and also by physicians in the early 20th century as a sialogogue, an antitussive, and a remedy for toothache.[41,42] The slight differences in the structures of the alkamides revealed significant differences in CB receptor activity, for instance the replacement of isobutylamide moiety of the dodeca-2E,4E,8Z,10Z/E-tetraenoic acid isobutylamide for 2-methylbutylamide moiety turned the G-protein stimulation property from inverse agonist to partial agonist.[43] However, the extracts of *E.purpurea* were not active in the antiacetylcholinesterase (antiAChE) assay.[44] The plant tincture increased the stimulation effect of L-3,4-dihydroxyphenylalanine (L-DOPA) and showed antidepressant activity in the clofelin-induced depression test in white rats.[45]

Cytotoxic activity

The extract of the flowers and cichoric acid inhibited both the human colon cancer cell lines Caco-2 and HCT-116 in a dose dependent manner after 48 h. Cichoric acid has decreased telomerase activity in HCT-116 cell line, which could be assumed as the molecular mechanism of apoptosis indication.[46] Moreover, an n-hexane extract of the plant root obtained from three *Echinacea* species showed potential anticancer activity.[47]

Mutagenicity

The extract of *E. purpurea* flower did not exhibit mutagenicity against *Salmonella typhimurium* TA98 and TA100 with or without the S9 fraction. It also showed a dose-dependent inhibition against the mutagenicity of 2-aminoantheracene, therefore it could be a good antimutagenic agent.[48] High concentrations of the plant (8 mg/ml) reduced sperm motility and sperm penetration of hamster oocytes attributed to sperm DNA denaturation, while these effects were not observed by administrating low concentrations.[49,50]

Toxicology

Generally, animal studies of various preparations of *Echinacea* species have shown low toxicity.[8] In a study of acute toxicity, LD50 value was calculated as 2500 mg/kg in an intraperitoneal injection of the polysaccharide fraction of the plant in female mice.[51] In other studies, the LD50 values of oral and intravenous administration of the plant juice evaluated more than 30 g/kg and 10 g/kg in mice, and 15 g/kg and 5 g/kg in rats, respectively.[52,53]

Clinical trials

The findings of clinical trials of the plant preparation are controversial. The results of a randomized blinded trial revealed no significant difference in the incidence and severity of colds and respiratory infection between *Echinacea* and placebo groups in 108 patients during 8 weeks of administration of the plant juice. Only a small decrease for the total lymphocyte counts was found, which may have occurred by chance rather than because of a true effect of the plant juice or placebo.[54] On the other hand, the results of a study showed that preparations of *E. purpurea* had a beneficial effect on adults with cold symptoms in clinical trials, especially in comparison to placebo, if the treatment is begun early.[55,56]

Adverse effects

In several clinical studies, the frequency of adverse effects in the *Echinacea* group and control group were similar and not statistically significant, while in some studies, rash and gastrointestinal symptoms were reported from the *Echinacea* group. Some adverse effects were also reported by the UK Committee on Safety of Medicines and the Medicines and Healthcare Products Regulatory Agency's spontaneous reporting scheme (the "yellow card" scheme), commonly including abdominal pain, angioedema, dyspnea, nausea, pruritus, rash, erythema, and urticaria.[7]

Contraindications

The *Echinacea* preparations are contraindicated in some patients including those with progressive systemic diseases such as tuberculosis, leukemia and leukemia-like diseases, collagen disorders, multiple sclerosis, and other autoimmune diseases.[57] Some *Echinacea* products are also contraindicated in AIDS and HIV infections. These statements are based on the theory of immunumodulatory activity of *Echinacea*, although there is an opposing idea that these products are not harmful in patients with autoimmune diseases.[58] There are no clinical data available supporting the mentioned views, but it is recommended to avoid these preparations in immune-related diseases.[7] The preparations of *Echinacea* should be administered with caution concomitantly with immunosuppressant drugs.[7] Effects of some preparations made from the root and herb of *E. purpurea* along with cichoric acid were tested on the human drug-metabolizing enzyme cytochrome P450 3A4 (CYP3A4). The results indicated that the preparations moderately inhibited the enzyme, while cichoric acid showed low inhibition activity.[59] The findings of a study of healthy nonsmoking volunteers showed that the plant root remedy inhibited cytochrome P450 1A2 (CYP1A2) and intestinal CYP3A4 but not cytochrome P450 2C9 (CYP2C9) or cytochrome P450 2D6 (CYP2D6), whereas induced hepatic CYP3A4 activity. The selective effects of the plant on CYP3A4 activity may be explained by several mechanisms: The constituents of the plant, that are responsible for CYP3A4 inhibition are not systematically available; the constituents that are responsible for CYP3A4 induction may rapidly be absorbed, leading to a lack of intestinal CYP3A4 induction; hepatic CYP3A4 induction may occur by the metabolites of the plant, or the CYP3A4 induction may involve tissue activators that are influenced by constituents of the plant.[60] Additionally, the plant inhibited metabolism of testosterone by CYP3A4 via nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reaction.[61]

