Review paper

Immunotropic activity of *Echinacea*. Part I. History and chemical structure

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Abstract

Use of Echinacea species (E. purpurea, E. angustifolia, E. pallida) has a long history in therapy, beginning from North American Indians. They were used internally and topically as antiseptic, anti-inflammatory, and analgesic drugs, for curing burns, snake and insect bites, and also as general immunoenhancer. Chemical compounds such as carbohydrates, fatty acids, derivatives of caffeic and tartaric acids, flavonoids, fitosterols, polisaccharides (heteroxylans and arabinoramnogalactans) and isobutyloamides, poliacetylenes, essential oils and other constituents were detected in fresh plants, and differences depended on the species. Chicoric and caftaric acids were the main phenolic compounds of E. purpurea herb, cynarin was a characteristic component of E. angustifolia roots, echinacoside was the main phenolic in E. angustifolia and E. pallida roots.

Key words: *Echinacea, immune system, antibacterial, anti-inflammatory, healing, antiangiogenic.*

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Introduction

Echinacea is a plant contained within the botanical family of *Asteraceae* (*Compositae*), commonly known as a coneflower. It is a perennial plant, reaching up to 40-150 cm of height, depending on the species. The most characteristic feature of *Echinacea genus* is the shape of its flower. These are basket-type inflorescentia, composed of tubular flowers, placed singly on the stalks.

The genus name stems from a Greek word echinos, meaning a hedgehog. The botaniser Conrad Moench in 1794 was the one, who gave the plant this name, due to the acanthous, prickly scales on a dry seed head. In fact, the flowers of *Echinacea* are spherical, with spiny flower bottoms and long, tongue-shaped petals of corolla, which resemble hedgehog. The basket-type inflorescences measure up to 12 cm in diameter. Pink-purple or white flowers surround a spiny, protuberant central part. *Echinacea* blooms from July to October. So far 9 different species of *Echinacea* have been recognised and described, all of them are naturally endemic only in North America (US, Canada), practically in its south-eastern part, including the Great Lakes area, the Appalachian Mountains and Rocky Mountains. *Echinacea* can be found there growing as perennial plants on the prairies and forests mainly in states of Missouri, Kentucky, Oklahoma and Arkansas.

There are three closely-related species of *Echinacea* being currently used for the pharmaceutical and medical purposes: *Echinacea purpurea* or purple coneflower, *Echinacea pallida* or pale coneflower and *Echinacea angustifolia* or narrow-leaved *Echinacea*. Originated from North America, however currently cultivated also in Europe, all three exhibit similar therapeutic effects. After the acclimatisation process, *Echinacea purpurea* (purple coneflower), can be cultivated also in Poland, providing a full-valued, medical herb. Since 1990 it is also cultivated in China [1-8].

History

The very first descriptions of *Echinacea appeared* in the 18th century, first in 1737, in the *Catalogue of Plants,*

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Fruits, *and Trees Native to Virginia*, written by John Clayton from Virginia and then in 1762, in *Flora Virginica*, published by Frederic Gronovius.

Later on, in 1787, the subject of *Echinacea* has been broached in *Materia Medica Americana*, as *Echinacea* was used in horses in cases of skin ulcers caused by saddles. In 1887, *Echinacea* was also acknowledged by the American Society of Pharmacists, and has been added to its medicine register [9]. However, the plant of *Echinacea* itself and its therapeutic properties were known and used much earlier, by numerous Indian tribes of North America.

They have been all using it commonly and successfully, from skin problems to alimentary tract illnesses, fevers, respiratory and urinary system disorders. The spectrum was even broader, as the plant was used also in snakes and venomous insects bites, contagious diseases, various infections, venereal diseases, wound healing [10, 11].

Subsequently, as soon as analgetic properties of *Echinacea* were discovered, it has been also used as a painkiller and a stimulant. Traditionally, chronic skin inflammations, burns and local infections were also treated with *Echinacea*. This tradition was later on passed to white settlers, who, due to the scarcity of other medicines, were somewhat forced to use the Indian remedy. Gradually, they deepened their knowledge of the medical utilisation of *Echinacea* and it was them in fact, who brought the remedy to Europe on the verge of 19th and 20th century [9, 12].