BIOLOGICAL ACTIVITY

Antioxidant activity

In some studies, radical-scavenging activities of the plant extracts were tested.[62,63] For this plant, antioxidant activity compared with the alkamides and cichoric acid that are the characteristic compounds of *Echinacea*. The free radical-scavenging activity of the extracts was related to their cichoric acid contents, while alkamides were inactive against free radicals.[44,64] Although in some studies it was revealed that the extract of the plant did not show any antioxidant property,[65] other investigations of the root extract of the plant revealed antioxidant activity,[48] which may be attributed to the phenolic contents and cichoric acid in the plant.[66] Cichoric acid shows an efficient radical-scavenging activity toward 2,2-diphenyl-1-picrylhydrazyl (DPPH), comparable to flavonoids and rosmarinic acid. Although alkamides have not exhibited antioxidant activity, they are able to increase the activity of cichoric acid through two mechanisms: First, surface activity that enables cichoric acid to have better access to inhibit lipid oxidation in the lipophilic droplets of emulsion, and second, regeneration of cichoric acid by donating allylic hydrogen to the one-electron oxidized cichoric acid.[64,67]

Antibacterial and antifungal activity

The extract of *E. purpurea* considerably inhibited growth of *Candida albicans* and *Saccharomyces cerevisiae*, but no inhibition zone was observed for *Aspergillus niger*. The extract obtained by classical method showed higher antimicrobial activity in comparison with those obtained by ultrasound-assisted extraction.[68] In another study, three pathogens *Streptococcus pyogenes, Haemophilus influenzae*, and *Legionella pneumophila* were sensitive to the preparation of the plant, although *Acinetobacter baumannii*, *Bacillus cereus, Bacillus subtilis, Enterococcus faecalis (*vancomycin-resistant), *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* were relatively resistant to the drug, while two fungi organisms, *C. albicans* and Trichoderma viride, were essentially resistant to the preparation.[32] The hexane extract of the root inhibited both yeasts *S. cerevisiae* and *C. albicans*. Additionally, 1-Tridecene-3,5,7,9,11-pentayne, a polyacetylenic compound from root of the plant has showed light-mediated inhibition toward *S. cerevisiae*.[69]

Antiviral activity

The aqueous extract of *E. purpurea* were active against acyclovir-susceptible and acyclovir-resistant strains of both *herpes simplex* virus 1 (HSV-1) and *herpes simplex* virus 2 (HSV-2) *in vitro*,[70] whereas the hexane extract of the plant root and cichoric acid inhibited HSV-1.[71] In addition, cichoric acid inhibited human immunodeficiency virus type 1 (HIV-1) integrase.[72,73] Mouse embryonic fibroblasts incubating with the plant juice and alcoholic

root extract were resistant to influenza A2, herpes, and vesicular stomatitis virus infection for 24 h.[74] The standard preparation of the plant showed strong inhibition against the influenza viruses A/Victoria/75 (H3N2) and A/Puerto Rico/8/1934 (H1N1), avian strains A/Thailand/1(KAN-1)/2004 (H5N1) and A/FPV/Dutch/1927 (H7N7), and the pandemic novel swine-origin influenza A (S-OIV) (H1N1) in direct contact. Moreover, the results of hemagglutination (HA) assays indicated that the preparation inhibited HA activity and could therefore block entry of a virus into treated cells.[75] The administration of a polysaccharide extract of the plant to infected mice with influenza A H1N1 (A/WSN/33) caused weight loss, but similar pulmonary viral titers were observed in comparison with untreated mice. Lower systemic and pulmonary keratinocyte chemoattract (KC) and interleukin 10 (IL-10) levels, and systemic levels of IFNγ were observed in treated mice, indicating that *E. purpurea* could modulate the clinical symptoms of influenza by altering cytokines.[76] Based on the results of the two latter studies, it seems that different components of the plant have beneficial effects on influenza patients through different mechanisms.

Mosquitocidal property

Purified alkamides from *E. purpurea* show mosquitocidal activity against *Aedes aegypti* larvae. The alkamides with isobutylamide moiety show stronger mosquitocidal activity compared to those with 2-methylbutylamide moiety, suggesting that isobutyl plays a role in the mosquitocidal property of alkamides.[35,77]

Phytochemical content

Various compounds belonging to different classes of secondary metabolites have been isolated and identified in *E. purpurea* extract. Alkamides, caffeic acid derivatives, and polysaccharides are the three major groups of secondary metabolites in the plant, which have been considered mostly in manuscripts [Figure 1]. In one study, 10 alkamides, mostly with isobutylamide and 2-methylbutylamide moieties, have been successfully purified from the n-hexane extract of the plant root.[78] Purification of the chloroform extract of the plant root also has resulted in the purification of some alkamides with isobutylamide and 2-methylbutylamide moieties and nitidanin-diisovalerianate as well as a sequesterpene, 1β-hydroxy-4 (15),5E,10 (14)-germacratriene, using chromatographic methods.[43] Isolated alkamides (with isobutylamides) mostly contained 2,4-dienoic units in their structures.[7] The results of a high-performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS) analysis of the roots and aerial parts of the plant showed that the storage conditions and methods of the extraction can significantly influence the contents of cichoric acid and alkamide derivatives.[79] Contents of alkamides and cichoric acid in *E. purpurea* (root and aerial parts) have been analyzed in different samples, and the results showed that high-quality root contains more than 6 mg/g alkamides, while cichoric acid content for root and aerial parts was evaluated as more than 15 mg/g. The aerial parts of the plant usually cannot be considered as a source of alkamides. For marketing, a minimum standard level could be assumed as > 3 mg/g for alkamides and > 5 mg/g for cichoric acid.[80] Polysaccharides and polyacetylenes along with glycoproteins were also purified from the aerial parts of the plant as well.[7] The juice of the plant contains heterogeneous polysaccharides (MW < 10 kDa), inulin-type fractions (MW = 6 kDa) and acidic highly branched arabinogalactan polysaccharide (MW = 70 kDa). Arabinogalactan-protein isolated from the aerial parts of the plant is composed of 83% polysaccharide (galactose/arabinose), 4-5% uronic acids, and 7% protein, with high concentrations of serine and hydroxyproline.[7,81] Other compounds including alkaloids, amides, and flavonoids (quercetin, kaempferol, isorhamnetin and their free phenolic acids including p-coumaric, p-hydroxybenzoic, and protocatechuic acids) have also been isolated and identified from the plant.[7,82]

ANALYSIS METHODS OF THE ECHINACEA EXTRACT

Alkamides have been analyzed with reverse-phase HPLC coupled with different detectors including UV spectrophotometric, coulometric electrochemical, and electrospray ionization mass spectrometric.[83,84] Furthermore, caffeic acid derivatives have been determined using reverse-phase HPLC or capillary electrophoresis (CE) with photodiode array (FDA) UV spectrophotometric detection.[85-87] Phenolic acids were analyzed by micellar benzoic acid electrokinetic chromatography (MEKC), both charged and uncharged analytes, based on the use of sodium deoxycholate (SDC), a surfactant in borate buffer (pH 9.2), as well as in the *E. purpurea* extract.[88] However, determination methods for both caffeic acid derivatives and alkamides have been developed in single analysis. Although it is a difficult process to separate these diverse constituents in one analysis, methods for the concurrent determination of caffeic acid derivatives and alkamides have the advantages of reduced time and sample size needed for the analysis.[85] Gradient elution on reverse-phase HPLC has been employed for concurrent analysis of caffeic acid derivatives and alkamides from *E. purpurea* using various detectors such as FDA UV spectrophotometric and electrospray ionization mass spectrometry (EIMS).[79,85] Simultaneous analysis of both mentioned derivatives has also been performed by electrophoresis with FDA UV spectrophotometric detector, together with sodium dodecyl sulfate and hydroxypropyl-β-cyclodextrin in Britton Robinson buffer (10 mM, pH 8.0).[89]