Originally, *Echinacea* was used in ethno medicine, as a broad-spectrum agent, including antidote against snail venoms. During the 18th and 19th century white inhabitants of US were using the herb and root of *Echinacea* in multiple kinds of afflictions, among which there were arthral pains, cough-related pleuric and intercostal pains, headaches and many others. With regard to its anti inflammatory, sudorific and wound healing properties, *Echinacea* was a remedy against high fevers, and abscessed, infected wounds during the American Civil War. Extracts proved to disinfect and improve the regeneration process of damaged tissues. The same extracts were administered as well in cases of burns, frostbites, bedsores and ulcerations. Moreover, *Echinacea* was administered preventively and therapeutically in patients with liver diseases and biliary tract dysfunction, as it exhibited biligenic, choleretic and spasmolytic actions. It also stimulates the secretion of digestive juices (gastric, pancreatic and intestinal) [13].

In 18th and 19th century, *Echinacea* preparations were used to treat many bacterial and virus infections, parasite diseases and mycosis (such as influenza, herpes simplex, measles, small-pox, herpes zoster, and water-pox, rubeola, angina, syphilis, diphtheria, scarlatina, malaria, upper respiratory tract diseases and sinusitis, vaginal yeast, cryptococcosis) or even cases of rabies. Taking into account the broad spectrum of therapeutic use, in the late 19th century *Echinaceas* were officially introduced to the US pharmacopoeia by John King and John Uri Lloyd. First clinical trials of *Echinacea* were conducted by Meyer and King in a Cincinnati hospital [14-16].

Consequently, an immensely broad medical utilisation, as well as a raising, public and scientific interest in *Echinacea* brought about such a popularity, that in the twenties of 20th century *Echinacea* had become the bestselling medical plant in the US.

In Europe *Echinacea* first appeared between 19th and 20th century as a homeopathic agent, after a while however, it became a commonly administered medicine. In the thirties of 20th century, Gerhard Madaus launched in Germany a cultivation of *Echinacea* on an industrial scale.

The development of chemotherapy in medicine led to the loss of interest in medical plants (including *Echinacea*), even though the research had been previously started on a larger scale. Intense trials conducted worldwide managed to restore the interest and create the scientific basics to use *Echinacea* as an immunomodulator. Since the seventies onwards, in Canada and US a great increase in the interest in *Echinacea* has been observed.

Today, numerous, immune-stimulating preparations produced from roots and herbs of *Echinacea purpurea* or *angustifolia* are available on the market. They contain various active substances and it is now well known that several groups of those compounds are concerned in the process of immune-stimulation [17-20].

Active compounds

The raw materials used for the manufacture of medicinal preparations from *Echinacea* are both blooming herbs and their roots. All three species of *Echinacea* being used in therapy can be characterised by an outstanding diversity of substances, which can affect the parameters of the immune system. Due to the abundance of those substances (various additionally in each species), obtaining the standardised preparations was highly problematic. Among many others, chemical compounds such as carbohydrates, fatty acids, derivatives of caffeic and tartaric acids, flavonoids, fitosterols, polisaccharides (heteroxylans and arabinoramnogalactans) and isobutyloamides (the last two being highly immune-stimulating), poliacetylenes and essential oils were detected in a fresh raw material. Chicoric and caftaric acids were the main phenolic compounds of *Echinacea purpurea* herb, cynarin was a characteristic component of *Echinacea angustifoli*a roots, echinacoside was the main phenolic in *Echinacea* angustifolia and *Echinacea pallida* roots [21-25].

Echinacea is well known and used as an immunostimulating agent. It has been proved to increase the number of white blood cells and potentiate the process of phagocytosis by macrophages and granulocytes. *Echinacea* is a wide-spectrum immunomodulator that modulates both innate and adaptive immune responses [26-29].

It is known that thanks to the inhibition of the enzyme hyaluronidase, the cell membranes structure, collagen and proteoglycans are strengthened, which impedes the penetration of pathogens into the vulnerable tissues [30].

It is also correlated with the increase of interferon (INF) release, which immunize the organism against viral infections and limits its proliferation. Moreover, thanks to a decrease in secretion of inflammatory mediators such as histamine, prostaglandins or leukotrienes, the anti-inflammatory and anti-exudative effect of *Echinacea* is exhibited. Keratinocytes and Langerhans-cells in epithelium are stimulated, they present the antigens to lymphocytes, which leads to the release of intercellular, signalling proteins – cytokines. Another regenerating activity of *Echinacea* is a stimulation of collagen and elastic fibres synthesis in chondroblasts, osteoblasts and fibroblasts. Metabolic conversion is generally intensified, the tonic and regenerating action is demonstrated both locally or throughout the whole organism, depending on the way of administration and chemical composition of the preparation [31-33].

Among many chemical compounds, playing the role of active substances contained in the extracts of *Echinacea* there are numerous, different, chemical groups ranging from carbohydrates, phenolic compounds being very common in the aboveground parts of the plant, all the way to polyterpens and antibiotic-like type of substances.

Diversity aside however, only the physiologically active, secondary metabolites are of high, therapeutic significance. They are all the products of special metabolic conversions and cannot be related to any basic life-function, unlike primary metabolites such as monosaccharides, amylum, lipids, chlorophyll, all the other pigments, amino acids, proteins and nucleic acids, which are all essential to the life of the plant.

Presently, it is acknowledged that some plants (including *Echinacea*) are able to synthesise aromatic compounds, mainly phenols and their derivatives. Phenols in their free form are often a component of essential oils. They can also occur in a glycoside-form. As phenolic acids, they are almost constantly present in plants, mainly as caffeic acid or its ester, chlorogenic acid. The antiseptic (antibacterial, disinfection) properties of natural phenols are commonly known. Polyphenols influence omnidirectionally the humoral immune response by modulating the release of various cytokines. Phenolic acids contribute also to the production of antibodies. Chlorogenic and caffeic acids intensify *in vitro* the migration activity of macrophages in mice. Yet, it is not to be forgotten that during the air-drying of fresh *Echinaceas* over 30% of active substances decompose. Many valuable components (including the chicoric acid) may be lost during the improper process of extraction [34-36].

In the last decade of 20th century the medical use of *Echinacea* was once again intensively examined. Substances obtained from this plant tempt the scientists with its antiviral, antibacterial, antifungal, anti inflammatory, immunestimulant and angiogenesis modulation effects. Phenols and phenolic acids are one of the simplest bioactive compounds present in *Echinacea*. Caffeic acid and its derivatives, which come under the caffetanins, exhibit antiviral, antibacterial and antifungal activity. It is likely that their toxicity against microorganisms stems from the presence of hydroxyl group, situated in phenolic ring. Some authors stated that with the increase of hydroxylation, the activity is also augmented but this remains to be clarified. On the other hand however, there are publications concerning the flavonoids, which despite the loss of hydroxyl group in the phenolic ring maintain intense and effective activity against microorganisms. This might be explained by higher lipophilicity and therefore greater affinity to pathogen's bilayer lipid membrane, which is the target of action.

Flavonoids are, in fact, able to form complex compounds with extracellular, soluble proteins and the elements of bacterial cell wall.

Phenolic acids and their esters have moreover strong anti inflammatory and antioxidant properties, that enables to maintain the right balance between oxidants and reductants, being essential to organism homeostasis. In a full competence of immunological system, the active oxidants destroy pathogens and then gradually undergo the inactivation by both extra and intracellular enzymes. All pathological conditions correlated with chronic inflammatory response favour the production of oxidants, which leads to their surplus, consequently – the neutrophil oxidase NADPH and myeloperoxidase are being activated excessively, producing constantly more and more different forms of active oxidants. Due to the superabundance of oxidants in organism, numerous detrimental reactions occur, such as the oxidation of lipids, intracellular organelles, nuclear membranes and unsaturated fatty acids.

Furthermore, inflammations with increased number of free radicals favour the promotion of tumours. Some of the substances contained in *Echinacea* are thus used in dermatology and cosmetology, as they inactivate the free oxygen radicals and by that, help to protect the nucleic acids, proteins and many other microstructures in living skin cells [37, 38].

Phenolic antioxidants inhibit the NF-κB (nuclear factor κB) and AP1, which participate in the production of proinflammatory cytokines interleukin 1α (IL-1α), IL-6 and tumor necrosis factor α (TNF-α).

Phenolic acids present in *Echinacea* may be used in prevention of inflammation. The molecular mechanism of anti inflammatory activity of CAPE (caffeic acid phenetyl ester) has been so far the best known and described.

It has been observed that CAPE completely block the NF-κB, produced in cells under the influence of inflammation process inductors – TNF-α and the esters of phorbol. Caffeic acid phenetyl ester in a specific way prevents the translocation of p65 subunit in NF-κB, and therefore inhibits the binding of NF-κB to DNA [39-43].

Flavonoids also directly inhibit lipooxygenase and cyclooxygenase thereby preventing the conversion of arachidonic acid to prostaglandins, leukotrienes and thromboxanes. Quercetin present in the herb of *Echinacea* has an ability to inhibit the viral replication by activating NF-κB. Just like hesperetin and catechin it counteracts Herpes simplex type-1 (HSV-1), polio type-1, RSV and human parainfluenza virus type-3 proliferation. The flavonol quercetin has an additional, universal, anti-infective action.