DISCUSSION

Echinacea purpurea has a worldwide reputation for its immunomodulatory and anti-inflammatory properties, capable of modulating of various immune system pathways. There are different classes of secondary metabolites of the plant showing immunostimulatory activity, such as alkamides, caffeic acid derivatives, polysaccharides, and glycoproteins.[7] Among the above, alkamides have been noticed in many studies, since they are absorbed through intestinal, buccal, and esophageal membranes.[14-17] Alkamides' binding with CB1

and CB2 has been considered the possible mechanisms for their immunomodulatory and psychoactive activities, respectively.[10-12,39,40] They increase NF-κB, which regulates the expression of critical genes in innate immune responses and is strongly linked with apoptosis regulation.[90-92] It seems that in the absence of a stimulant (e.g. injury, stress, LPS, viral and microbial pathogens, cytokines and growth factors), the root extract and its compounds have no effect on NF-κB expression.[37,88,89] Based on the results of various experiments, the plant extract and its compounds activate the main players of innate immunity like macrophages, neutrophils, and DCs.[7,25,31,93]

The plant and its compounds have shown different effects in various studies. For instance, alkamides could enhance TNF-α release from alveolar macrophages but have showed no effect on TNF-α in splenocytes,[4] while lozenges of *E. purpurea* decreased TNF-α and IL-6 in six healthy volunteers.[17] However, polysaccharides of the plant could increase production of IL-1, IL-6, and TNF-α by macrophage.[22] The plant extract (in high concentration; 8 mg/ml) also showed potential anticancer activity and antimutagenicity but caused reduction in sperm motility and sperm penetration, *in vitro*.[46-50] The beneficial effect of the plant preparations for patients with cold symptoms remains debatable, as the results of different studies are not entirely in agreement.[54-56] Preparations of *E. purpurea* are contraindicated in autoimmune diseases like HIV infection, and AIDS, but these overviews have not been supported by clinical data.[7,57,58] Cichoric acid has been considered a major antioxidant agent in the plant, whose activity is strengthened with the presence of alkamides.[64,67] Antibacterial and antiviral activities of the plant, especially against influenza viruses, and its larvicidal activity have been reported, too.[35,68,69,75]

Alkamides of *E. purpurea* are mostly purified from nonpolar extracts and they are mainly concentrated in the root of the plant. Aerial parts of the plant are usually not considered a source of alkamides. As can be seen in Figure 1, their structures contain some ethylenic and/or acetylenic bonds with an amide part, which has anisobutylamide or a 2-methylbutylamide moiety.[43,78] The polar extracts of the plant usually contain more polar metabolites, including polysaccharides and glycoproteins.[7,80] Other compounds like cichoric acid, alkaloids, flavonoids, phenolic acids, and amides have also been isolated from the plant[7,82] As alkamides and caffeic acid derivatives have been considered the main and particular secondary metabolites of the plant, different methods have been employed for concurrent or separate analysis of them, such as HPLC.[83,85,86]

The results of the studies.which were controversial suggested that in spite of many successfully completed experiments on *E. purpurea*, many questions remain unanswered regarding beneficial effects of the plant in relieving cold symptoms and how the plant and its compounds stimulate the immune system. The results of a review paper, which focused on common cold treatment with *Echinacea* to assess essential factors of experimental design, indicated that most of the studies reporting positive effects of the plant preparation for use in cases of common cold failed to meet the criteria of experimental design.[94] In particular, there are some more important questions, such as "How does the plant differentially affect NF-κB, TNF-α, and IL-6 in the various functional cells?" and "When are these effects helpful in the body during treatment?"[95] Prominent concerns for future investigations should be the complete recognition of the plant's mechanisms of action, using new complementary methods, which could be essential for promoting the rational use of the plant as well as providing leads for new drugs.

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