Compound such as quercetin, CAPE and few other flavonoids present in *Echinacea* inhibit the HIV-1 integrase. Some authors state in their publications, that administering the remedies based on *Echinacea* to patients with AIDS is risky, others however, that it is moderately hazardous whereas the clinical benefits are significant.

Immunological effect of *Echinacea* is mainly an immunomodulation, that is, a stimulation of the production of antibodies, cytokines, growth factors and a direct modification of effector cells functions (i.e. phagocytosis, cytotoxicity). Substances, originated from this plant, can also influence both the thymus itself (central immunological organ) and its cells – thymocytes, as well as various subpopulations of thymus-dependent T-lymphocytes.

In *Echinacea angustifolia* it is a root, which is the most commonly used raw-material, while in *Echinacea purpurea* the aboveground parts play a more important role. Despite the differences in type and content of the immunologically active substances in both of the species, they happen to exhibit similar anti-oxidative action, which stems from the synergistic action of their ingredients [44].

The herb of *Echinacea* contains up to 0.32% of volatile, liquid compounds, being called ethereal (or essential) oils. These are the secondary metabolites, structurally based on isoprene.

Apart from the influence (fragrance) on human psyche, they demonstrate somewhat an antimicrobial activity. Ethereal oils are mixtures of dozens or even hundreds of chemical substances of numerous functions, which are not all structurally explored.

The predominant ingredient of ethereal oils are monoterpenes or their oxygen derivatives – alcohols, aldehydes and ketones. Di-, tri-, tetra- or sesqiterpenes (in the root) can be also present.

Over 1000 compounds has been so far discovered in oils and sesqiterpenes resins and the number is still growing. They inhibit the proliferation of Gram-positive bacteria such as: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Propionibacterium acnes* and *β-hemolitic Streptococcus*, as well as Gram-negative bacteria such as: *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Acinetobacter*, *Escherichia coli*, *Shigella sonnei*, *Proteus mirabilis*, *Legionella* sp. Ethereal oils prevent additionally the proliferation of fungi: *Candida albicans*, *Aspergillus niger.* The antiviral activity against influenza virus was also observed. Substances present in ethereal oils proved themselves to be effective in the treatment of acne, herpes, dandruff, psoriasis, burns and others. The antibacterial, antiviral, antifungal and anti-protozoa activity is a result of the presence of active, chemical compounds in ethereal oils.

The bioactivity of the *Echinacea* extracts and their stimulating immune system properties are closely correlated with alkamides, mainly the isobutyloamides of dodecatetraenic acid, polisaccharides, glycoproteins, phenolic acids and their derivatives. The roots of *Escherichia angustifolia* contain echinacoside and cinarine, which both exhibit antibacterial and antiviral properties. Alkamides, phenolic acids (caffeic, chlorogenic and chicoric) and other thermo-stable fractions of *Escherichia purpurea* extracts also act antivirally and antifungally.

Alkamides are thermo-labile, hence the use of high temperature during the process of their production may inactivate them and change the bioactivity of the whole, obtained preparation.

It could be perhaps the explanation of differences between the activity of preparations manufactured by different producers and between liquid and powdered preparations, especially if the process of drying requires higher temperatures.

Echinaceas react on many elements of immune response, stimulating both specific and non-specific immunity. The main mechanisms of their action are to stimulate phagocytosis and to augment the antibacterial activity of macrophages, then to inhibit the production of both endo and exogenous hyaluronidase, thereby limiting the spreading of infection and inflammation. They may also stimulate the T-lymphocytes-dependent cell-type immunity, the cytotoxic cells and the cytokines production including interferons, TNF-α, IL-1 and others. Another mechanism can be either to activate the alternative route of complement system by increasing the production of P-factor (Properdin), or to inhibit the lipooxygenesis, or finally either to stimulate the production of antibodies, or to intensify the immunological angiogenesis (which could be of high significance in the treatment of the impairments of recovery processes in wound healing, bone fractures and ulcerations or even in future cardiology as a prevention against ischaemic heart disease or myocardial infarctions).

Glycoproteins, being present in similar quantities in roots of both *Echinacea purpurea* and *Echinacea angustifolia*, stimulate the activity of type-B lymphocytes and the production of cytokines by macrophages [45-53].

However, one should remember, that many herbal remedies (among them extracts of *Rhodiola* and *Echinacea* plants), being stimulatory in lower doses, in higher doses presented inhibitory effects, affecting proliferation and mobility of lymphocytes [54].

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